Phloeodictines A and B : new antibacterial and cytotoxic bicyclic amidinium salts from New Caledonian sponge, Phloedictyon sp

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Phloeodictines A and B: New Antibacterial and Cytotoxic Bicyclic Amidinium Salts from the New Caledonian Sponge, *Phloeodictyon* sp.[1]

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Two new alkaloids, phloeodictine A (1) and phloeodictine B (2), possessing an unprecedented 6-hydroxy-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrimidinium skeleton have been isolated from an undescribed species of the deep sponge *Phloeodictyon*. The structures were determined by extensive spectroscopic analysis particularly two-dimensional NMR experiments. Both compounds exhibited in vitro antibacterial activity against Gram-positive and Gram-negative bacteria and were moderately cytotoxic against KB cells.

As an outgrowth of our search for biologically active compounds from marine organisms, we report here that extracts from an undescribed species of the deep water sponge *Phloeodictyon*[2] (family Nepheliospongia, order Nepheliospongidae), collected in the south of the New Caledonian lagoon, strongly inhibit the growth of bacteria and are moderately cytotoxic. Bioassay-guided purification of the crude extract resulted in the purification of two novel bicyclic amidinium salts with a unique 6-hydroxy-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrimidinium skeleton which we have named phloeodictine A (1) and phloeodictine B (2). This paper describes the isolation and structure elucidation of 1 and 2.

Phloeodictyon sp. was kept frozen until workup. The lyophilized sponge was homogenized and consecutively extracted with heptane and methanol. The antimicrobial methanolic extract was desalted over Amberlite XAD-7 and subsequently subjected to medium-pressure reversed-phase liquid chromatography (H_2O–MeOH step gradient). Final purification of 1 and 2 was accomplished by repetitive preparative and semipreparative RP-HPLC [Delta-Pak C18, MeOH–NaCl (0.2 M)–THF (56:43:1 for

(1) Presented in part at the Third Pacific-Asia Symposium on Biologically Active Natural Products, Noumea, New Caledonia, 26–30 Aug 1991.

(2) The sponge was identified by Prof. C. Levi, Museum National d'Histoire Naturelle, 75005 Paris. To our knowledge, specimens of this genus have not been chemically studied previously.

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Table I. ^{13}C (62.5-MHz) and ^{1}H (400-MHz) NMR Data of Phloeodictine A (1)[a] and Long-Range Correlations from HMBC Experiments

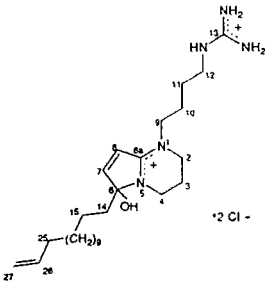
position	δ ^{13}C (m)	δ ^{1}H (m, J, Hz)	HMBC (^{1}H)
2	46.7 (t)	a 3.52 (m)	H-9ab,[b] H-3ab[a,c]
		b 3.50 (m)	
3	20.1 (t)	a 2.17 (m)	H-2ab,[d] H-4ab[d]
		b 2.00 (m)	
4	37.0 (t)	a 3.50 (m)	H-2ab,[c] H-2ab,[a] H-3ab[a,c]
		b 3.34 (m)	
6	98.5 (s)		H-7,[d] H-8,[c] H-14ab,[d] OH[b]
7	153.1 (d)	7.30 (d, 6.5)	H-14a,[a,c] H-8[a]
8	121.0 (d)	7.05 (d, 6.5)	H-7,[d] OH[c]
8a	160.1 (s)		H-2ab,[d] H-4ab,[d] H-7,[d] H-8,[d] H-9ab[d]
9	53.3 (t)	a 3.57 (m)	H-10,[a,c] H-11[d]
		b 3.48 (m)	
10	26.2 (t)	1.76 (m)	H-9ab,[d] H-11,[c] H-12[d]
11	26.1 (t)	1.65 (m)	H-9ab,[d] H-10,[c] H-12[d]
12	42.0 (t)	3.25 (m)	H-10,[d] H-11[a,c]
13	158.0 (s)		H-12[d]
14	36.0 (s)	a 2.00 (m)	H-15,[a] OH[b]
		b 1.76 (m)	H-14[a,c]
15	24.8 (t)	1.14 (m)	H-14[a,c]
16–24	30.1–30.9 (t)	1.25–1.35 (br s)	H-14,[a] H-15,[d] H-25[d]
25	34.9 (t)	2.00 (m)	H-27ab[c]
26	139.9 (d)	5.80 (ddt, 10,17,7)	H-25[d]
27	115.4 (t)	a 5.01 (dd, 2, 17)	H-25[d]
		b 4.92 (dd, 2, 10)	
OH		6.95 (s)[b]	
NH_2		6.90–7.70 (br s)[b]	
NH		8.20 (br s)[b]	

[a] In CD_3OD at rt except as noted. [b] In DMSO-d_6 at rt. [c] In DMSO-d_6 at 70 °C. [d] Correlations observed under all conditions run.

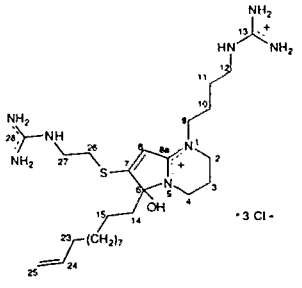
1 and 66:33:1 for 2), pH adjusted to 2.2 with HCl]. Typical yields were 0.004% for 1 and 0.02% for 2 (dry weight sponge). Preliminary comparison of the spectral data of 1 and 2 showed that the two compounds were closely related, and structure elucidation was carried out primarily on the most abundant 1.

Phloeodictine A (1) was obtained as a colorless amorphous solid. The molecular formula $C_{26}H_{48}N_5O$, calculated from the parent ion (M^+, m/z 446.3891, −3.2 mmu error) in the HRFAB mass spectrum and confirmed by ^{13}C NMR methods, required 5.5 unsaturations and pointed out that 1 must be a quaternary salt. This indication was corroborated by elemental analysis (C, H, N, and Cl) which showed that 1 was isolated as a dichloride. The UV spectrum exhibited absorptions at 224 (ε 6700) and 274 (2200) nm suggesting a heterocyclic chromophore. A positive coloration with Sakaguchi reagent and a ^{13}C NMR signal at δ 158.0 (s) established 1 as a guanidine derivative.[3] The IR spectrum was of limited value but did confirm the presence of a guanidine moiety[4] by absorbances at ν_{max} 3100–3440 and 1665 cm^{-1}. The ^{1}H and ^{13}C NMR spectrum (Table I) revealed the presence of three additional unsaturations: a terminal allyl group [δ_C 139.9 (d) and 115.4 (t); δ_H 5.80 (ddt, 1 H), 5.01 (dd, 1 H) and 4.92, dd, 1 H], a disubstituted olefin [δ_C 153.1 (d) and 121.0 (d); δ_H 7.30 (d) and 7.05 (d)], and an unpaired sp^2 carbon (δ 160.1, s). The remaining unsaturations required by the molecular formula had to be satisfied by two rings.

The ^{1}H NMR spectrum of 1 also disclosed a deuterium exchangeable signal for hydroxyl (δ 6.95, br s) and five exchangeable protons [δ (DMSO-d_6) 8.20, br s, 1 H; 6.90–7.70, br s, 4 H] exhibiting cross peaks in the ^{1}H-detected heteronuclear one-bond ^{1}H–^{15}N correlation



1



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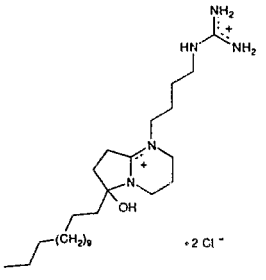

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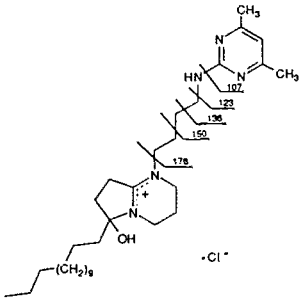

4

(HMQC)[5] spectrum and therefore assigned to the NH and NH_2 protons of the protonated guanidine functionality. This suggested that the two remaining nitrogens are either tertiary or quaternary. The assignments of all protonated carbons were established by the ^{1}H-detected heteronuclear one-bond ^{1}H–^{13}C correlation experiment (HMQC).[5]

Careful analysis of double quantum filtered phase-sensitive COSY (DQF-COSY)[6,7] spectra in CD_3OD, simultaneous with the evaluation of the homonuclear Hartmann–Hann (HOHAHA)[8,9] spectrum in DMSO-d_6, verified four spin-coupled networks. The six methylenic protons on C-2–C-4 constituted an isolated system and were involved in a six-membered heterocyclic ring from chemical shift considerations; the protons on C-7 and C-8 exhibited an AX spin system and were present in a pentacyclic ring according to the value of $^{1}J_{H-H}$ (6.5 Hz). Additional features included an aliphatic long chain with a terminal allyl group from C-14 to C-27 and an *N*-butylguanidine side chain from C-9 to C-13. Key long-range ^{1}H–^{1}H COSY[10,11] correlation peaks (DMSO-d_6) from H-12 to NH (δ 8.30) and HOHAHA peaks from H-9ab, H-10, H-11, and H-12 to NH verified the location of the guanidino group. The structural subunit from C-9 to C-13 was also supported by diagnostic peaks in the HRFAB mass spectrum of 1 at m/z 333.1965, Δ 0.2 mmu, $C_{22}H_{25}N_2O$, formed by α cleavage of this side chain with concomitant hydrogen rearrangement and of an ion of low mass at m/z 114.1038, Δ −0.7 mmu, $C_5H_{12}N_3$, ascribed to the molecular pic CH_2=CH-$(CH_2)_2$NHC=$N^+H_2(NH_2)$.

Further information on the structural framework was obtained through a series of ^{1}H-detected heteronuclear multiple-bond correlation (HMBC)[12] spectra (Table I).

(3) Carter, G. T.; Rinehart, K. L. *J. Am. Chem. Soc.* **1978**, *100*, 4302–4304.
(4) Sharma, G.; Magdoff-Fairshild, J. *J. Org. Chem.* **1977**, *42*, 4118–4124.
(5) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565–569.
(6) Shaka, A. J.; Freeman, R. *J. Magn. Reson.* **1983**, *51*, 169–173.
(7) The DQF-COSY spectrum of 1 (CD_3OD) clearly showed the following connectivities (H–H): 2ab–3ab, 3ab–4ab, 7–8, 9ab–10, 10–11, 11–12, 14a–15, 15–(16 to 24), (16 to 24)–25, 25–26, 26–27ab.
(8) Bax, A.; Davies, D. G. *J. Magn. Reson.* **1985**, *65*, 355–360.
(9) The HOHAHA spectrum of 1 (DMSO-d_6) afforded the following correlations (H–H): 2ab–3ab, 2ab–4ab, 3ab–4ab, 7–8, 9ab–10, 9ab–11, 9ab–12, 10–11, 10–12, 10–NH, 11–12, 11–NH, 12–NH, 14ab–15, 14ab–(16 to 24), 15–(16 to 24), (16 to 24)–25, 26–26, 25–27, 26–27.
(10) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542–561.
(11) The LR-COSY (with an additional evolution time of 75 M) of 1 afforded the following connectivities (H–H): 2ab–3ab, 2b–4b, 3a–4b, 7–8, 9ab–10, 10–11, 11–12, 12–NH, 14ab–15, 15–(16 to 24), (16 to 24)–25, 25–26, 25–27, 26–27.

The positions of C-2 and C-4 with respect to C-8a were deduced from the long range ^1H-^{13}C correlations observed between H-2ab and C-8a and between H-4ab and C-8a. On the basis of the lower field resonance of C-2 (δ 46.7), C-4 (δ 37.0), and C-8a (δ 160.1), and in order to satisfy the molecular composition determined by positive ion FABMS, two nitrogen atoms were inserted at positions 1 and 5, thus allowing the tetrahydropyrimidine ring A to be closed. However, at this point, it was not clear which of C-2 or C-4 should be placed at the α-position to N-1. Vital new insights were provided from the *N*-butylguanidine fragment where the methylene protons on C-9 showed HMBC couplings to both C-2 and C-8a. Considering the ^1H and ^{13}C chemical shift of CH_2-9 (δ_C 53.3; δ_H 3.57 and 3.48, H-9ab) which suggested nitrogen substitution, this methylene was attached to the N atom at position 1, thus defining C-2 as vicinal to N-1.

Another ring was still needed by the molecular formula. The allylic side chain C-14–C-27 was deduced to be attached to the sp^3 carbon at position 6, since long-range ^1H–^{13}C correlations were observed between H-14ab and C-6. Cross peaks of C-6 and C-8a to H-7 and of C-6 and C-8a to H-8 in the HMBC spectrum of **1** allowed the $\Delta^{7(8)}$ double bond to be located between the quaternary carbons C-6 and C-8a. A cross peak between H-14ab and C-7 indicated that the latter was at the α-position to C-6. The chemical shift of C-6 argued for substitution by two heteroatoms. The best fit would be achieved by substitution with both oxygen and nitrogen rather than two nitrogens.[13] Oxygen substitution was confirmed by a two-bond coupling between the tertiary hydroxyl (δ 6.95, s) and C-6. Given the chemical composition of **1**, nitrogen substitution can be achieved by connecting C-6 to either N-5 or N-1. The presence of an intense NOE cross peak between H-8 and H-9ab in the ROESY[14,15] spectrum (DMSO-d_6) of **1** established the C-6/N-5 bond, leading to the obtention of an amidinium group in **1** and allowing B ring to be closed. The skeletal framework shown in **1** was therefore proposed. The CD spectrum showed that **1** was optically active [CD (MeOH) λ 215 ($\Delta\epsilon$ −1.91) nm]; the absolute stereochemistry of the chiral center at C-6 was not assigned.

Catalytic hydrogenation of **1** over palladium/charcoal in MeOH led to the tetrahydro derivative **3** [FABMS, *m/z* 450, M^+] with a modified UV [λ_{max} (MeOH) 219 nm (ϵ 9700)]. Compound **3** was converted to its 4,6-dimethylpyrimidine derivative **4** [HRFABMS *m/z* 514.4486, Δ −0.2 mmu, M^+, $C_{31}H_{56}N_5O$] by treatment with acetylacetone in a sodium bicarbonate solution.[16,17] The electron-impact mass spectrum of **4** showed a series of peaks from *m/z* 107 to 178 (see formula **4**), which establishes the [(4,6-dimethyl-2-pyrimidyl)amino]butane unit.[18]

Phloeodictine B (**2**), found to be more polar than **1**, was also obtained as a colorless amorphous solid. The molecular formula, $C_{27}H_{51}N_8OS$, was derived from HRFABMS (M^+, *m/z* 535.3900, Δ 0.6 mmu, $C_{27}H_{51}N_8OS$). Elemental analysis (S, Cl) confirmed the presence of sulfur and indicated that **2** was isolated as a trichloride salt. The UV spectrum of **2** exhibited maxima at 279 (ϵ 7100) and 202 (5500) nm, suggesting the presence of a distinct UV chromophore than **1**.

Table II. ^{13}C (50-MHz) and ^1H (400-MHz) NMR Data of Phloeodictine B (2)[a] and Long-Range Correlations from HMBC Experiments

position	δ ^{13}C (m)	δ ^1H (m, *J*, Hz)	HMBC (^1H)
2	46.6 (t)	3.60 (m)	
3	20.6 (t)	a 2.20 (m)	
		b 2.05 (m)	
4	37.3 (t)	a 3.58 (m)	
		b 3.34 (m)	
6	98.8 (s)		H-8,[a,b] H-14ab,[b] OH[b]
7	169.8 (s)		H-14a,[b] OH,[b] H-26[a,b]
8	108.6 (d)	6.95 (s)	
8a	159.8 (s)		H-8,[a,b] H-9[a,b]
9	53.1 (t)	3.75 (m)	
10	26.6 (t)	1.83 (m)	H-12[b]
11	26.2 (t)	1.68 (m)	H-12[b]
12	42.2 (t)	3.25 (m)	
13	158.6 (s)		H-12[b]
14	36.4 (t)	a 2.05 (m)	
		b 1.83 (m)	OH[b]
15	23.6 (t)	1.14 (m)	H-14ab[b]
16–22	30.1–30.6 (t)	1.25–1.35 (br s)	H-23[a,b]
23	34.8 (t)	2.05 (m)	H-24,[b] H-25ab[b]
24	140.1 (d)	5.80 (ddt, 10,17,7)	H-23[a,b]
25	114.7 (t)	a 5.01 (dd, 2, 17)	H-23[a,b]
		b 4.92 (dd, 2, 10)	
26	31.7 (t)	3.40 (m)	
27	40.9 (t)	3.60 (m)	
28	158.6 (s)		
OH		7.13 (s)[b]	
NH_2		6.70–7.70 (br s)[b]	
NH		8.25 (br s)[b]	

[a] In CD_3OD except as noted. [b] In DMSO-d_6.

The ^1H and ^{13}C NMR spectra of compound **2** (Table II) were almost identical to those of **1** except for differences related to the length of the allylic chain (C-14–C-27 in **1**, C-14–C-25 in **2**) and to the B ring portion of the molecule. The resonances associated with the $\Delta^{7(8)}$ double bond were conspicuously absent and were replaced by a quaternary signal at δ_C 170.0 (C-7) and by a methine signal at δ_C 108.8 (C-8) associated with a proton singlet at δ_H 6.95. This is best explained by substitution at C-7. The respective positions of C-7 and C-8 were determined based on the following: a long-range HMBC correlation was seen from the hydroxyl proton signal (δ 7.13, s, 1 H) to C-6 and C-7; furthermore, a three-bond correlation was observed from H-8 to the C-8a amidinium carbon (δ 159.9, s) which was further correlated to the H-9 protons. An additional difference in the NMR spectra of **2** lied in the presence of two methylene resonances (δ_C 38.7 and 30.4; δ_H 3.65 and 3.45) and a new quaternary overlapping signal (δ_C 158.5) assigned to a guanidino group. Evaluation of the HMQC spectrum of **2**, simultaneous with the analysis of DQF-COSY[19] and HOHAHA[20] correlations [COSY (DMSO-d_6) cross peaks for H-26/H-27, H-27/NH and HOHAHA (CD_3OD) cross peaks H-26/H-27] allowed an *N*-ethylguanidine moiety to be proposed, which must be the substituent at position 7. The connection from C-7 to C-26, therefore, remained to be elucidated. From chemical shift arguments and as required by the molecular formula, these two carbons were suggested to be connected through a

(12) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
(13) Kalinowski, H. O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: New York, 1988.
(14) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–812.
(15) The ROESY spectrum of **1** showed the following correlations (H–H): 7–14a, 8–9ab, 8–10, 8–11.
(16) Shemyakin, M. M.; Ovchinnikov, Yu. A.; Vinogradova, E. I.; Feigina, M. Yu.; Kiryushkin, A. A.; Aldanova, N. A.; Alakhov, Yu. B.; Lipkin, V. M.; Rosinov, B. V. *Experientia* **1967**, *23*, 428–430.
(17) Attempts to obtain the 4,6-dimethylpyrimidine derivative directly from **1** without previous hydrogenation was unsuccessful because **1** was unstable under basic conditions.
(18) Cheng, M. T.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1978**, *100* (23), 7409–7411.
(19) The DQF-COSY of **2** revealed the following correlations (H–H); 2–3ab, 3b–4b, 9–10, 10–11, 11–12, 12–NH, 14ab–15, 15–(16 to 22), (16 to 22)–23, 23–24, 24–25ab, 26–27, 27–NH.
(20) The HOHAHA spectrum of **2** afforded the following correlations (H–H): 2–3ab, 2–4ab, 3ab–4a, 9–10, 9–11, 9–12, 10–11, 10–12, 11–12, 14ab–15, 15–(16 to 22), (16 to 22)–23, 23–24, 23–25ab, 24–25ab, 26–27.

sulfur atom. This connectivity was supported by the observation of a cross peak of H-26 to C-7 in the HMBC spectrum. The structure of phloeodictine B was, consequently, concluded to be **2**.

Compounds **1**, **2**[21] and **4** have been tested against several bacteria using the standard microdilution plate assay and were found to possess significant activity with the following respective MIC's (μg/mL): *Streptococcus fecalis* (5, >15, >15), *Staphylococcus aureus* (1, 3, 3), *Escherichia coli* (1, 30, >30), and *Pseudomonas aeruginosa* (10, >30, >30). These substances also exhibited in vitro cytotoxicity toward KB human nasopharyngeal carcinoma cells with IC_{50}'s of 1.5, 11.2, and 7.0 μg/mL for **1**, **2** and **4**, respectively. Phloeodictine A and phloeodictine B represent the first naturally occurring members of the bicyclic 1,2,3,4-tetrahydro-6*H*-pyrrolo[1,2-*a*]pyrimidinium ring system. The isolation from *Phloeodictyon* sp. of other bioactive compounds belonging to this class of alkaloids is currently under way in our laboratory. The origin and biosynthetic pathway of these metabolites remain to be resolved.

Experimental Section

Collection, Extraction, and Separation. *Phloeodictyon* sp., a firm sponge possessing a high spicule content, was collected in the course of the dragging campaigns of the ORSTOM-CNRS Programme "Substances Marines d'Intérêt Biologique" (SMIB) in the south of the New Caledonian lagoon at a depth of 235 m, at Kaimon Maru Mountain. A taxonomic voucher specimen is deposited at the ORSTOM Centre in Noumea, under the reference R1411. The animals (1.5 kg fresh weight) were lyophilized immediately and transferred to Gif-sur-Yvette. The sponge (450 g dry weight), stored at −20 °C, was homogenized and consecutively extracted with heptane (3 L × 2) and MeOH (3 L × 3). Evaporation of the methanol soluble portion under reduced pressure afforded a crude extract (50.2 g) exhibiting antibacterial activity. Desalting of the methanolic extract over Amberlite XAD-7 using a H_2O–CH_3OH system afforded an active fraction (18.5 g) which was chromatographed under RP medium-pressure liquid chromatography by using a C-18 stationary phase (55–105 μm, 25 cm × 30 mm) and a step gradient of H_2O–CH_3OH as eluent. Fractions 2 (1.6 g) and 4 (1.5 g) eluting with H_2O–CH_3OH (6:4) and (2:8), respectively, were subjected to preparative HPLC using a Delta Prep 3000 chromatography system [Prepak cartridge, Delta-pak C18, 15 μm, 100 Å, 47.0 mm × 30.0 cm; flow rate 100 mL/min; UV double detection at 230 and 280 nm; eluent MeOH–NaCl (0.2 M)–THF (56:43:1 for fraction 2 and 66:33:1 for fraction 4), pH 2.2 with HCl]. Final purification (Delta-Pak C-18, 15 μm, 100 Å, 25.0 mm × 10.0 cm, flow rate 8 mL/min, UV detection 230 and 280 nm) using the same solvent systems afforded compound **1** (94 mg, $k' = 15.5$) from fraction 2 and compound **2** (18 mg, $k' = 12$) from fraction 4.

Phloeodictine A (1): colorless amorphous solid; CD (MeOH) λ 215 (Δε −1.91) nm; UV (MeOH) λ_{max} 224 (ε 6700) and 274 (2200) nm; FTIR (film) ν_{max} 3440–3080, 2930, 2850, 1665, 1590 cm^{-1}; ^1H and ^{13}C NMR (Table I); FABMS [poly(ethylene glycol) + glycerol + MeOH matrix] *m/z* 446 (M^+, 45.6), 333 ($M^+ - C_5H_{11}N_3$, 14.2), 114 (100); HRFABMS *m/z* 446.3891 ($C_{26}H_{48}N_5O$ requires 446.3858), 333.1965 ($C_{22}H_{25}N_2O$ requires 333.1966), 114.1038 ($C_5H_{12}N_3$ requires 114.1031). Anal. Calcd for $C_{26}H_{48}N_5O$, 2HCl: C, 60.09; H, 9.69; N, 13.47; Cl, 13.64. Found: C, 59.72; H, 9.60; N, 13.31; Cl, 13.52.

Phloeodictine B (2): colorless amorphous solid; CD λ 220 (Δε −0.32) nm; UV (MeOH) λ_{max} 279 (ε 7100) and 202 (5500) nm; FTIR (film) ν_{max} 3440–3080, 3020, 2930, 1665 cm^{-1}; ^1H and ^{13}C NMR (Table II); FABMS (glycerol + HCl matrix) *m/z* 535 (M^+, 9), 114 (70); HRFABMS *m/z* 535.3900 ($C_{27}H_{51}N_8OS$ requires 535.3906), 114.1060 ($C_5H_{12}N_3$ requires 114.1031). Anal. Calcd for $C_{27}H_{51}N_8OS$, 3HCl: S, 4.97; Cl, 16.48. Found: S, 4.91; Cl, 16.30.

Hydrogenation Derivative 3. A methanolic solution (15 mL) of **1** (38 mg, 0.085 mmol) and 10% Pd/C (ca. 20 mg) were shaken for 3 h under an atmosphere of hydrogen. After removal of the catalyst and the solvent, the hydrogenated derivative **3** was obtained (35 mg, 92% theoretical yield) as a colorless amorphous solid: UV (MeOH) λ_{max} 219 nm (ε 9700); FTIR (film) ν_{max} 3440–3176, 3019, 2928, 2855, 1668 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 3.16 (m, H-7a), 3.06 (m, H-7b), 2.38 (m, H-8a), 2.09 (m, H-8b), 1.85–1.71 (m, H-10/14ab), 1.42 (m, H-26), 1.35–1.25 (br s, H-16 to 25), 0.89 (m, Me-27); ^{13}C NMR (D_2O, 62.5 MHz) δ 164.7 (s, C-8a), 158.0 (s, C-13), 98.5 (s, C-6), 53.3 (t, C-9), 46.1 (t, C-12), 38.4 (t, C-14), 38.0 (t, C-4), 33.1 (t, C-7), 32.5 (t, C-25), 31.1-30.6 (t, C-16 to C-24), 28.8 (t, C-8), 26.4 (t, C-10), 25.2 (t, C-11), 24.8 (t, C-15), 23.8 (t, C-26), 20.0 (t, C-3), 15.0 (q, C-27); FABMS (glycerol + HCl matrix) *m/z* 450 (M^+, 100), 337 (18) and 114 (50).

4,6-Dimethylpyrimidine Derivative 4. Compound **3** (30 mg, 0.07 mmol) was dissolved in 95% EtOH (1 mL) and H_2O (0.5 mL) containing $NaHCO_3$ (0.03 g, 0.36 mM). Acetylacetone (70 μL, 0.68 mM) was added, and the mixture was refluxed for 3 h. The solution was then neutralized with HCl, filtered, and evaporated until dryness. After removal of NaCl by precipitation in $CHCl_3$–EtOH (85:15), filtration, and evaporation, the residue was purified by chromatography on silica gel eluted with CH_2Cl_2–MeOH (8:2) affording compound **4** (12.5 mg, 42% theoretical yield) as a colorless amorphous solid: UV (MeOH) λ_{max} 235 (ε 5500) and 299 (1070) nm; FTIR (film) ν_{max} 3440–3176, 3019, 2928, 2855, 1658, 1630, 1588; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.12 (exchangeable br s, NH), 6.35 (s, 1 H), 2.36 (br s, 6 H); ^{13}C NMR (D_2O, 62.5 MHz) δ 168.9 (s, 2 C), 164.2 (s), 161.0 (s), 110.5 (d), 98.3 (s), 53.4 (s), 46.0 (t), 41.7 (t), 38.4 (t), 37.9 (t), 33.1 (t), 32.4 (t), 31.2–30.7 (t, 9 C), 28.8 (t), 26.6 (t), 25.2 (t), 24.9 (t), 23.8 (t, 1 C; q, 2 C), 19.9 (t), 15.1 (q); HRFABMS *m/z* 514.4486 ($C_{31}H_{56}N_5O$ requires 514.4484); EIMS *m/z* 495 (83), 312 (62), 288 (85), 248 (24), 178 (74), 150 (40), 136 (100), 123 (24), 107 (23).

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Supplementary Material Available: All spectra of 1–4 (49 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(21) Before the antibiotic and cytotoxicity tests, Phloeodictine B (**2**) was further purified by HPLC to remove the impurities contained in the samples of the NMR experiments (signals at δ_H 0.95–0.85 and δ_C 14.5, see supplementary material).