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Phloeodictines A1-A7 and C1-C2, Antibiotic and Cytotoxic Guanidine Alkaloids from the New Caledonian Sponge, *Phloeodictyon* sp.

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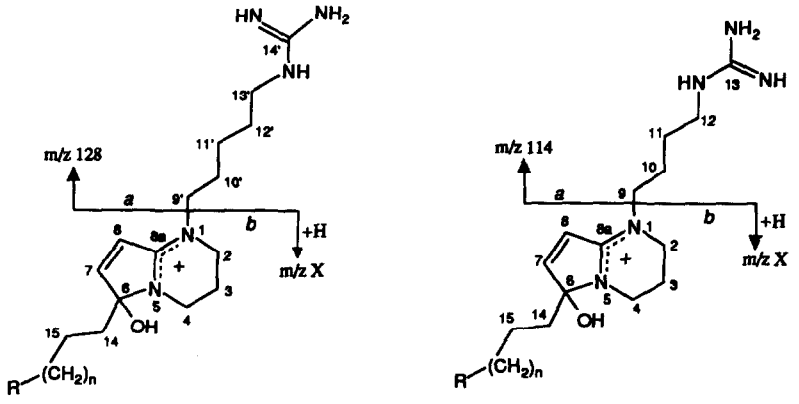
Abstract. - Phloeodictines A1-A7 (**3a, 3b, 4a, 4b, 4c, 5a, 5b**) and phloeodictines C1-C2 (**6a, 6b**), new antibacterial and cytotoxic guanidine alkaloids, have been isolated from the sponge *Phloeodictyon* sp. Their structures were established essentially by mass spectrometry utilizing B/E linked scanning and by 2D NMR experiments.

We have recently reported the structure elucidation of two antibacterial and cytotoxic guanidine derivatives containing an unprecedented 6-hydroxy-1,2,3,4-tetrahydropyrrolo[1,2-a] pyrimidinum skeleton, phloeodictines A (**1**) and B (**2**), isolated from the New-Caledonian deep water sponge *Phloeodictyon* sp.[1] (family Nepheliospongia, order Nepheliospongidae). Further search for bioactive agents from the same sponge resulted in the isolation of new structurally related pyrrolo[1,2-a]pyrimidines named phloeodictines A1-A7 (**3a, 3b, 4a, 4b, 4c, 5a, 5b**) and C1-C2 (**6a, 6b**). The structures of these compounds were established essentially by comparison of their collisionally activated dissociation (CAD) mass spectra obtained using FAB ionisation and B/E linked scanning[2] with those of **1**. All compounds exhibited in vitro antibacterial activities and were moderately cytotoxic against KB cells.

The lyophilized sponge was extracted with 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 using preparative and semi-preparative RP-HPLC [Delta-Pak C18, MeOH- NaCl (0.2M) -THF, pH adjusted to 2.2 with HCl] yielded a ca 2.6:1 mixture (**3**) of phloeodictines A1 (**3a**) and A2 (**3b**), a ca 2.6:0.7:0.3 mixture **4** of phloeodictines A3 (**4a**), A4 (**4b**) and A5 (**4c**), a ca 1:1.4 mixture (**5**) of phloeodictines A6 (**5a**) and A7 (**5b**) and a ca 1:1 mixture **6** of phloeodictines C1 (**6a**) and C2 (**6b**) as colorless amorphous solids. Typical yields were 0.55% for **3**, 0.02% for **4**, 0.02% for **5** and 0.54% for **6** (dry weight sponge).

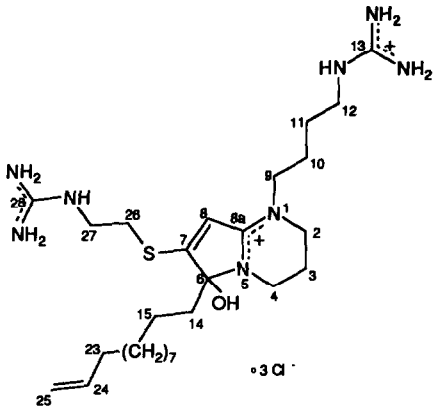
The UV absorption of mixture **3** was the same as that of the previously reported phloeodictine A (**1**), exhibiting maxima at 224 (ε 6700) and 274 (ε 2200) nm. The positive ion FAB mass spectrum of **3** revealed two M^+ peaks at *m/z* 432 and 418 corresponding to phloeodictine A1 (**3a**) and A2 (**3b**) respectively. The molecular formulas $C_{25}H_{46}N_5O$ (M^+, *m/z* 432.3712, Δ -1.0 mmu) for **3a** and $C_{24}H_{44}N_5O$ (M^+, *m/z*



3a n = 7, R = -$CH_2CH=CH_2$, X = 305
4a n = 5, R = -$CH_2CH=CH_2$, X = 277
4c n = 4, R = -$CH_2CH=CH_2$, X= 263
5a n = 8, R = -$CHMe_2$, X = 321

1 n = 9, R = -$CH_2CH=CH_2$, X = 333
3b n = 7, R = -$CH_2CH=CH_2$, X =305
4b n = 5, R = -$CH_2CH=CH_2$, X = 277
5b n = 8, R = -$CHMe_2$, X = 321

Fig.1. Major fragmentation by B/E CAD of the molecular ions of phloeodictine A (**1**) and phloeodictines A1-A7 (**3a, 3b, 4a, 4b, 4c, 5a, 5b**).



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418.3545, Δ 0.1 (Δ 0.1 mmu) for **3b**, established by HRFABMS, differed only by 14 and 28 amu respectively from **1** ($C_{26}H_{48}N_5O$), suggesting that the mass difference could correspond to fewer methylene units in the side chains. Since **3a** and **3b** appeared to be homologues of **1**, it was desirable to see if CAD spectra could be used to pinpoint the location of the homology. To this end, the product ion mass spectra of the M^+ ions of **1**, **3a** and **3b** were acquired. The two major cleavage processes obtained are shown in figure 1. For **1**, the mass of the ion arising from path *a* is at *m/z* 114, while the ion from path *b* appears at *m/z* 333[3]. Collisional activation of the M^+ ion of **3a** indicates that the N-butylguanidine side chain in **1** is replaced, in **3a**, by a N-pentylguanidine moiety (ion from path *a* at *m/z* 128). In the case of **3b**, the ion arising from path *b* cleavage is at *m/z* 305, while the ion from path *a* is unshifted as compared to **1**, at *m/z* 114, revealing that the difference between **1** and **3b** resides in the allyl side chain.

The ^1H and ^{13}C NMR spectral data of **3** (Table 1) further supported the structural assignment of the alkaloids. Two different sets of ^{13}C signals were observed, in a ratio of 2.6 to 1, for the guanidine side chain methylene groups of **3a** and **3b** respectively. All other ^1H and ^{13}C resonances were virtually unchanged from the corresponding signals of **1**. The assignment of all protonated carbons were confirmed by DQF-COSY[4] and HMQC experiments.

Catalytic hydrogenation of **3** led to mixture **7** of the tetrahydroalkaloids **7a** and **7b**, which were further converted using acetylacetone in HCO_3Na/H_2O/EtOH (3h, reflux) to mixture **8** of their 4,6-dimethylpyrimidine derivatives **8a** and **8b**, respectively. The HREIMS spectrum of **8** confirmed that **8a** and **8b** differed by the chain lenght at N-1. In addition to the molecular ions (M^+) at *m/z* 500.4305 ($C_{30}H_{54}N_5O$, Δ 2.4 mmu) and 486.4144 ($C_{29}H_{52}N_5O$, Δ 2.8 mmu), the spectrum displayed typical fragmentations (Fig. 2) at *m/z* 375.3330 ($C_{24}H_{43}N_2O$, Δ 4.0 mmu), 361.3191 ($C_{23}H_{41}N_2O$, Δ 2.7 mmu), 330.2283 ($C_{18}H_{28}N_5O$, Δ 1.0 mmu), 316.2100 ($C_{17}H_{26}N_5O$, Δ 3.7 mmu), 192, 178, 150 and 136.

The UV spectrum of mixture **4** was the same as that of **1** and **3**, indicative of the same absorbing chromophore. The HRFABMS of **4** exhibited two M^+ ions at *m/z* 404.3415 (Δ -2.6 mmu) and 390.3265 (Δ -3.2 mmu) corresponding to the molecular formulas $C_{23}H_{42}N_5O$ and $C_{22}H_{40}N_5O$, respectively. However, CAD spectra led to the identification of two isomers, phloeodictines A4 (**4b**) and A5 (**4c**) occurring at m/z 390 and differing in the lengths of their guanidine and allyl side chains. This identification is based on the observed product ions at m/z 263 and 277 arising from the collisional activation of the M^+ ion at m/z 390 (Fig. 1). The CAD spectrum of the ion at *m/z* 404 displayed the major fragmentations shown in figure 1 (product ions at m/z 277 and 128), allowing to assign the structure of phloeodictine A4 (**4a**). The ^1H and ^{13}C NMR spectra of **4** were almost identical to those of **3**, in agreement with the proposed structures **4a**, **4b** and **4c**.

The UV spectrum of mixture **5** was the same as those of **1**, **3** and **4**. The FAB mass spectrum of **5** showed two M^+ peaks at *m/z* 448 and 434. The molecular formulas $C_{26}H_{50}N_5O$ (M^+, *m/z* 448.4003, Δ 1.2 mmu) for phloeodictine A6 (**5a**) and $C_{25}H_{48}N_5O$ (M^+, *m/z* 434.3848, Δ 1.1 mmu) for phloeodictine A7 (**5b**) were deduced from HRFABMS. On the basis of the information obtained from the CAD spectra of both molecular ions (Fig. 1), the difference of 14 amu between **5a** and **5b** could be located in the guanidine side chain. Comparison of the ^1H and ^{13}C NMR data of **5** with those of **4** revealed that the molecules were almost identical, the only difference being the absence, in **5**, of the signals due to the allyl group [δ_C 139.9 (d) and 115.4 (t); δ_H 5.80 (ddt, 1H), 5.01 (dd, 1H) and 4.92 (dd, 1H)] and their replacement by signals at δ_C 23.1 (t,

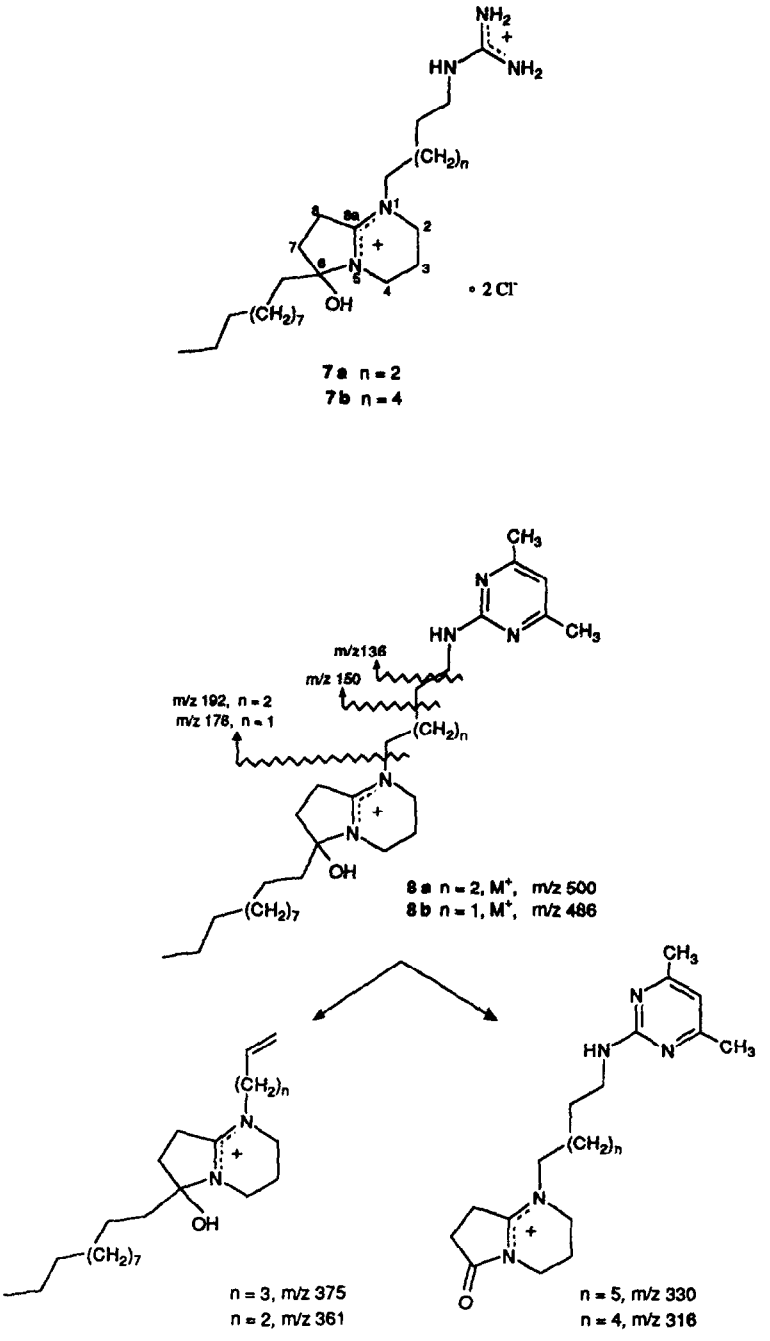


Fig. 2. EIMS fragmentation of **8a** and **8b**.

2C) and 29.1(d) and δ_H 0.88 (d) due to a terminal isopropyl group. The structures of phloeodictines A5 and A6 were, consequently, concluded to be **5a** and **5b** respectively.

The UV spectrum of **6** exhibited a maximum at 219 nm (ε 9100) suggesting the presence of a distinct UV chromophore than the previously described phloeodictines. The FAB mass spectrum of **6** contained two weak M^+ peaks at *m/z* 551 (12%) and 537 (5%). The molecular formulas $C_{28}H_{55}N_8OS$ (M^+, *m/z* 551.4220, Δ 0.0 mmu) for phloeodictine C1 (**6a**) and $C_{27}H_{53}N_8OS$ (M^+, m/z 537.4083, Δ -2.0 mmu) for phloeodictine C2 (**6b**) were established by HRFABMS. Elemental analysis (S, Cl) confirmed the presence of sulfur and indicated that the compounds were isolated as trichloride salts.

Comparison of the ^1H and ^{13}C NMR spectra of **6** with those of phloeodictine B[1] (**2**) showed that ring B $\Delta^{7,8}$ double bond [δ_C 170.0 (s) and 108.8 (d); δ_H 6.95 (s)] had been replaced by a methylene resonance at δ_C 38.9 (t, C-8) and δ_H 3.80 (m, H-8a) and 3.17 (m, H-8b) and by a methine signal at δ_C 46.3 (C-7) associated with a proton multiplet at δ_H 3.65. An additional difference lay in the presence of two sets of ^{13}C resonances, in a ratio of 1 to 1, corresponding to the guanidine side chain methylene groups of **6a** and **6b** (Tables 2 and 3, respectively). The remainder of the spectra were essentially identical with those of **2**. Evaluation of the HMQC spectrum of **6**, together with comparison of the COSY[5] and HOHAHA[6] correlations with those of **2**, allowed the substitution pattern about the pyrrolo[1,2-a]pyrimidine ring to be confirmed. Finally, analysis of the HMBC spectrum (Tables 2 and 3) led to the assignment of structures **6a** and **6b** for phloeodictines C1 and C2, respectively. Particularly relevant were the correlations observed between H-7 (δ 3.65) and C-26 (δ 31.6) as well as the complementary cross peak between H-26 (δ 2.79) and C-7 (δ 46.3), thus establishing the connectivity of the sulfur side chain at position 7. However, no NOE correlations were observed and the stereochemistry at C-7 was not determined.

The structure of the sulfur side chain was also supported by diagnostic peaks in the HRFAB mass spectrum of **6** at m/z 432.3712 ($C_{25}H_{46}N_5O$, Δ -1.0 mmu) and m/z 418.3545 ($C_{24}H_{44}N_5O$, Δ 0.1 mmu) formed by the loss of $(NH_2)NH{=}C\text{-}NH(CH_2)_2\text{-}SH$ from the molecular ions of **6a** and **6b**, respectively. The CAD spectra of the M^+ ions of **6a** and **6b** (Fig. 3) provided confirmation of this fragmentation (product ions at m/z 432 and 418). Moreover, collisional activation of the positive ions at m/z 432 and 418 yielded fragmentation patterns similar to those observed for the molecular ion of phloeodictines A1 (**3a**) and A2 (**3b**), respectively, thus confirming that the difference of one methylene unit between **6a** and **6b** resided in the guanidine side chain moiety.

Alkaline treatment of **6** afforded the disulfide **9**. Compound **9** most probably results from the dimerisation of the radical $NH_2(NH{=})C\text{-}NH\text{-}(CH_2)_2\text{-}S^{\bullet}$ (**12**) which could be formed as suggested in scheme 1[7,8,9].

Mixtures **3**, **4**, **5** and **6** have been tested against several bacteria using the standard microdilution plate assay and were found to possess a wide spectrum of activity with the following respective MIC's (μg/ml): *Staphylococcus aureus* (3, 30, 1, 3), *Escherichia coli* (3, 30, 3, >30), *Pseudomonas aeruginosa* (30, >30, 30, >30), *Clostridium perfringens* (30, >30, 1, >100), *Bacteroides fragilis* (10, >30, 3, >100) and *Peptococcus assaccharolyticus* (10, >30, 3, >100). These substances also exhibited *in vitro* cytotoxicity towards KB human nasopharyngeal carcinoma cells with IC_{50}'s of 2.2, 3.5, 0.6 and 1.8 μg/ml for **3**, **4**, **5** and **6** respectively.

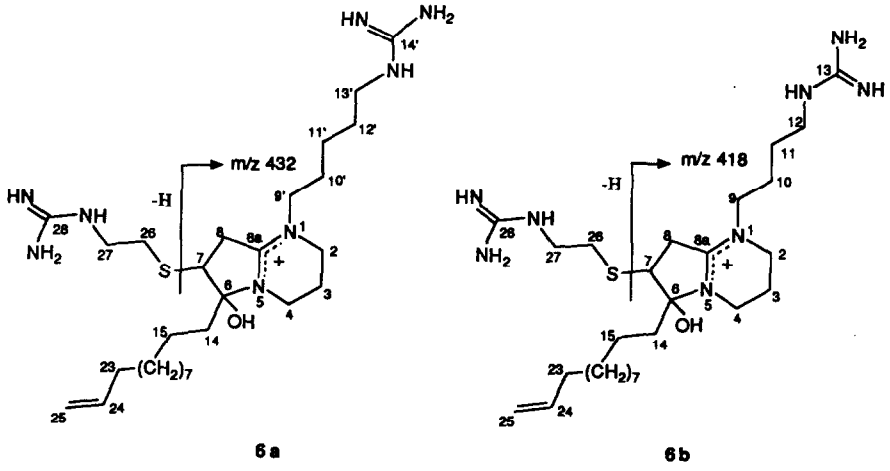
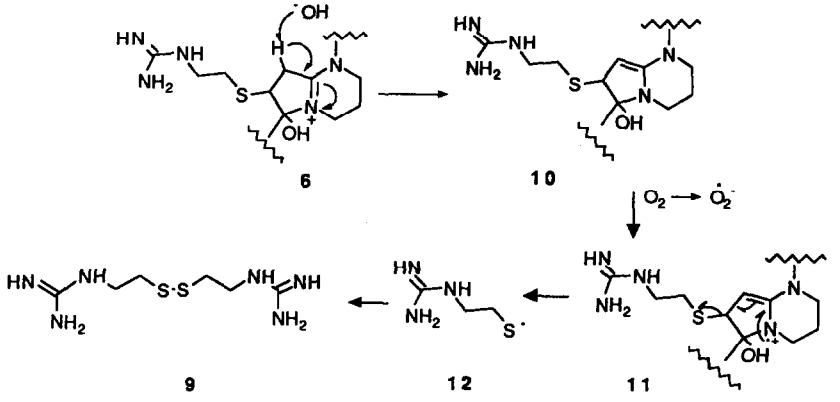


Fig. 3. Fragmentation by B/E CAD of the molecular ions of phloeodictines C1 (**6a**) and C2 (**6b**).



Scheme 1

TABLE 1. ^{13}C (H_2O, 62.5MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR Data of Phloeodictines A1 (**3a**) and A2 (**3b**)

3a			**3b**		
Position	δ ^{13}C (m)	δ ^{1}H (m, *J*, Hz)	Position	δ ^{13}C (m)	δ ^{1}H (m, *J*, Hz)
2	46.6 (t)	3.62 (m)	2	46.7 (t)	3.62 (m)
3	20.1 (t)	a 2.20 (m) b 2.05 (m)	3	20.1 (t)	a 2.20 (m) b 2.05 (m)
4	37.0 (t)	a 3.55 (m) b 3.25 (m)	4	37.0 (t)	a 3.55 (m) b 3.25 (m)
6	98.4 (s)		6	98.4 (s)	
7	153.0 (d)	7.30 (d, 6.5)	7	153.0 (d)	7.30 (d, 6.5)
8	121.0 (d)	7.05 (d, 6.5)	8	121.0 (d)	7.05 (d, 6.5)
8a	160.0 (s)		8a	160.0 (s)	
9'	53.6 (t)	3.62 (m)	9	53.2 (t)	3.62 (m)
10'	28.8 (t)	1.76 (m)	10	26.2 (t)	1.76 (m)
11'	24.1 (t)	1.45 (m)	11	26.0 (t)	1.65 (m)
12'	28.4 (t)	1.65 (m)	12	42.0 (t)	3.25 (m)
13'	42.2 (t)	3.30 (m)	13	157.9 (s)	
			14	36.0 (t)	a 2.05 (m) b 1.76 (m)
14'	157.9 (s)				
14	36.0 (t)	a 2.05 (m) b 1.76 (m)			
15	24.8 (t)	1.15 (m)	15	24.8 (m)	1.15 (m)
16-22	30.9-30.1 (t)	1.25-1.35 (br s)	16-22	30.9-30.1 (t)	1.25-1.35 (br s)
$\underline{CH_2}$-CH=CH_2	34.8 (t)	2.05 (m)	$\underline{CH_2}$-CH=CH_2	34.8 (t)	2.05 (m)
CH_2-$\underline{CH}$=CH_2	139.8 (s)	5.80 (ddt, 10,17,7)	CH_2-$\underline{CH}$=CH_2	139.8 (s)	5.80 (ddt, 10,17,7)
CH_2-CH=$\underline{CH_2}$	115.4 (t)	a 5.01 (dd, 2, 17) b 4.92 (dd, 2, 10)	CH_2-CH=$\underline{CH_2}$	115.4 (t)	a 5.01 (dd, 2, 17) b 4.92 (dd, 2, 10)

EXPERIMENTAL

General. UV spectra were recorded on a Shimadzu UV-160 spectrophotometer; IR on a Nicolet 205 FT-IR spectrometer; EIMS (70 eV) on a Kratos MS 50; HREIMS, FABMS (bombardment gas : xenon; matrix : glycerol + HCl) and B/E linked scan spectra on a Kratos MS 80. Collisional activation was obtained using argon as collision gas; the collision gas pressure was set to give a 30% attenuation of the parent ion beam measured at the final collector. HRFABMS were acquired on a VG-ZAB-SEQ spectrometer; NMR on Bruker AM 250 (^{1}H and ^{13}C NMR spectra) and AM 400 (^{1}H and 2D-NMR spectra). All NMR spectra were recorded with TMS as

internal standard. 2D-NMR experiments were performed with standard pulse sequences. HPLC was carried out on Waters Associated instruments. Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

Extraction and isolation. Specimens of *Phloeodictyon* sp. (1.5 Kg fresh weight) were collected, extracted and desalted as described earlier[1]. The desalted active fraction (18.5 g) was chromatographed under RP medium-pressure liquid chromatography by using a C-18 stationary phase (55-105 μm, 25 cm x 30 mm) and a step gradient of H_2O-CH_3OH as eluent. Purification was achieved by repetitive preparative and semi-preparative HPLC using Waters Delta Prep 3000 chromatography system [Delta-Pak C-18, 15 μ, 100 Å, 47.0 mm x 30.0 cm, flow rate 100 ml/min followed by final purification on Delta-Pak C-18, 15 μ, 100 Å, 25.0 mm x 10.0 cm, flow rate 8 ml/min, UV double detection at 230 and 280 nm, eluent MeOH-NaCl (0.2M) -THF (56:43:1 followed by 66:33:1, pH adjusted to 2.2 with HCl), to afford, in order of increasing polarity, mixtures **4** (9 mg), **6** (244 mg), **3** (250 mg) and **5** (11 mg) as amorphous solids.

Mixture 3 of phloeodictines A1 (3a) and A2 (3b). UV (MeOH) λ_{max} 224 (ε 6700) and 274 (2200) nm; FTIR (film) υ_{max} 3400-3100, 3019, 2928, 2855, 1665, 1589, 1462 cm^{-1} ; FABMS *m/z* 432 (M^+, 100), 418 (M^+, 38), 305 (27), 128 (48), 114 (73); HRFABMS *m/z* 432.3712 ($C_{25}H_{46}ON_5$ requires 432.3702), 418.3545 ($C_{24}H_{44}ON_5$ requires 418.3545); CAD spectrum of *m/z* 432 *m/z* 415, 414, 390, 305, 128 ; CAD spectrum of *m/z* 418 *m/z* 401, 400, 357, 305, 114; CAD spectrum of *m/z* 128 *m/z* 111, 86; CAD spectrum of m/z 114 *m/z* 97, 72. ^1H and ^{13}C NMR : Table 1.

Mixture 4 of phloeodictines A3 (4a), A4 (4b) and A5 (4c). UV (MeOH) λ_{max} 224 (ε 6700) and 274 (2200) nm; FTIR (film) υ_{max} 3400-3100, 2928, 2850, 1665, 1589, 1462 cm^{-1} ; FABMS *m/z* 404 (M^+, 100), 390 (M^+, 38), 128 (48), 114 (73); HRFABMS m/z 404.3415 ($C_{23}H_{42}N_5O$ requires 404.3389), 390.3265 ($C_{22}H_{40}N_5O$ requires 390.3233); CAD spectrum of *m/z* 404 *m/z* 387, 386, 362, 345, 277, 128; CAD spectrum of *m/z* 390 *m/z* 372, 277, 263, 128, 114; CAD spectrum of m/z 128 *m/z* 111, 86; CAD spectrum of m/z 114 *m/z* 97, 72; ^1H NMR (CD_3OD) for **4a** : δ 5,80 (CH_2-C<u>H</u>=CH_2, ddt, J = 10, 17 and 7 Hz), 5,01 (CH_2-CH=C<u>H$_a$</u>H_b, dd, J = 2,17 Hz), 4,92 (CH_2-CH=C<u>H$_a$H$_b$</u>, dd, J = 2,10 Hz), 3.62 (H-9' and H-2, m), 3.55 (H-4a, m) 3.20 (H-4b and H-13', m), 2.20 (H-3a, m), 2.05 (H-3b, H-14a and C<u>H</u>$_2$-CH=CH_2, m), 1.74 (H-10' and H-14b, m), 1.62 (H-12', m), 1.45 (H-11', m), 1.32 (H-16 to H-20, br s); 1.14 (H-15, m); ^1H NMR (CD_3OD) for **4b** : same as for **4a** except δ 3.62 (H-9 and H-2, m), 3.20 (H-4b and H-12, m), 1.74 (H-10 and H-14b, m), 1.62 (H-12, m); ^1H NMR (CD_3OD) for **4c** : same as for **4a** except δ 1.32 (H-16 to H-19, br s); ^{13}C NMR (D_2O) for **4a** : δ 160.2 (C-8a), 157.9 (C-14'), 153.2 (C-7), 141.6 (CH_2-<u>C</u>H=CH_2), 120.7 (C-8), 115.2 (CH_2-CH=<u>C</u>H_2), 98.8 (C-6), 53.3 (C-9'), 46.5 (C-2), 42.0 (C-13'), 36.9 (C-4), 34.8 (C-14), 34.3 (<u>C</u>H_2-CH=CH_2), 29.4 (C-16 to C-20), 28.8 (C-10'), 28.0 (C-12'), 24.0 (C-15), 23.7 (C-11'), 20.0 (C-3); ^{13}C NMR (D_2O) for **4b** : same as for **4a** except δ 157.9 (C-13), 53.2 (C-9), 42.0 (C12), 26.2 (C-10), 26.2 (C-11);^{13}C NMR (D_2O) for **4c** : same as for **4a** except δ 29.4 (C-16 to C-20).

Mixture 5 of phloeodictines A6 (5a) and A7 (5b). UV (MeOH) λ_{max} 224 (ε 6800) and 274 (2400) nm; FTIR (film) υ_{max} 3400-3100, 2928, 2850, 1665, 1589, 1462 cm^{-1} ; FABMS *m/z* 448 (M^+, 38), 434 (M^+, 53), 321 (20), 128 (17), 114 (100); HRFABMS *m/z* 448.4003 ($C_{24}H_{50}N_5O$ requires 448.4015), 434.3848 ($C_{23}H_{48}N_5O$ requires 434.3859); CAD spectrum of *m/z* 448 *m/z* 431, 430, 406, 387, 321, 128; CAD spectrum of *m/z* 434 *m/z* 417, 416, 373, 321, 114; CAD spectrum of *m/z* 128 *m/z* 111, 86; CAD spectrum of *m/z* 114 *m/z* 97, 72; ^1H NMR (CD_3OD) for **5a** : δ 7.30 (H-7, d, J = 6.5 Hz), 7.05 (H-8, d, J =

TABLE 2. ^{13}C (62.5MHz) and ^{1}H (400 MHz) NMR Data of Phloeodictine C1 (**6a**)[a] and Long-Range Correlations from HMBC experiments.

Position	δ ^{13}C (m)	δ ^{1}H (m, *J*, Hz)	HMBC (^{1}H)
2	46.5 (t)	3.48 (m)	H-4a
3	20.1 (t)	a 2.08 (m) b 1.90 (m)	
4	37.9 (t)	a 3.48 (m) b 3.17 (m)	
6	98.5 (s)		H-7, H-8a, H-14ab
7	46.3 (d)	3.65 (m)	H-8ab, H-26
8	38,9 (t)	a 3.80 (m) b 3.17 (m)	H-7
8a	164.1 (s)		H-2, H-4a, H-8ab
9'	53.8 (t)	3.48 (m)	
10'	29.1 (t)	1,70 (m)	H-13'
11'	24.1 (t)	1.52 (m)	H-12'
12'	27.6 (t)	1.52 (m)	H-9', H-11'
13'	42.4 (t)	3.17 (m)	H-11'
14'	158.3 (s)		H-13'
14	36.5 (s)	a 1.90 (m) b 1.70 (m)	
15	24.5 (t)	1.14 (m)	H-14ab
16-22	29.1-30.2 (t)	1.25-1.35 (br s)	H-15, H-23
23	34.7 (t)	1.90 (m) 1.70 (m)	H-24
24	141.5 (d)	5.80 (ddt, 10,17,7)	H-23
25	115.5 (t)	a 5.01 (dd, 2, 17) b 4.92 (dd, 2, 10)	H-23, H-24
26	31.6 (t)	2.79 (m)	H-7, H-25
27	42.2 (t)	3.48 (m)	H-26
28	158.3 (s)		H-27
OH		6.90 (s)[b]	
NH_2		7.05-7,75 (br s)[b]	
NH-28		8.02 (br s)[b]	
NH-14'		8.12 (br s)[b]	

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

6.5 Hz), 3.70 (H-9' and H-2, m), 3.55 (H-4a, m), 3.25 (H-4b and H-13', m), 2.22 (H-3a, m), 2.00 (H-3b and H-14a, m), 1.79 (H-10' and H-14b, m), 1.65 (H-12', m), 1.45 (H-11'), 1.30 (H-16 to H-23, br s), 1.14 (H-15, m), 0.90 (CH<u>Me</u>$_2$, d); ^{1}H NMR spectrum for **5b** : same as for **5a** except δ 3.70 (H-9 and H-2, m), 3.25 (H-12 and H-4b, m), 1.79 (H-10 and H-14b, m), 1.65 (H-11, m); ^{13}C NMR (D_2O) for **5a** : δ 160.2 (C-8a), 158.1 (C-14'), 153.2 (C-7), 121.1 (C-8), 98.8 (C-6), 53.6 (C-9'), 46.6 (C-2), 42.2 (C-13'), 40.1 (C-23), 37.4 (C-4), 35.4 (C-14), 31.1-30.5 (C-16 to C-22), 29.1 (<u>C</u>HMe$_2$), 28.5 (C-10'), 28.2 (C-12'), 24.8 (C-15), 24.1 (C-11'), 23.1 (CH<u>Me</u>$_2$), 20.0 (C-3); ^{13}C NMR (D_2O) for **5b** : same as for **5a** except δ 158.1 (C-13), 53.3 (C-9), 46.6 (C-2), 42.3 (C-12), 26.2 (C-10), 26.1 (C-11).

Mixture 6 of phloeodictines C1 (6a) and C2 (6b). UV (MeOH) λ_{max} 219 nm (ε 9400) ; FTIR (film) υ_{max} 3400-3100, 3019, 2855, 1668, 1462 cm^{-1}; FABMS *m/z* 551 (M^+, 5), 537 (M^+, 10), 432 (48), 418 (37) 305 (14), 128 (27), 114 (54); HRFABMS *m/z* 551.4220 ($C_{28}H_{55}N_8OS$ requires 551.4220), 537.4083 ($C_{27}H_{53}N_8OS$ requires 537.4063), 432.3712 ($C_{25}H_{46}N_5O$ requires 432.3702), 418.3545 ($C_{24}H_{44}N_5O$ requires 418.3546); Anal. Calcd for $C_{27.5}H_{54}N_8OS$, 3HCl: S, 5.0; Cl, 16.5. Found: S, 4.9; Cl, 16.3; CAD spectrum of *m/z* 551 *m/z* 432; CAD spectrum of *m/z* 537 *m/z* 418; CAD spectrum of *m/z* 432 *m/z* 415, 414, 401, 390, 371, 305, 238, 128; CAD spectrum of *m/z* 418 *m/z* 401, 400, 357, 305, 224, 114; CAD spectrum of *m/z* 128 *m/z* 111, 86; CAD spectrum of *m/z* 114 *m/z* 97, 72; ^{1}H and ^{13}C NMR : Tables 2-3.

Mixture 7 of hydrogenation derivatives 7a and 7b. A methanolic solution (15 ml) of **3** [70 mg, 10% Pd/C (ca 40 mg) was shaken for 3 h under an atmosphere of hydrogen. After removal of the catalyst and the solvent, a mixture of the hydrogenated derivatives **7a** and **7b** was obtained (60 mg, 86% theoretical yield) as a colorless amourphous solid. UV (MeOH) λ_{max} 219 nm (ε 9800); FTIR (film) υ_{max} 3400-3100, 3019, 2930, 2855, 1668, 1467 cm^{-1}; FABMS *m/z* 436 (M^+, 65), 422 (M^+, 30); ^{1}H NMR (CD_3OD) : δ 3.40 (m, 5H), 3.30 (m, 3H), 3.12 (m, 1H), 3.00 (m, 1H), 2.19 (m, 2H), 2.00 (m, 2H), 1.84-1.71 (m, 3H), 1.60 (m, 2H), 1.45 (m, 2H), 1.32 (m, 2H), 1.29-1.20 (m, 16 H), 0.89 (t, 3H); ^{13}C NMR (D_2O) : δ 164.7 (s), 158.0 (s), 98.5 (s), 53.6 (t), 46.0 (t), 38.3 (t), 38.0 (t), 33.1 (t), 32.4 (t), 30.9-30.5 (t, 9 C), 28.9 (t), 28.7 (t), 27.5 (t), 26.3 (t), 25.2 (t), 24.7 (t), 24.3 (t), 24.3 (t), 23.8 (t), 20.1 (t), 15.0 (q); FABMS *m/z* 436 (M^+, 65) and 422 (M^+, 30).

Mixture 8 of 4,6-dimethylpyrimidine derivatives 8a and 8b. Mixture **7** (60 mg) was dissolved in 95% EtOH (1 ml) and H_2O (0.5 ml) containing $NaHCO_3$ (0.04g). Acetylacetone (70 µl) was added and the mixture was refluxed for 3 h. The solution was then neutralized with HCl, filtered and evaporated till 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 (8:0.2 to 7:3) affording compounds **8a** and **8b** as an inseparable mixture (17 mg, 43% theoretical yield). UV (MeOH) λ_{max} 235 (ε 6000) and 299 (1080) nm; FTIR (film) υ_{max} 3440-3100, 3019, 2920, 2855, 1658,1588 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 7.12 (exchangeable br s, NH), 6.30 (s, 1H), 2.50 (br s, 6H); ^{13}C NMR (D_2O) : δ 167.0 (s, 2C), 163.7 (s), 161.9 (s), 109.3 (d), 98.1 (s), 52.4 (t), 44.8 (t), 40.4 (t), 37.6 (t), 36.4 (t), 31.5 (t), 31.0 (t), 28.8-29.3 (t, 9 C), 28.8 (t), 26.6 (t), 23.5 (q, 2C), 23.4 (t), 22.3 (t), 18.6 (t), 13.7 (q); HREIMS *m/z* 500.4305 (M^+, $C_{30}H_{54}N_5O$ requires 500.4329), 486.4144 (M^+, $C_{29}H_{52}N_5O$ requires 486.4172), 375.3330 ($C_{24}H_{43}N_2O$ requires 375.3375), 361.3191 ($C_{23}H_{41}N_2O$ requires 361.3218), 330.2283 ($C_{18}H_{28}N_5O$ requires 330.2293), 316.2100 ($C_{17}H_{26}N_5O$ requires 316.2137), 192.1820 ($C_{11}H_{18}N_3$ requires 192.1835), 178.1558 ($C_{10}H_{16}N_3$ requires 178.1567), 150.1020 ($C_8H_{12}N_3$ requires 150.1031), 136.0872 ($C_7H_{10}N_3$ requires 136.0875).

TABLE 3. ^{13}C (62.5MHz) and ^{1}H (400 MHz) NMR Data of Phloeodictine C2 (**6b**)[a] and Long-Range Correlations from HMBC experiments.

Position	δ ^{13}C (m)	δ ^{1}H (m, *J*, Hz)	HMBC (^{1}H)
2	46.5 (t)	3.48 (m)	H-4a
3	20.1 (t)	a 2.08 (m) b 1.90 (m)	
4	37.9 (t)	a 3.48 (m) b 3.17 (m)	
6	98.5 (s)		H-7, H-8a, H-14ab
7	46.3 (d)	3.65 (m)	H-8ab, H-26
8	38.9 (t)	a 3.80 (m) b 3.17 (m)	H-7
8a	164.1 (s)		H-2, H-4a, H-8ab
9	53.8 (t)	3.48 (m)	
10	26.5 (t)	1.70 (m)	H-11
11	25.3 (t)	1.52 (m)	H-12
12	42.4 (t)	3.17 (m)	H-11
13	158.3 (s)		H-12
14	36.5 (s)	a 1.90 (m) b 1.70 (m)	
15	24.5 (t)	1.14 (m)	H-14ab
16-22	29.1-30.2 (t)	1.25-1.35 (br s)	H-15, H-23
23	34.7 (t)	1.90 (m)	H-24
24	141.5 (d)	5.80 (ddt, 10,17,7)	H-23
25	115.5 (t)	a 5.01 (dd, 2, 17) b 4.92 (dd, 2, 10)	H-23, H-24
26	31.6 (t)	2.79 (m)	H-7, H-27
27	42.2 (t)	3.48 (m)	H-26
28	158.3 (s)		H-27
OH		6.90 (s)[b]	
NH_2		7.05-7.75 (br s)[b]	
NH-28		8.02 (br s)[b]	
NH-13		8.20 (br s)[b]	

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

2,2'-diguanidinodiethyldisulfide (9) To a solution of **6** (100 mg) in MeOH (8 ml) was added NaOH 8N (1.3 ml) and the mixture was stirred for 16 h at room temperature. The solution was then neutralized with HCl, and after removal of NaCl, the residue was purified by CC on reversed phase silica gel (Waters Preparative C18 55-105 µm, 2.4 g). Compound **9** (15 mg, 15% theoretical yield) was eluted at first with water: FABMS *m/z* 273 [(MH + $H^{35}Cl$)$^+$, (28)], 237 [(MH)$^+$, (58)], 120 (85), 93 (100); ^{1}H NMR (DMSO-d_6): δ 8.0 (exchangeable br s, NH), 7.80-7.35 (exchangeable br s, NH_2), 3.44 (t, J = 6.5 Hz, CH_2-N), 2.90 (t, J = 6.5 Hz, CH_2-S); ^{13}C NMR (D_2O) : δ 158.0 [s, -NH-C=NH(NH_2)], 41.0 (t, -CH_2-NH-), 37.3 (t, -CH_2-S-).

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3. The structures of the ions at *m/z* 114 and *m/z* 333 in the FAB mass spectrum of **1** were confirmed by high-resolution mass measurements (see reference 1).
4. The DQF-COSY spectrum of **3** (CD_3OD) showed the following correlations (H-H): 2ab/3ab, 3ab/4ab, 7/8, 9ab/10, 10/11, 11/12, 9'/10', 10'/11', 11'/12', 12'/13',14a/15, 15/(16 to 22), (16 to 22)/23, 23/24, 24/25.
5. The DQF-COSY spectrum of **6** (CD_3OD) showed the following connectivities (H-H): 2/3ab, 3ab/4ab, 7/8ab, 25/26,14ab/15, 15/(16 to 22), (16 to 22)/23, 23/24, 24/25ab, 26/27 as well as 9/10, 10/11, 11/12 for the N-butylguanidine moiety of **6b** and 9'/10', 10'/11', 11'/12', 12'/13' for the pentylguanidine side chain of **6a**.
6. The HOHAHA spectrum of **6** (DMSO-d_6) afforded the following connectivities (H-H): 2/3ab, 2/4ab, 3ab/4ab, 7/8ab, 25/26,14ab/15, 15/(16 to 22), (16 to 22)/23, 23/24, 23/25ab, 24/25ab, 26/27, 26/NH, 27/NH as well as 9'/10', 9'/11', 10'/11', 10'/12', 11'/12', 12'/13', 11'/13', 12'/NH and 13'/NH for the pentylguanidine side chain of **6a** and 9/10, 9/11, 10/11, 10/12, 11/12, 12/NH, 11/NH for the N-butylguanidine moiety of **6b**.
7. It is known that an enamine can easily transfer one electron to oxygen (see references 8 and 9). One can assume that **10**, which is the enamine form of an amidinium (and thus a stronger electron-donating group), easily gives rise to **11** in the presence of atmospheric oxygen.
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