PHLOEODICTINE AND THIOPHLOEODICTINE, NOVEL ANTIMICROBIAL AND CYTOTOXIC GUANIDINE ALKALOIDS FROM THE NEW CALEDONIAN SPONGE, *PHLOEODICTYON* SP.

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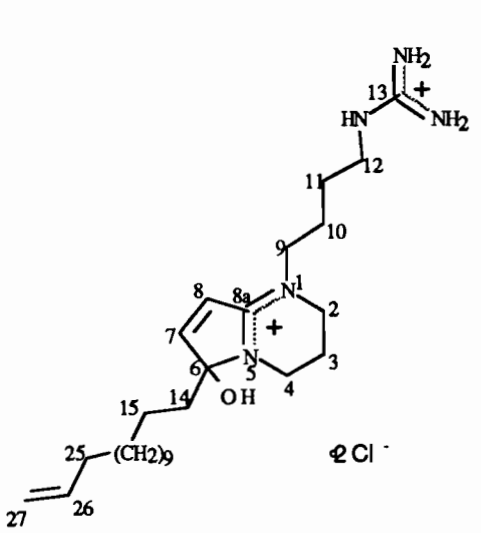
***Résumé** : deux composés antibiotiques et cytotoxiques dénommés phloeodictine (1) et thiophloeodictine (2) ont été isolés de l'éponge d'eau profonde* Phloeodictyon *sp. originaire de Nouvelle-Calédonie. Leur structures ont été établies par une étude spectroscopique approfondie incluant les RMN 1D et 2D (HMQC, DQF-COSY, HOHAHA et HMBC).*

***Abstract** : two antibiotic and cytotoxic bicyclic amidinium guanidine containing alkaloids, phloeodictine (1) and thiophloeodictine (2) have been isolated from the New-Caledonian deep water sponge* Phloeodictyon *sp. The structures were determined by extensive spectroscopic analysis especially one and two dimensional NMR including HMQC, DQF-COSY, HOHAHA and HMBC.*

In the course of the dragging campains of the ORSTOM-CNRS programme "Substances Marines d'Interêt Biologiques" (SMIB), it was found that crude extracts of the deep water sponge *Phloeodictyon* sp., collected in the south of New Caledonia on the Kaimon Maru sea-mount, at a depth of 235 m, exhibited *in vitro* biological activity in antibacterial and cytotoxicity assays. The isolation and structural elucidation of the active principles which we have named phloeodictine **1** and thiophloeodictine **2** are described here.

The purification of **1** and **2** was bioassay guided using antimicrobial activity and was achieved as follows: a) the lyophilized sponge was homogenized and sequentially extracted with methanol and water; b) the methanolic extract exhibiting the antibacterial activity was desalted using Amberlite XAD-7; c) the active fraction was subsequently subjected to medium pressure reversed-phase liquid chromatography using a water-methanol gradient; d) finally, two antibacterial alkaloids **1** and **2** were obtained pure after repetetive preparative and semi-preparative RP-HPLC. These compounds possess an unprecedented bicyclic amidinium skeleton substituted with both a lipophilic alkyl chain and a hydrophilic N-butyl guanidine chain. Thiophloeodictine possesses, in addition, a guanidine side chain biosynthetically derived from taurocyamine.



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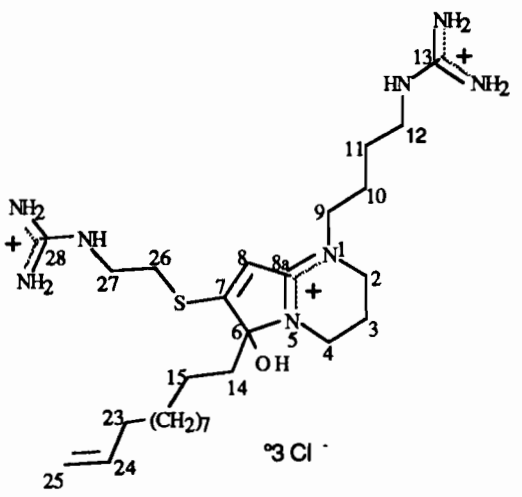

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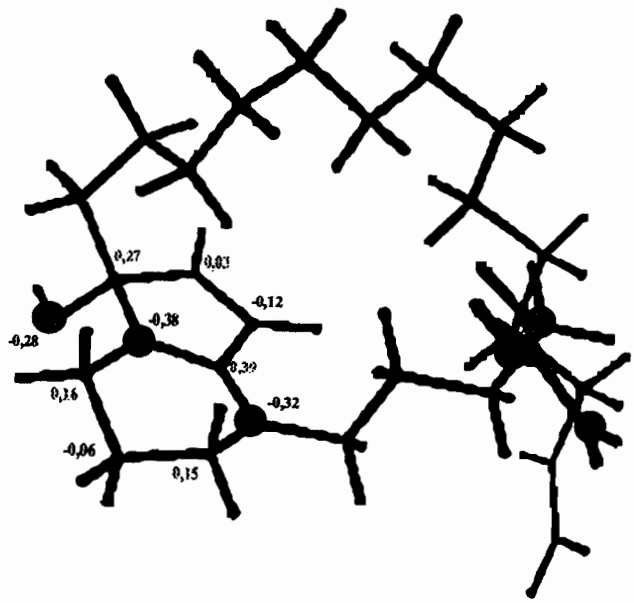
The structure elucidation of both compounds was determined by extensive spectroscopic analysis. The high resolution positive FAB mass spectrometry yielded the molecular formulas $C_{26}H_{49}N_5O$ and $C_{27}H_{51}N_8OS$ for **1** and **2** respectively. Elemental analysis revealed the presence of chloride atoms, indicating that **1** and **2** were dichloride and trichloride salts, respectively. A positive coloration with Sakaguchi reagent revealed the existence of guanidine functionalities confirmed by the presence in the ^{13}C NMR spectrum of a quaternary resonnance at 158.8 ppm. ^1H and ^{13}C NMR data of both compounds also revealed the presence of a terminal allyl group with characteristic chemical shift values and a long alkyl chain evidenced by the observation of an intense signal in the saturated region of both spectra (δ_H around 1.25 ppm; δ_C around 30.3 ppm). An exchangeable proton singlet at around δ_H 7 ppm corresponding to a tertiary hydroxyl group was also observed when the ^1H spectra of **1** and **2** were registered in DMSO-d_6. The main difference in the spectral data of both compounds lied in the following: a) structure **1** but not **2** possessed an AX spin system corresponding to a disubstituted double bond; b) thiophloeodictine **2** possessed a quaternary carbon at 159.9 ppm and a methine at 108.8 ppm associated with a singlet at 6.9 ppm which were absent in **1**; c) compound **2** contained a supplementary guanidino carbon at 158.8 ppm and two supplementary methylenes carbons in the saturated region of the ^{13}C spectrum.

But the rest of the molecules presented a more difficult assignment challenge and required 2D NMR methods. The direct ^1H -^{13}C connectivities were first assigned by analyzing the^1H detected multiple quantum coherence (HMQC) spectra. The different spin systems of **1** and **2** were subsequently identified by using a combination of double quantum filtered phase sensitive COSY (DQF-COSY) and homonuclear Hartmann-Hann (HOHAHA) experiments in CD_3OD and DMSO-d_6. The reasonable connection between the spin systems through the heteroatoms or the quaternary carbons present was later provided by extensive ^1H detected heteronuclear multiple-bond correlation (HMBC) experiments.

Computer modeling of **1** was carried out using the MACROMODEL program (version 3.1). Structure **1** was energy minimized by MM2 calculation to yield one of the most stable conformations shown in figure 1. An MNOD calculation of the charge distribution using the MOPAC software demonstrated that the amidinium charge of ring A was equally distributed between the two nitrogen atoms at positions 1 and 5.

Phloeodictine **1** and thiophloeodictine **2** exhibited interesting antibacterial activities, as determined using the microdilution plate method. Compound **1** was the most potent, with MIC values of 1 µg/ml against *Escherichia coli* and *Staphylococcus aureus*, and of 10 µg/ml against *Pseudomonas aeruginosa*.. Compound (**2**) was less active with MIC's of 30, 3 and > 30 µg/ml

against *E. coli*, *S. aureus* and *P. aeruginosa*, respectively. Both substances also possessed *in vitro* cytotoxic activity against KB buccal carcinoma cells with IC_{50}'s of 1.5 and 11.2 µg/ml for **1** and **2** respectively. Other biological activities are presently being investigated, but further *in vivo* studies are required in order to determine if these compounds present potential medical uses. The present report described two unusual, guanidine-containing compounds pertaining to a new class of naturally occuring alkaloids which merits further chemical and biochemical studies.



Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie

Nouméa, Nouvelle-Calédonie, 26-30 Août 1991

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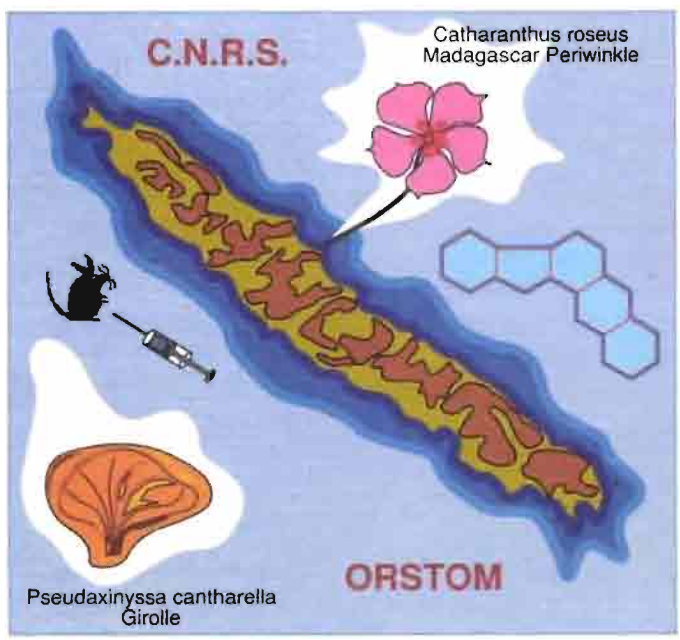

Editeurs : Cécile DEBITUS, Philippe AMADE,
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