

**7-HYDROXYPLEUROCORINE,  
A NEW ALKALOID ISOLATED FROM *PLEUROSTYLIA OPPOSITA***

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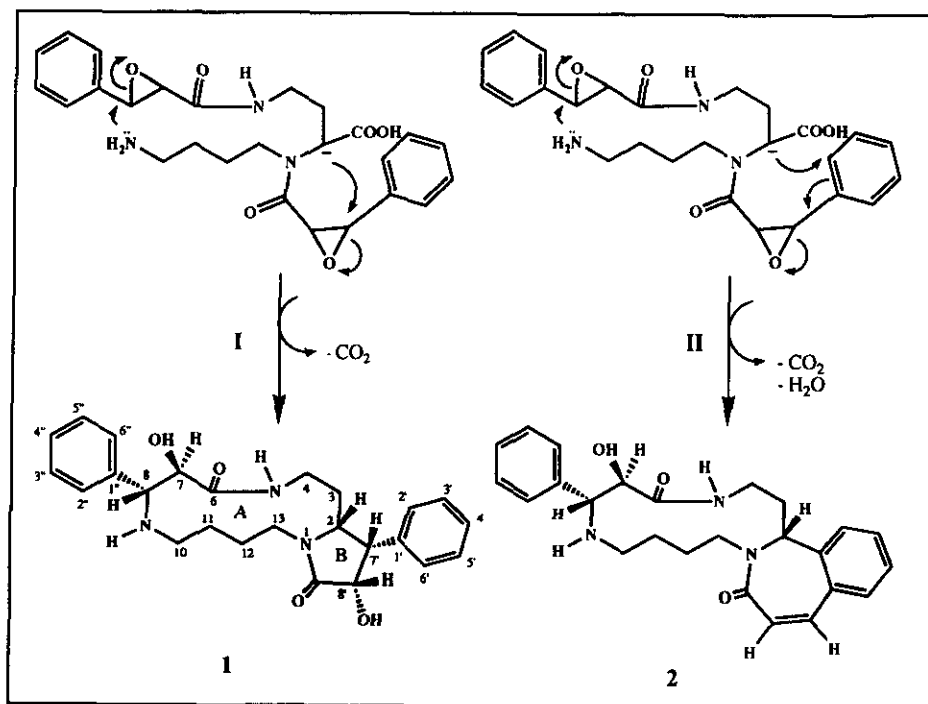
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**Abstract** -7-Hydroxypleurocorine (**1**), a novel spermidine alkaloid was isolated from the leaves of *Pleurostyliya opposita* and its structure was elucidated by means of extensive  $^1\text{H}$  and  $^{13}\text{C}$  nmr studies involving DQF COSY, HOHAHA, HMBC and HMQC experiments. The relative stereochemistry of the molecule was also probed using NOE difference spectroscopy. 7-Hydroxypleurocorine represents a new type of spermidine derivative including a pyrrolidinone.

We recently described the isolation of new hydroxylated spermidine alkaloids from a New Caledonian celastraceous species, namely *Pleurostyliya opposita* (Wall.) Merrill-Metcalf.<sup>1</sup> As outlined in Scheme 1 (II), a biosynthetic pathway involving epoxy derivatives of dicinnamoyl spermidines was then proposed to explain the origin of the pleurostyline-type skeleton. Accordingly, macrocyclic lactams including a five membered ring could also be derived from the same precursor as shown in Scheme 1 (I).

Hitherto, however, there was no report of the isolation of such compounds from a natural source. Extensive nmr studies were thus undertaken to elucidate the structure of the new alkaloid 7-hydroxypleurocorine (**1**) isolated from the same plant. Nmr experiments included DEPT, NOED, DQF COSY, HOHAHA, HMBC<sup>2</sup> and HMQC<sup>3</sup> spectroscopy. HOHAHA technique especially provided an invaluable help in establishing unambiguously the presence of a 3-hydroxy-2-pyrrolidinone moiety in **1**.



Scheme 1

Interlocking  $^{13}\text{C}$  and DEPT spectra (see Table 1) showed that 1 ( $\text{C}_{25}\text{H}_{31}\text{N}_3\text{O}_4$ ; anal.: 437.2314; found: 437.2306) possessed two aromatic cycles, two carbonyls, two secondary alcohols, six  $\text{CH}_2$ 's and three  $\text{CH}$ 's. Typical signals for the macrocyclic ring were in evidence in the  $^1\text{H}$  nmr spectrum (Table 2) and the DQF-COSY contour plot. Indeed, the key resonance for the 5-NH appeared as a double doublet centered at  $\delta$  7.10 ppm and correlating with signals at  $\delta$  3.45 (1H, m, H4A) and 1.78 ppm (1H, m, H4B). As for 7-hydroxypleurostyline (2), H7 and H8 appeared as two weakly coupled doublets respectively at 3.90 and 4.27 ppm ( $J_{7,8} = 1.0$  Hz). Proton resonances at  $\delta$  3.76 (1H, m, H2),  $\delta$  2.16 (1H, m, H3A) and  $\delta$  1.64 ppm (1H, m, H3B) were then readily assigned through cross-peak correlations. Finally identification of the H13A and H13B resonances at  $\delta$  3.56 and  $\delta$  2.66 ppm allowed us to complete the  $^1\text{H}$  assignments of the macrocycle of 1 through the coupling network of the molecule (see Table 2).

Table 1.  $^{13}\text{C}$  Nmr chemical shifts of 1

carbon	$\delta$ (ppm)
C2	52.6
C3	24.8
C4	36.1
C6	173.3
C7	73.3
C8	63.7
C10	47.2
C11	26.9
C12	25.2
C13	36.6
C1'	140.6
C2'	129.0
C3'	129.0
C4'	126.6
C5'	129.0
C6'	129.0
C7'	57.6
C8'	71.6
C9'	168.6
C1''	141.6
C2''	126.7 <sup>a</sup>
C3''	127.4 <sup>a</sup>
C4''	127.8
C5''	127.4 <sup>b</sup>
C6''	126.7 <sup>b</sup>

a,b-Values with the same superscript are interchangeable.

Table 2.  $^1\text{H}$  Nmr chemical shifts of 1

proton	$\delta$ (ppm)	$J$ (Hz)
H2	3.76 m	
H3A	2.16 m	
H3B	1.64 m	
H4A	3.45 m	
H4B	1.78 m	
H5	7.10 dd	$J_{5,4a} = J_{5,4b} = 6$
H7	3.90 d	$J_{7,8} = 1$
H8	4.27 d	$J_{8,7} = 1$
H10A	2.80 m	
H10B	2.25 m	
H11A	1.41 m	
H11B	1.41 m	
H12A	1.78 m	
H12B	1.41 m	
H13A	3.56 ddd	$J_{13a,13b} = 15$ $J_{13a,12a} = J_{13a,12b} = 5.5$
H13B	2.66 m	
H7'	3.69 dd	$J_{7',8'} = 8.5$ $J_{7',2} = 5.5$
H8'	4.86 d	$J_{8',7} = 8.5$
H <sub>ar</sub>	7.31 m	

The most striking features of the  $^{13}\text{C}$  nmr spectrum of **1** were on the one hand the evidence for the presence of two  $\text{CHOHC}=\text{O}$  groups in the molecule with typical signals at  $\delta$  173.3, 168.6, 73.3 and 71.6 ppm. On the other hand, only two quaternary aromatic carbons appeared on the same spectrum. The combination of these elements suggested the existence of an unusual  $\text{C}2\text{-C}7'$  bond at ring B and the presence of a pyrrolidinone ring in **1** was then postulated.

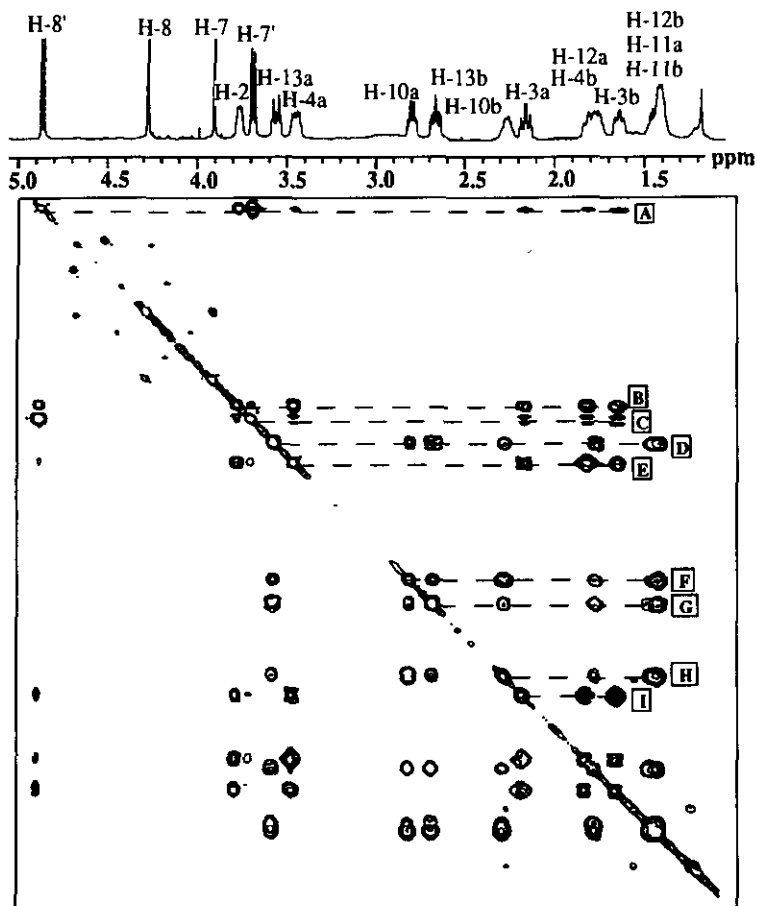


Figure 1. Part of the contour plot of the phase sensitive HOHAHA of **1** showing the following main spin-spin connectivities :

- (A) H-8', H-2, H-7', H-4a, H-3a, H-4b, H-3b;
- (B) H-2, H-7', H-4a, H-3a, H-4b, H-3b;
- (C) H-7', H-4a, H-3a, H-4b, H-3b;
- (D) H-13a, H-10a, H-13b, H-10b, H-12a, H-11a, H-12b, H-11b;
- (E) H-4a, H-3a, H-4b, H-3b;
- (F) H-10a, H-13b, H-10b, H-12a, H-11a, H-11b, H-12b;
- (G) H-13b, H-10b, H-12a, H-11a, H-11b, H-12b;
- (H) H-10b, H-12a, H-11a, H-11b, H-12b;
- (I) H-3a, H-4b, H-3b.

According to this hypothesis, the proton resonances at  $\delta$  4.86 (1H, d,  $J_{8,7}=8.5$  Hz) and 3.69 ppm (1H, dd,  $J_{7,8}=8.5$  Hz,  $J_{7,2}=5.5$  Hz) were attributed to H8' and H7', respectively, and the carbon resonances at 71.6 and 57.6 ppm were assigned to C8' and C7'. The assignment of the remaining  $^{13}\text{C}$  resonances (Table 1), matching this structure, was finally completed using HMQC and HMBC experiments.

Therefore, due to the originality of this ring B, further experiments were needed to establish unambiguously the structure of **1**. In order to confirm through spin-spin connectivities the aforementioned assignments, we chose to run a phase-sensitive HOHAHA experiment (Figure 1) where, for long mixing times, magnetisation from one proton is distributed over most of the other protons sharing the same coupling network.<sup>2</sup>

The transfers observed from H8' to H7', H2, H3A, H3B, H4A and H4B as the one observed from H2 to H3A, H3B, H4A, H4B and H7' then clearly demonstrated the presence of the 3-hydroxy-2-pyrrolidinone ring in 7-hydroxypleurocorine (**1**). The remaining correlation peaks shown in Figure 1 are also in total accordance with the proposed structure for **1**. An NOE difference spectroscopy experiment was finally undertaken in order to extract some information on the chiral centers of the molecule. The results of this study are summarized in Table 3. H7 and H8 are weakly coupled (1.0 Hz) so their dihedral angle must be close to  $90^\circ$ . Presaturation of the aromatic protons causes a 14% NOE on H8 and a 18% NOE on H7. The intensities of the signals for H8 and for the phenyl protons are enhanced by 12% and 9% respectively on irradiation of H7. Therefore, H7 and the phenyl group at C8 must lie on the same side of the molecule (Figure 2a). The vicinal coupling constant between H7' and H8' is 8.5 Hz, pointing to a dihedral angle ca  $20^\circ$  since presaturation of H7' enhances H8' by 14%. On presaturation of H8', a 30% NOE is observed for the aromatic protons while a 14% NOE is observed for H7', and irradiation of the aromatic protons enhances H8' by 26%. Thus H7' and H8' are *syn* relative to the C7'-C8' bond (Figure 2b). The phenyl at C7' and H2 are *anti* since presaturation of the aromatic protons causes a 24% NOE on H7' and no measurable effect is observed on H2. The magnitude of  $^3J_{2,7}$  (5.5 Hz) is thus associated with a dihedral angle ca  $60^\circ$  (Figure 2c). We then propose for 7-hydroxypleurocorine one possible relative stereochemistry presented in structure (**1**).

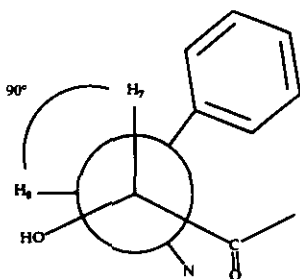


Figure 2a

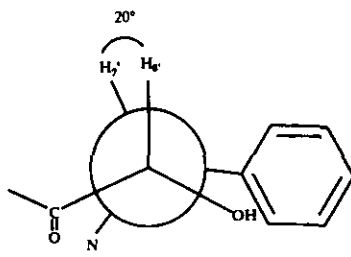


Figure 2b

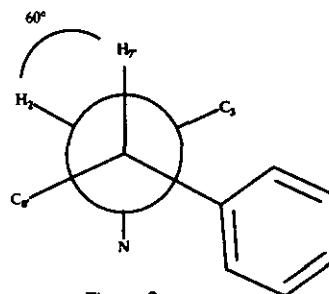


Figure 2c

To the best of our knowledge, this is the first report on a spermidine derivative alkaloid including a pyrrolidinone ring. Therefore, 7-hydroxypleurocorine represents a new type of spermidine alkaloids which potential therapeutical interest should be evaluated in view of the broad biological activity generally associated with macrocyclic lactams.<sup>5-7</sup>

irradiated signals	signals showing enhanced intensity
Har	H8' 26% H7' 24% H8 14% H7 18%
H8'	Har 30% H7' 14%
H7'	H8' 14%
H8	Har 11% H7 10% H10A 11% H10B 5%
H7	Har 9% H8 12% NH5 5%

Table 3. Summary of NOE difference results for 1

## EXPERIMENTAL

6 mg of **1** were dissolved in 0.5 ml of CDCl<sub>3</sub> and then degassed through a freeze-thaw cycle and flame sealed in a 5 mm nmr tube. The nmr experiments were performed on a JEOL  $\alpha$  500 (500.125 MHz) except for NOE difference spectroscopy which was run on a JEOL GSX 270 WB (270.5 MHz). The DEPT, HMQC and HMBC experiments were performed and processed using standard JEOL software. The parameters used in the phase sensitive 2D homonuclear correlation experiments were as follows: DQF-COSY: 17.7 ms 90° <sup>1</sup>H pulse width; 2 x 512 x 512 data matrix size with a spectral width of 4380.2 Hz in f<sub>1</sub> and f<sub>2</sub> (two separate sets of data with 512 data points in t<sub>1</sub> and t<sub>2</sub> were zero filled to 1 K data points); 8 scans for each t<sub>1</sub> value, the first t<sub>1</sub> value was preceded by 4 dummy scans; total acquisition time = 0.12 s; pulse delay = 0.9 s; a sine bell filter shifted by 30% was applied in each dimension before Fourier transformation.

HOHAHA : rf power corresponding to 34.5 ms 90° <sup>1</sup>H pulse width; 2 x 512 x 512 data matrix size, 8 scans per t<sub>1</sub> values, pulse delay = 0.9 sec, MLEV-17 mixing time 90 ms preceded and followed by 2.5 ms trim pulses; The data was zero filled and processed using a complex function of exponential and shifted sine bell windows. A presaturation delay of 10 s was used in the NOE difference spectroscopy with an accumulation of 8 scans and 4 dummy scans for each frequency for a total number of 512 scans. Prior to FT, a 2 Hz line broadening function was applied to each FID.

### Isolation of 1

Extraction and purification of the spermidine alkaloids have already been described.<sup>1</sup>

7-Hydroxypleurocorine 1: amorphous, C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O<sub>4</sub> (calcd:437.2314, found:437.2306) [ $\alpha$ ]<sub>D</sub><sup>20</sup>(C=1, CHCl<sub>3</sub>), ir (KBr) 3400, 1726, 1645, 1510, 765, 705 cm<sup>-1</sup>. <sup>1</sup>H-Nmr (500 MHz, CDCl<sub>3</sub>, TMS=0):see Table 1, <sup>13</sup>C-nmr (125 MHz, CDCl<sub>3</sub>, TMS=0): see Table 2.

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