171. New Hydroxylated Spermidine Alkaloids from *Pleurostylia opposita* (WALL.) MERILL-METCALF

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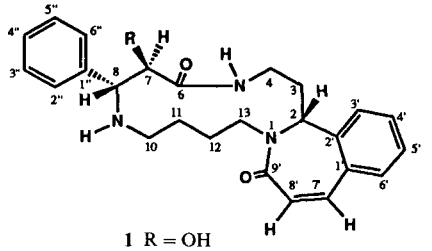
The leaves of a New Caledonian celastraceae, *Pleurostylia opposita* (WALL.) MERRILL-METCALF, have yielded three new spermidine alkaloids, 7-hydroxypleurostyline (**1**), 7-hydroxycelacinnine (**2**), and 7′-hydroxy-7′,8′-dihydropleurostyline (**3**), as minor basic constituents, together with three related alkaloids pleurostyline (**4**), celacinnine (**5**), and celallocinine (**6**).

Introduction. – Macrocyclic lactams derived from spermidine are of real therapeutical interest due to the broad biological activity which has been well established for this kind of molecules [1]. In the wake of a general survey of New Caledonian plants as new sources of therapeutical agents[1]), we have investigated the alkaloid content of a celastraceous species, namely *Pleurostylia opposita*.



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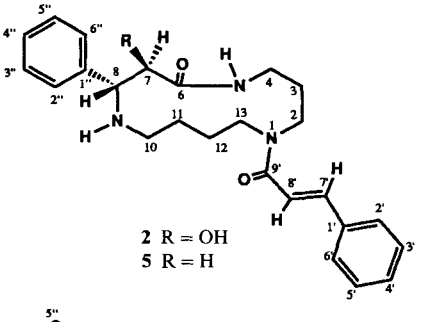
4 R = H



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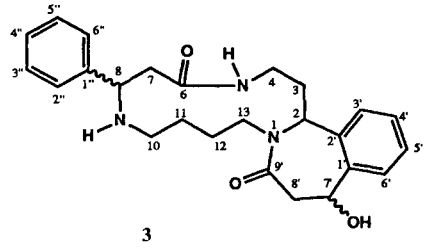
5 R = H



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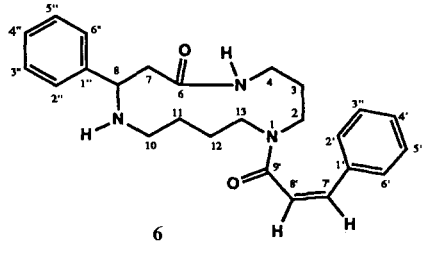

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[1]) Part 143 of the series 'Plantes de Nouvelle Calédonie'. Part 142: [2].

Compounds **1–6** presented in this report were obtained by standard acid-base workup of a $CHCl_3$ extract followed by medium-pressure liquid chromatography and preparative TLC.

Results. – Direct comparison of different spectral data for 7-hydroxypleurostyline (**1**) and pleurostyline (**4**) [3][4] was first made to identify the skeleton type of **1**. The high-resolution mass spectrum of **1** revealed the molecular formula $C_{25}H_{29}N_3O_3$ (calc.: 419.2220; found: 419.2208), while the IR spectrum showed strong absorption at 3400 and 3300 cm^{-1} (OH and NH), 1640 and 1580 cm^{-1} (amide), 760 and 710 cm^{-1} (aromatic).

^{1}H-NMR (*Table 1*) and COSY experiments run for this compound indicated a close relationship between **1** and **4**, featuring key signals of the macrocyclic ring at 4.73

Table 1. *^{1}H-NMR Data* (270 MHz) *of* **1–3** ($CDCl_3$, TMS as internal reference)

	1		**2**		**3**	
H-Atom	δ [ppm]	J [Hz]	δ [ppm]	J [Hz]	δ [ppm]	J [Hz]
H_A–C(2)	4.73 (*dd*)	J(2,3A) = 10.2 J(2,3B) = 3.4	3.75 (*m*)		4.49 (*dd*)	J(2,3A) = 5.9 J(2,3B) = 6.1
H_B–C(2)			3.43 (*m*)			
H_A–C(3)	2.48 (*m*)	J(3A,2) = 10.2	2.25 (*m*)		2.26 (*dd*)	J(3A,2) = 5.9
H_B–C(3)	2.48 (*m*)	J(3B,2) = 3.4	1.70 (*m*)		2.26 (*dd*)	J(3B,2) = 6.1
H_A–C(4)	3.50 (*m*)		3.80 (*m*)		3.40 (*m*)	
H_B–C(4)	2.46 (*m*)		3.00 (*m*)		3.40 (*m*)	
NH	7.21 (*t*)		7.00 (*t*)		7.72 (*m*)	
H_A–C(7)	4.20 (*d*)	J(7,8) = 1.5	4.16 (*d*)	J(7,8) = 1.2	2.65 (*m*)	J(7A,8) = 6.2
H_B–C(7)					2.65 (*m*)	J(7B,8) = 8.6
H–C(8)	4.26 (*d*)	J(8,7) = 1.5	4.27 (*d*)	J(8,7) = 1.2	3.99 (*dd*)	J(8,7A) = 6.2 J(8,7B) = 8.6
H_A–C(10)	2.78 (*m*)		2.86 (*m*)		2.65 (*m*)	
H_B–C(10)	2.46 (*m*)		2.31 (*m*)		2.42 (*m*)	
H_A–C(11)	1.58 (*m*)		1.65 (*m*)		1.67 (*m*)	
H_B–C(11)	1.39 (*m*)		1.45 (*m*)		1.40 (*m*)	
H_A–C(12)	1.75 (*m*)		1.92 (*m*)		1.92 (*m*)	
H_B–C(12)	1.63 (*m*)		1.62 (*m*)		1.67 (*m*)	
H_A–C(13)	3.67 (*m*)		3.57 (*m*)		3.52 (*m*)	
H_B–C(13)	3.38 (*m*)		3.38 (*m*)		3.52 (*m*)	
H–C(2′)			7.52 (*m*)			
H–C(6′)			7.52 (*m*)		7.55 (*d*)	
H–C(7′)	6.94 (*d*)	J(7′,8′) = 12.2	7.66 (*d*)	J(7′,8′) = 15.4	5.12 (*dd*)	J(7′,8′A) = 1.9 J(7′,8′B) = 7.3
H_A–C(8′)	6.25 (*d*)	J(8′,7′) = 12.2	6.30 (*d*), 6.32 (*d*)	J(8′,7′) = 15.4	3.33 (*dd*)	J(8′A,8′B) = 15.4 J(8′A,7′) = 1.9
H_B–C(8′)					2.94 (*dd*)	J(8′B,7′) = 7.3 J(8′B,8′A) = 15.4
arom. H	7.35 (*m*)		7.35 (*m*)		7.30 (*m*)	

(H–C(2)), 4.26 (H–C(8)), 3.50 (H_A–C(4)), and 2.46 ppm (H_B–C(4)), and the (*Z*)-cinnamoyl protons at 6.94 (H–C(7′)) and 6.25 ppm (H–C(8′), J(7′,8′) = 12.2 Hz). However, the *dd* due to H–C(7) in the spectrum of **4** was there replaced with a deshielded *d* at 4.20 ppm (J(7,8) = 1.5 Hz). The ^{13}C-NMR spectrum of **1** (*Table 2*) displayed among 23

Table 2. *^{13}C-NMR Data* (67.5 MHz) *of* **1–3** ($CDCl_3$, TMS as internal reference)

C-Atom	**1** δ [ppm]	**2** δ [ppm]	**3** δ [ppm]
C(2)	59.41	43.03, 44.59[b])	60.83
C(3)	30.94	27.92, 30.39	38.29
C(4)	37.30	36.72, 36.16	36.51
C(6)	172.73	172.50, 172.34	171.94
C(7)	76.52	76.07, 76.16	44.36
C(8)	62.75	64.01, 64.38	59.29
C(10)	43.24	46.55, 46.46	42.57
C(11)	23.68	24.22[a]), 26.06	23.55
C(12)	22.52	24.88[a]), 25.12	22.20
C(13)	46.91	44.01, 46.80[b])	47.20
C(1′)	133.51	135.41	136.25
C(2′)	138.41	128.78	139.17
C(3′)	128.34[a])	128.78	128.08[a])
C(4′)	130.90[a])	129.49	129.79[a])
C(5′)	127.93[a])	128.78	128.55[a])
C(6′)	129.68[a])	128.78	130.61
C(7′)	136.13	140.79	67.26
C(8′)	128.36	117.53	43.50
C(9′)	165.69	165.16, 165.82	170.07
C(1″)	140.15	142.35, 142.44	142.11
C(2″)	129.78[b])	127.76[c])	126.21[b])
C(3″)	127.22[b])	126.99[c])	128.92[b])
C(4″)	127.78[a])	127.56	127.68[a])
C(5″)	127.22[b])	126.99[c])	128.92[b])
C(6″)	127.78[b])	127.76[c])	126.21[b])

[a]) [b]) [c]) Values with the same superscript are interchangeable.

distinct signals two amide C=O signals at 165.69 and 172.73 ppm. DEPT and ^{1}H,^{13}C heteronuclear-correlation experiments confirmed the structure elements deduced from aforementioned data and showed that **1** and **4** only differ in the number of CH and CH_2 moieties: two CH and seven CH_2 groups were present according to the ^{13}C-NMR spectrum of **4**, while the spectrum of **1** indicated three CH groups, one of which being of the $>$CHOH type (signal highly deshielded at 76.5 ppm) and six CH_2 groups.

Irradiation of H–C(8) at 4.26 ppm collapsed the *d* at 4.20 ppm to a *s*. A key one-bond heteronuclear correlation was then observed between this *d* and the ^{13}C signal at 76.52 ppm. Indeed, on the basis of these elements, an OH group could be located at C(7).

In the high-resolution MS of **1**, the base peak at m/z 362 was attributed to a double cleavage of the amide and the C(7)–C(8) bonds ($[M - C_2HO_2]^+$) [5]. Accordingly, the structure of **1** was established as 7-hydroxypleurostyline.

A difference NOE experiment was then carried out to assess the relative configurations at C(7) and C(8). Significant NOE's were observed on one hand as follows: H–C(7) to H–C(2″) and H–C(6″) (6%), H–C(8) to H–C(2″) and H–C(6″) (16%), H–C(2″) and H–C(6″) to H–C(7) (7%), and H–C(2″) and H–C(6″) to H–C(8) (11%). On the other hand, the dihedral angle between H–C(7) and H–C(8) was found to be close to 90°, demonstrating their weak vicinal coupling (1.5 Hz). These results, thus, indicate that the

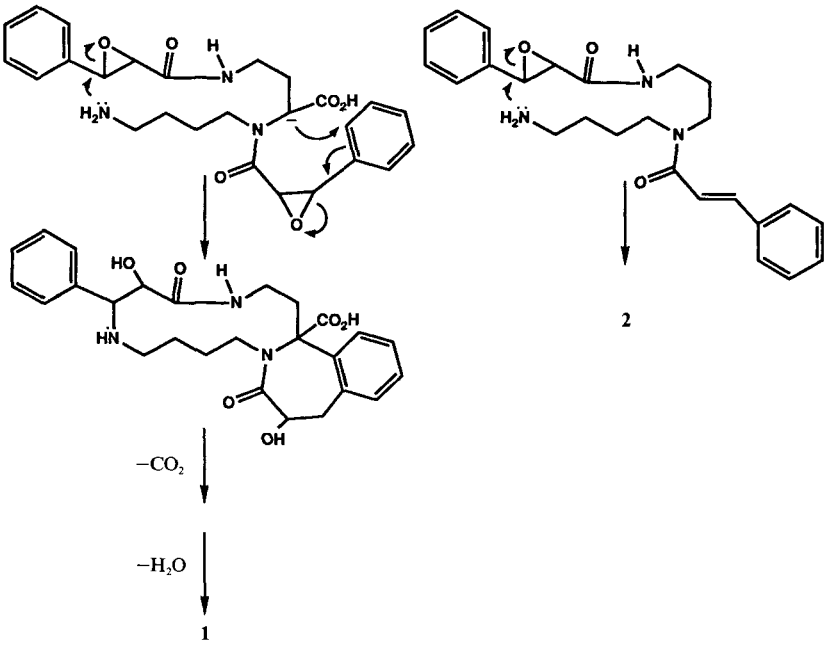
OH group at C(7) and the Ph group at C(8) must lie on two opposite sides of the molecule as shown for **1**. The relative configuration at C(2) is proposed on the basis of the same experiment, since irradiation at 4.73 ppm (H–C(2)) led to enhancements at 2.48 (H_A–C(3) and H_B–C(3), 7%) and at 7.35 ppm (H–C(3'), 16%).

A similar structural relationship between **5** and **2** ($C_{25}H_{31}N_3O_3$; calc.: 421.2365, found: 421.2380) enabled the identification of the latter. As for **5**, the rotational isomerism of one amide group in **2** was deduced from its ^{1}H-NMR spectrum showing an (*E*)-cinnamoyl *AB* system at 7.66 (H–C(7')), 6.32, and 6.30 ppm ($J(7',8') = 15.4$ Hz, H–C(8')) [3] [6]. H–C(7) was also evidenced as a *d* at 4.16 ppm ($J(7,8) = 1.2$ Hz), one-bond correlated with a deshielded tertiary C-atom appearing at 76.07 and 76.16 ppm in the ^{13}C-NMR spectrum of **2**, in which almost all signals for both rotamers were distinguishable.

The complete assignments of ^{1}H and ^{13}C resonances of **2** were then easily completed (see *Tables 1* and *2*), allowing unambiguous identification of **2** as 7-hydroxycelacinnine. As for **1**, the relative configuration at C(7) and C(8) was deduced from NOE difference experiments, and the observed enhancements (see *Exper. Part*) led to the relative configuration indicated for **2**.

A biosynthetic pathway for 7-hydroxycelacinnine is proposed in the *Scheme*. Compound **2** is probably obtained as a racemic mixture, since the molecule show neither optical rotation nor CD effect.

Scheme



A molecular formula of $C_{25}H_{31}N_3O_3$ (calc.: 421.2365; found: 421.2380) was established for **3** on the basis of high-resolution MS and ^{13}C-NMR. No cinnamoyl chromophore was evidenced in the UV spectrum, and signals for olefinic protons were lacking

in the ^{1}H-NMR spectrum, DEPT and ^{1}H,^{13}C heteronuclear-correlation experiments indicated that **3** had a pleurostyline-type skeleton with two CH and seven CH_2 groups as well as three aromatic quaternary C-atoms and two C=O groups (*Table 2*). The ^{1}H-NMR spectrum exhibited signals for H_A–C(7) and H_B–C(7) (2.65 ppm), H–C(8) (3.99 ppm, J(7A,8) = 6.2 Hz, J(7B,8) = 8.6 Hz) and H–C(2) (4.49 ppm, J(3A,2) = 5.9 Hz, J(3B,2) = 6.1 Hz). A *dd* (J(7′,8′A) = 1.9 Hz, J(7′,8′B) = 7.3 Hz), centered at 5.12 ppm and one-bond correlated to a C-atom resonating at 67.26 ppm in the ^{13}C-NMR spectrum, was then considered to correspond either to a *H*–C(7′)–OH or to a *H*–C(8′)–OH group.

Finally, the location of the OH group could be determined NOE difference spectroscopy. Indeed, irradiation of H–C(7′) led to 11% enhancement of H–C(6′) and 3% enhancement of H_A–C(8′), while no NOE appeared between H_A–C(8′) and H–C(6′) or H_B–C(8′) and H–C(6′). From these results. The OH group was, thus, undoubtedly located at C(7′). However, relative configurations for the different chiral centers of the molecule could not be deduced from this experiment, though the structure of **3** was clearly established as 7-hydroxy-7′,8′-dihydropleurostyline.

Discussion. – The new spermidine alkaloids **1**–**3** exhibit unusual OH substitutions at C(α) or C(β). It follows that the biosynthesis of macrocyclic alkaloids **1**–**6** probably involves epoxy derivatives of appropriately substituted dicinnamoyl spermidines. As outlined in the *Scheme*, a double nucleophilic attack would so generate the pleurostyline-type skeleton, and successive decarboxylation and dehydroxylation would then lead to compound **1**. An epoxide derivative of pleurostyline would also generate **3** as minor product.

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Experimental Part

General. All known compounds were identified by comparison of their IR, ^{1}H- and ^{13}C-NMR, and MS data with those published in [3–8]. ^{1}H- and ^{13}C-NMR Spectra: *Jeol GSX 270* spectrometer. MS: *Varian MAT 311* mass spectrometer in the EI mode at 70 eV.

Collection, Extraction, and Purification. The leaves of *P. opposita* (2.7 kg, dry) were collected near the Dothio river on the East coast of New Caledonia in 1983. A voucher specimen (PUSSET-CHAUVIERE 533) has been deposited at the herbarium of the 'Laboratoire de Phanérogamie, Museum National d'Histoire Naturelle, Paris'. The powered material was first defatted, then made basic with aq. NH_3 and extracted with $CHCl_3$ in a *Soxhlet* apparatus. Standard acid-base workup using 1% HCl and NH_4OH was followed by MPLC with $CHCl_3$ gradually enriched with MeOH as eluant. Final purification was accomplished by TLC on silica-gel plates. TLC bands were differentiated under short wave-length UV light and by means of *Dragendorff*'s reagent. TLC bands were desorbed from silica gel using $CHCl_3$/MeOH 4:1. Compounds obtained in this way were 7-hydroxypleurostyline (**1**; 800 mg), 7-hydroxycelacinnine (**2**; 250 mg), 7′-hydroxy-7′,8′-dihydropleurostyline (**3**; 15 mg) pleurostyline (**4**; 2.5 g), celacinnine (**5**; 1 g, and celallocinine (**6**; 500 mg).

7-Hydroxypleurostyline (**1**): amorphous, $C_{25}H_{29}N_3O_3$. $[\alpha]_D = -110$ (c = 1, $CHCl_3$). UV (MeOH): λ_{max} 266. IR (thin film): 3400, 3300, 1640, 1580, 760, 710. ^{1}H-NMR ($CDCl_3$): see *Table 1*. ^{13}C-NMR ($CDCl_3$): see *Table 2*. Significant NOE's: H–C(2) to H_A–C(3) and H_B–C(3) (7%), H–C(2) to H_A–C(4) and H_B–C(13) (7%), H–C(2) to NH (4%), H–C(2) to H–C(12) (9%), H–C(2) to H_A–C(13) (4%), H–C(2) to H_A–C(13) (4%), H–C(2) to H–C(3) (16%), H–C(7) to NH (3%), H–C(7) to H–C(2″) and H–C(6″) (6%), H–C(8) to H–C(3) (9%), H–C(8) to NH (6%), H–C(8) to H–C(11) (13%), H–C(8) to H–C(2″) and H–C(6″) (16%), H–C(3′) to H–C(2) (19%),

H–C(7′) to H–C(2′) (22%), H–C(7′) to H–C(8′) (39%), H–C(2″) and H–C(6″) to H–C(7) (7%), H–C(2″) and H–C(6″) to H–C(8) (11%), H–C(6′) to H–C(7′) (48%). HR-MS (70 eV): 419 (12, $M^{+\cdot}$), 363 (32), 362 (100), 160 (47), 159 (22), 158 (15), 70 (21), 28 (27).

7-Hydroxycelacininne (**2**): amorphous, $C_{25}H_{31}N_3O_3$. $[\alpha]_D = 0$ ($c = 1$, $CHCl_3$). CD (EtOH): no measurable effect. UV (MeOH): λ_{max} 217, 224, 281. IR (thin film): 3400, 3330, 1650, 1590, 760, 710. ^{1}H-NMR ($CDCl_3$): see *Table 1*. ^{13}C-NMR ($CDCl_3$): see *Table 2*. Significant NOE's: H–C(7) to H–C(8) (5%), H–C(7) to H–C(2′) and H–C(6′) (4%), H–C(8) to H–C(7) (4%), H–C(8) to H–C(2″) and H–C(6″) (10%), H–C(2′) and H–C(6′) to H–C(7′) (22%), H–C(2′) and H–C(6′) to H–C(8′) (8%), H–C(2″) and H–C(6″) to H–C(7) (3%), H–C(2″) and H–C(6″) to H–C(8) (8%). HR-MS (70 eV): 421 (24, $M^{+\cdot}$), 364 (100), 160 (69), 159 (16), 131 (77), 106 (16), 103 (44), 91 (25), 28 (17), 18 (19).

7-Hydroxy-7′,8′-dihydropleurostyline (**3**): amorphous, $C_{25}H_{31}N_3O_3$. $[\alpha]_D = 0$ ($c = 1$, $CHCl_3$). CD (EtOH): $\Delta\varepsilon$ −9 (223), −0.1 (273). UV (EtOH): λ_{max} 217. IR (thin film): 3400, 3330, 1650, 1590, 760, 710. Significant NOE's: H–C(2) to H–C(4) (7%), H–C(2) to NH (5%), H–C(2) to H–C(12) (3%), H–C(2) to H–C(3′) (17%), H–C(8) to NH (3%), H–C(8) to H–C(10) (3%), H–C(8) to H–C(2″) and H–C(6″) (10%), H–C(3′) to H–C(2) (9%), H–C(6′) to H–C(7′) (6%), H–C(7′) to H–C(6′) (11%), H–C(7′) to H_A–C(8′) (2%), H–C(8′) to H–C(7′) (5%), H_A–C(8′) to H_B–C(8′) (9%), H_B–C(8′) to H_A–C(8′) (18%), H_B–C(8′) to H–C(7′) (9%), H–C(2″) and H–C(6″) to H–C(7) (3%), H–C(2″) and H–C(6″) to H–C(8) (7%). HR-MS (70 eV): 421 (16, $M^{+\cdot}$), 203 (22), 172 (22), 160 (41), 159 (37), 158 (21), 147 (23), 146 (100), 132 (26), 131 (45), 118 (33) 117 (30), 106 (37), 105 (26), 104 (46), 103 (24), 91 (55), 79 (28), 77 (29), 70 (98), 69 (29), 56 (24), 55 (57), 31 (28), 30 (61), 28 (66), 27 (25), 18 (48).

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