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PETROSIN-A AND -B, TWO NEW BIS-QUINOLIZIDINE ALKALOIDS FROM THE SPONGE *PETROSIA SERIATA*[1]

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Dedicated to Prof. R.H. MARTIN for his 70th birthday

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Abstract : This paper describes the structure determinations of two new ichthyotoxic bis-quinolizidine alkaloids from the marine sponge *Petrosia seriata*. The structural elucidations are mostly based on homonuclear 2D-^{1}H NMR experiments.

In a previous paper we reported that the methanolic extract of the marine sponge *Petrosia seriata* is ichthyotoxic[3]. This toxicity is associated with a basic fraction which contains at least eight different alkaloids. A bis-quinolizidine alkaloid, named petrosin (1), could be isolated from this complex mixture as a crystalline compound. Its structure was established by X-ray diffraction analysis[3]. In this note we amplify upon our earlier work by reporting more complete spectral data for petrosin along with the structure determinations of two further ichthyotoxic stereoisomers of 1, petrosin -A (2) and petrosin -B (3). The structural elucidations are mostly based on homonuclear J-resolved and shift-correlated 2D-^{1}H NMR experiments.

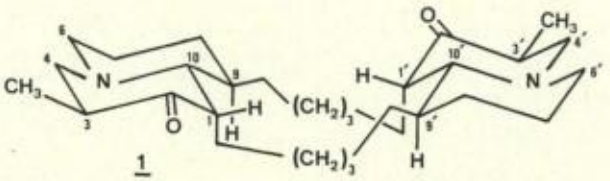
Repetitive alumina column chromatographies of the basic extract of *P. seriata* resulted in the isolation of the major alkaloid 1 and of a mixture of 2 and 3, the separation of which was achieved by preparative tlc on alumina. In contrast to petrosin (1), derivatives 2 ($[\alpha]_{579}$ -5°(CH_2Cl_2, c = 0.71)) and 3 ($[\alpha]_{579}$ - 12° (CH_2Cl_2, c = 0.79)) could not be induced to crystallize.

All three compounds have almost identical mass spectra (M^+ at m/z 470), only small variations of peaks intensity being observed. This fact and the similarity of their ^{1}H and ^{13}C NMR spectra (see table 1 and 2) indicate that petrosin -A (2) and -B (3) are stereoisomers of petrosin (1). The latter is racemic and possess a two-fold rotation axis (C_2) passing through the middle of the 16-membered ring and perpendicular to the mean plane of the same ring[3]. Accordingly, although petrosin (2) is a C_{30}-molecule only 15 peaks are observed in the proton noise-decoupled ^{13}C NMR spectrum. Petrosin -A (2) behaves in the same way, showing 15 peaks in the ^{13}C NMR spectrum and only one 6H doublet at δ 0.96 attributable to the two methyl groups, in the ^{1}H NMR spectrum. This indicates that 2 also has a two-fold rotation axis. In contrast, petrosin -B (3) has no element of symmetry. All three alkaloids exhibit a characteristic series of IR bands between 2700 and 2850 cm^{-1} (Bohlmann bands), in accordance with a *trans*-fused ring conformation for the quinolizidine systems[4]. The equatorial position of the methyl groups in 2 and 3 follows from the comparison of their ^{1}H and ^{13}C chemical shifts and J_{3-14} coupling constants with those of 1 (see table 1 and 2). Axial methyl groups should

afford much more different values[5, 6]. These data indicate that the three petrosins differ only by the configuration at C-9, C-9', C-1 and/or C-1'.

The assignments of the signals in the ^{1}H NMR spectrum of 1 (see table 1) are based on the spin-spin couplings observed in the 2D-COSY 45 spectrum and on the fact that in *trans*-fused quinolizidines the H-4 and H-6 equatorial protons appear at lower field (∿ 2.8 ppm) than the corresponding axial protons (∿ 2.0 ppm)[5]. The J-resolved 2D-^{1}H NMR allows to measure the coupling constants and in particular J_{1-10} (≡ $J_1'_{-10}'$; 3.5 Hz) and J_{9-10} (≡ $J_9'_{-10}'$; 10.0 Hz), wich are in agreement with the configurations at C-1 (C-1') and C-10 (C-10') determined by X-ray diffraction analysis[3].

Signal assignment in the ^{13}C NMR spectrum of 1 (see table 2) is based on chemical shift comparison with the model compounds 4[7], 5[6] and 6[6] and by using the DEPT pulse sequence[8].



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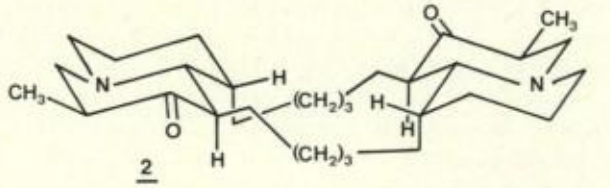

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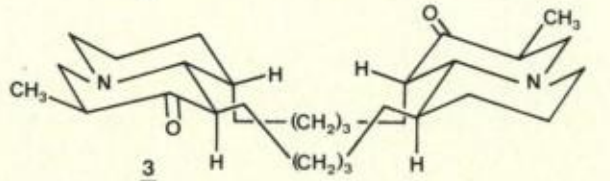

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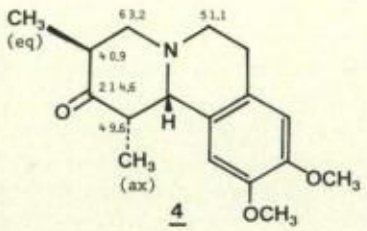

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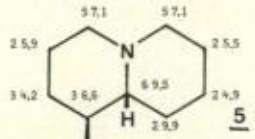

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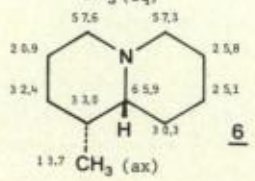

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Table 1 : Characteristic signals in the 250 ^{1}H NMR spectra of 1, 2, and 3 ($CDCl_3$, TMS, δ (J)).

Assignment	1	2	3
H_3C-16	0.96 6H	0.96 6H	0.95 3H d (6.5)
H_3C-16'	d (6.5)	d (6.5)	0.96 3H d (6.5)
H-1	2.53 2H	2.56 2H	2.42 1H ddd (11, 11, 1)
H-1'	ddd (3.5, 5, 11)	ddd (4, 5, 10.5)	2.55 1H ddd (11, 3.5, 3.5)
H-3	2.89 2H	2.95 2H	2.76 1H ddq (6, 6.5, 12.5)
H-3'	ddq (6, 6.5, 12.5)	ddq (6.5, 6.5, 12.5)	2.94 1H ddq (6, 6.5, 12.5)
H-4 (eq)	3.01 2H	3.07 2H	3.06 2H
H-4' (eq)	dd (6, 11)	dd (6.5, 11)	dd (6, 11)
H-4 (ax)	1.90 2H	1.88 2H	1.91 2H
H-4' (ax)	dd (11, 12.5)	dd (11, 12.5)	dd (11, 12.5)
H-6 (eq)	2.96 2H	3.01 2H	∿ 3.0 2H
H-6' (eq)	m	m	m
H-6 (ax)	1.92 2H	1.94 2H	∿ 1.9 2H
H-6' (ax)	m	m	m
H-10	1.84 2H	1.82 2H	1.94 1H dd (4.5, 11)
H-10'	dd (3.5, 10)	dd (3, 10.5)	1.86 1H dd (3.5, 9.5)

Table 2 : 62.8 MHz ^{13}C NMR spectra of 1, 2 and 3 ($CDCl_3$, TMS, δ)

Assignment	Multiplicity*	1	2	3
2 + 2'	C	213.8	213.9	214.1 + 212.4
10 + 10'	CH	70.4	70.8	70.6 + 72.0
4 + 4'	CH_2	64.8	64.8	64.9 + 65.8
6 + 6'	CH_2	56.0	55.9	56.0 + 56.7
1 + 1'	CH	51.9	51.6	51.8 + 51.7
3 + 3'	CH	40.5	40.3	40.3 + 44.6
9 + 9'	CH	37.0	36.0	36.2 + 34.8
	CH_2	29.5 28.8	32.0 30.3	29.5 29.4 28.2 27.7
		27.4 25.1	29.1 26.3	26.6 26.5 25.0 25.0
		24.4 24.1	26.1 25.6	24.4 24.4 24.4 23.2
		23.9	25.0	22.7 20.0
16 + 16'	CH_3	11.3	11.3	11.3 + 11.3

* Assigned by using the DEPT pulse sequence.

The spectral properties of 2 are closely related to those of 1 and, as already mentioned, it also possesses a two-fold rotation axis. 2D-^{1}H NMR[9] experiments (J-RES and COSY 45)[10] demonstrate that the signal attributable to H-10 (H-10') is a double doublet with coupling constants of 3 and 10.5 Hz. This implies that it is coupled to both an axial and an equatorial proton. The only way to satisfy these requirements is to admit that petrosin -A (2) has the reverse configuration at C-1 (C-1') and C-10 (C-10') with respect to those of petrosin (1).

Because of the absence of symmetry, the ^{1}H and ^{13}C NMR spectra of 3 are more complex. As for the two other derivatives, the assignments of the signals in the ^{1}H NMR spectrum of 3 (see table I), are mainly based on homonuclear 2D-^{1}H NMR experiments (J-RES, COSY 45 and COSY 90)[10]. Although these experiments allowed us to measure the δ and J of most of the relevant protons, H-6 and H-6' did not come out clearly, thus precluding to measure with precision their chemical shifts. The H-10 appears in the COSY 90 spectrum as a double doublet with $J_{1-10} = 11$ Hz and $J_{9-10} = 4.5$ Hz. The coupling constants $J_{1'-10'}$ (3.5 Hz) and $J_{9'-10'}$ (9.5Hz) could not be measured by this way, but were obtained from spin decoupling and pseudo-INDOR experiments. Thus, 3 appears as a combination of the quinolizidine ring systems found in 1 and 2.

We have to point out that the spectroscopic values obtained for 3 do not rule out the structure where the two quinolizidine ring systems are linked tête-bêche (C-1 to C-1' and C-9 to C-9'). But, this alternative may be considered as much less probable on the basis of biogenetic arguments.

ACKNOWLEDGEMENTS

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