AXINELLAMINES A AND B, NEW PYRROLE ALKALOIDS OF THE MARINE SPONGE *AXINELLA* SP.

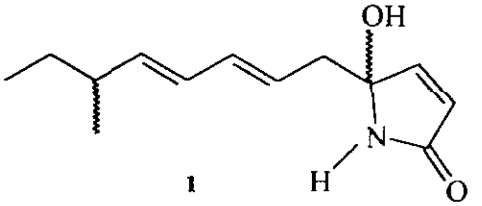
Karen C. Bascombe,[a] Sonia R. Peter,[a] Winston F. Tinto,[a,*]
Suzanne M. Bissada,[b] Stewart McLean,[b] and William F. Reynolds[b,*]

[a]Laboratory of Bioorganic Chemistry, Department of Biological and Chemical Sciences, University of the West Indies, Cave Hill Campus, Barbados
[b]Department of Chemistry, University of Toronto, Toronto, Ontario, M5S 1A1, Canada

Abstract - Two new pyrrole alkaloids, axinellamines A and B, were isolated from the Caribbean marine sponge *Axinella* sp. and their structures were established on the basis of spectroscopic data, including 2D NMR spectroscopy. Axinellamine A was determined to be 2-((*E*,*E*)-6-methyl-2,4-octadienyl)pyrrole while axinellamine B was a dimer of axinellamine A, but containing an additional Me_2C unit between the C-5 and C-5' carbons of the two pyrrole rings.

Marine sponges of the genus *Axinella* have been the subject of extensive chemical investigations, wherein a number of compounds with interesting biological properties have been isolated.[1] Some of these compounds contain nitrogen and possess cytotoxic and antineoplastic activity.[2-4] We recently reported an investigation of a Caribbean *Axinella* sp. from which a new pyrrole alkaloid, axinellamide (**1**) was isolated.[5] We have investigated further specimens of this sponge that were collected in the same location and report here, the isolation of two new pyrrole alkaloids, axinellamines A (**2**) and B (**3**). The structures of **2** and **3** were established by 2D nmr spectroscopy using ^1H-^1H COSY, HMQC and HMBC experiments.

Axinellamine A (**2**), was isolated as a pale yellow gum, $[\alpha]_D$ -45.4°. The molecular formula, $C_{13}H_{19}N$, was established by high resolution MS. The IR spectrum had absorptions at 3391 cm^{-1} and 1662 cm^{-1}, characteristic of amine and olefin residues, respectively. The UV spectrum exhibited an absorption maximum at 227 nm due to the presence of a conjugated diene. The ^1H-NMR spectrum displayed resonances at δ 5.95 (1H, m, H-3), δ 6.14 (1H, m, H-4) and δ 6.68 (1H, m, H-5), which were attributed to a 2-substituted pyrrole. The presence of a 1,4-disubstituted butadiene system was evident from resonances at δ 5.68 (1H, dt, J = 15.0, 7.2 Hz, H-7), δ 6.09 (1H, dd, J = 15.0, 10.4 Hz, H-8), δ 6.00 (1H, dd, J = 15.2, 10.4 Hz, H-9) and δ 5.52 (1H, dd, J = 15.2, 7.5 Hz, H-10). The stereochemistry of the double bonds was determined to be E due to the sizes



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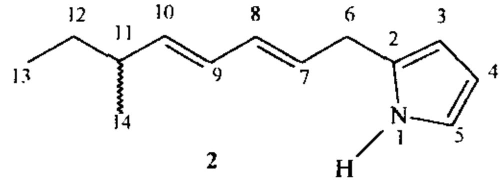

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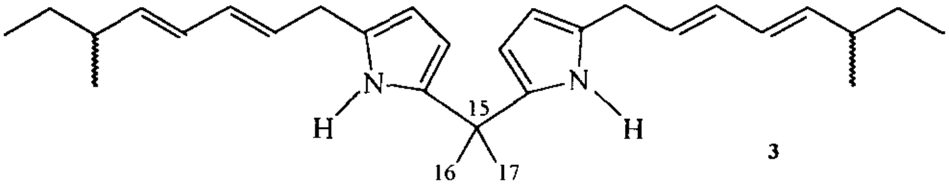

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of the vicinal ^1H-^1H coupling constants. Resonances for two methyl groups occurred at δ 0.86 (3H, t, $J = 7.0$ Hz, H-13) and δ 0.95 (3H, d, $J = 6.9$ Hz, H-14). The ^{13}C-NMR spectrum had signals for two tertiary, one secondary and two primary sp^3 carbons in addition to eight sp^2 carbons, one of which was non-protonated. The structure of the 6-methyl-2,4-octadienyl side chain was established by analysis of an ^1H-^1H COSY spectrum, while the proton-carbon correlations were established by an HMQC experiment; the proton and carbon assignments are recorded in Table I.

Axinellamine B (**3**), was also isolated as a pale yellow gum, $[\alpha]_D$ - 8.9°. The IR spectrum had absorptions at 3401 cm^{-1} (amine) and 1652 cm^{-1} (olefin) while the ultraviolet spectrum had an absorption maximum at 228 nm due to the presence of the conjugated diene. The molecular formula, $C_{29}H_{42}N_2$ (M^+ 418.3329), suggested that **3** was a dimer of **2**, but possessing an extra three-carbon unit. This was supported by the ^{13}C-NMR spectrum which had resonances for only fifteen carbon atoms. The ^1H-NMR spectrum had resonances due to the presence of six methyl groups - two were chemically equivalent and occurred at δ 1.61, another two were chemically equivalent and occurred as a triplet at δ 0.85 ($J = 7.2$ Hz) while the other two were also chemically equivalent and was a doublet at δ 0.98 ($J = 6.8$ Hz). A 2,5-disubstituted pyrrole had resonances at δ 5.81 (m, H-3) and δ 5.94 (m, H-4). A 1,4-disubstituted butadiene system as in **2** had resonances at δ 5.62 (1H, dt, $J = 15.0, 7.0$ Hz, H-7), δ 6.05 (1H, dd, $J = 15.0, 9.8$ Hz, H-8), δ 6.00 (1H, dd, $J = 14.3, 9.8$ Hz, H-9) and δ 5.48 (1H, dd, $J = 14.3, 7.1$ Hz, H-10). In the HMBC spectrum, the two methyl singlets at δ 1.61 (H-16/H-17) showed long-range correlations to the carbon resonance at δ 29.3 (C-16/C-17), a quaternary carbon at δ 35.3 (C15) and the pyrrole carbon at δ 138.1 (C-5). The foregoing evidence led to structure (**3**) for axinellamine B.

Table I. ^{13}C- and ^{1}H- NMR Spectral Data for Axinellamines A (**2**) and B (**3**).[a]

	(**2**)		(**3**)	
position	δ_C	δ_H	δ_C	δ_H
1	---	7.98 (br s)	---	7.51 (br s)
2	130.1	---	129.5	---
3	105.6	5.95 (m)	104.9	5.81 (m)
4	108.4	6.14 (m)	103.6	5.94 (m)
5	116.7	6.68 (m)	138.1	---
6	31.1	3.40 (br d, 7.2)	31.1	3.28 (br d, 7.0)
7	128.3	5.68 (dt, 15.0, 7.2)	128.2	5.62 (dt, 15.0, 7.0)
8	132.3	6.09 (dd, 15.0, 10.4)	132.0	6.05 (dd, 15.0, 9.8)
9	128.0	6.00 (dd, 15.2, 10.4)	128.0	6.00 (dd, 14.3, 9.8)
10	139.8	5.52 (dd, 15.2, 7.5)	139.5	5.48 (dd, 14.3, 7.1)
11	38.4	2.05 (m)	38.5	2.05 (m)
12	29.7	1.32 (m)	29.7	1.33 (m)
13	11.8	0.86 (t, 7.0)	11.8	0.85 (t, 7.2)
14	20.1	0.95 (d, 6.9)	20.1	0.98 (d, 6.8)
15	---	---	35.4	---
16/17	---	---	29.3	1.61 (s)

[a] Multiplicity and coupling constants (in Hz) are in parenthesis.

EXPERIMENTAL

The IR spectra were obtained on a Perkin-Elmer 1725X FT-IR spectrophotometer. UV spectra were recorded on a Hewlett-Packard 8452A spectrophotometer in MeOH solutions. Optical rotations were measured on a Perkin-Elmer 341 polarimeter in $CHCl_3$ solutions. All NMR spectra were obtained on a Varian Unity 500 MHz spectrometer in $CDCl_3$ solutions using TMS as an internal standard. MS were recorded on a VG 70-25S mass spectrometer operating at 70 eV; *m/z* values are reported for significant peaks.

Animal material The sponge was collected around Nelson Island, off Trinidad's Western peninsula in July 1996 and identified by Mr. Richard Hubbard, Institute of Marine Affairs, Trinidad and Tobago, where a voucher specimen was kept.

Extraction and Isolation The sponge (300 g, dried weight) was extracted with acetone (2000 mL) for 24 h at 28 °C and the solvent evaporated to give a dark brown solid (9.5 g). Flash chomatography on silica gel using hexane/acetone (9:1) followed by PLC using the same solvent mixture, gave **2** and **3**.

Axinellamine A (2): Pale yellow gum (48 mg); $[\alpha]_D$ - 45.4° (c= 0.24, $CHCl_3$); IR(film) 3391, 1662, 1597 cm^{-1}; UV (MeOH) 227 nm (є 8000); EIMS *m/z* (rel. int.) 189 (M^+, 36), 132 (100), 118 (32), 93 (68), 80 (84), 69 (45); HRMS: $[M]^+$ 189.1525 ($C_{13}H_{19}N$ requires 189.1518).

Axinellamine B (3): Pale yellow gum (18 mg); $[\alpha]_D$ - 8.9° (c= 0.18, $CHCl_3$); IR (film) 3401, 1652, 1473 cm^{-1}; UV (MeOH) 228 nm (є 6500); EIMS *m/z* (rel. int.) 418 (M^+, 31), 404 (33), 403 (95), 230 (65), 172 (48), 149 (70), 71 (73), 57 (100); HRMS: $[M]^+$ 418.3329 ($C_{29}H_{42}N_2$ requires 418.3348).

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REFERENCES

1. P. Ciminiello, E. Fattorusso, S. Magno, and L. Mayol, *Can. J. Chem.*, 1987, **65**, 518.
2. R. Herb, A. R. Carroll, W. Y. Yoshida, P. J. Scheuer, and V. J. Paul, *Tetrahedron*, 1990, **46**, 3089.
3. G. R. Pettit, F. Gao, and R. Cerny, *Heterocycles*, 1993, **35**, 711.
4. G. R. Pettit, F. Gao, D. L. Doubek, M. R. Boyd, E. Homel, R. Bai, J. M. Schmidt, L. P. Tackett, and K. Rützler, *Gazz. Chim. Ital.*, 1993, **123**, 371.
5. S. L. Miller, W. F. Tinto, J-P. Yang, S. McLean, and W. F. Reynolds, *Tetrahedron Lett.*, 1995, **36**, 5851.

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