BATZELLINES A, B, AND C. NOVEL PYRROLOQUINOLINE ALKALOIDS FROM THE SPONGE *Batzella Sp.*

Shinichi Sakemi and Hao H. Sun*

Harbor Branch Oceanographic Institution, Inc., 5600 Old Dixie Highway, Fort Pierce, Florida 34946, USA

Charles W. Jefford* and Gérald Bernardinelli

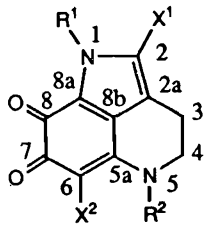
Department of Organic Chemistry and Laboratory of Crystallography, University of Geneva,

1211 Geneva 4, Switzerland

Abstract. Three highly functionalized pyrroloquinoline alkaloids, batzelline A (1), B (2), and C (3) were isolated from the deep water Bahamas sponge *Batzella* sp. The structure of 1 was determined by X-ray and those of 2 and 3 deduced by comparison of their spectral data with that of 1 and by chemical transformations.

In search of herbicidal agents from marine organisms, three nitrogenous pigments, batzellines A (1), B (2), and C (3) were found in the sponge *Batzella* sp. In view of their unusual nature, we now describe details of their isolation and the elucidation of their structures.

A sample was collected in July 1984 using the Johnson-Sea-Link submersible between Freeport and West End in the Grand Bahama Islands at a depth of 120 m. The frozen sponge (68 g) was repeatedly extracted with methanol-chloroform (2:1). The extract was concentrated *in vacuo* to give an aqueous suspension which was further extracted with ethyl acetate. This last extract was fractionated on an Ito multi-layer coil planet centrifuge (CPC) with heptane-EtOAc-MeOH-H_2O (4:7:4:3, lower phase stationary). The stationary phase was re-chromatographed on the CPC (heptane-EtOAc-MeOH-H_2O, 2:7:6:3, lower phase stationary) to yield a pure compound, batzelline A (1, 107 mg), and a fraction containing two minor components. Subsequent countercurrent chromatography on CPC employing $CHCl_3$-iPr_2NH-MeOH-H_2O (7:1:6:4, lower phase stationary) afforded pure batzelline B (2, 26 mg) and C (3, 12 mg). All three compounds were obtained as dark brown solids, however 1 and 3 gave purple solutions and 2 a greenish yellow one.



	R^1	R^2	X^1	X^2
1	Me	H	SMe	Cl
2	H	H	SMe	Cl
3	Me	H	H	Cl
4	Me	Me	SMe	Cl
5	Me	H	H	H

Batzelline A (1) was recrystallized from $CHCl_3$-MeOH as black prisms, melting point 205°C. High resolution EIMS and ^{13}C NMR established the molecular formula as $C_{12}H_{11}N_2O_2SCl$.[1] Its methanolic solution displayed UV absorbance at 214 (ϵ 8,900), 269 (ϵ 22,100), 356 (ϵ 7,900), 376 (ϵ 8,000), and 548 nm (ϵ 600) indicative of an aromatic molecule. The presence of an S-methyl, N-methyl, N-methylene, and an allylic methylene group including eight non-protonated sp^2 carbon atoms in 1 was deduced from its ^1H and ^{13}C NMR data (Table).

Table. ^{13}C and ^{1}H NMR Assignments of Batzellines A (1), B (2), and C (3)[a]

	1		2		3	
Atom #	^{13}C δ	^{1}H δ (m, JHz)	^{13}C δ	^{1}H δ (m, JHz)	^{13}C δ	^{1}H δ (m, JHz)
1						12.85 (br s)
2	132.3		131.3		129.4	7.14 (s)
2a	121.9		123.5		122.9	
3	19.1	2.80 (t, 7.0)	18.7	2.72 (t, 7.0)	18.9	2.73 (t, 7.0)
4	41.5	3.60 (td, 7.0, 2.6)	41.6	3.60 (td, 7.0, 1.7)	41.7	3.56 (td, 7.0, 1.9)
5		8.34 (br s)[b]		8.35 (br s)[b]		8.28 (br s)[b]
5a	148.4		148.7		148.9	
6	97.1		97.0		96.9	
7	168.6[c]		167.3[c]		169.1[c]	
8	170.9[c]		171.7[c]		171.4[c]	
8a	124.8		125.1		123.4	
8b	121.9		119.7		116.7	
1-Me	32.7	3.87 (s)			35.5	3.82 (s)
2-SMe	18.0	2.36 (s)	17.3	2.49 (s)		

[a]Recorded in $(CD_3)_2SO$, δ in ppm from TMS. [b]D_2O exchangeable. [c]Value assigned either to C7 or C8.

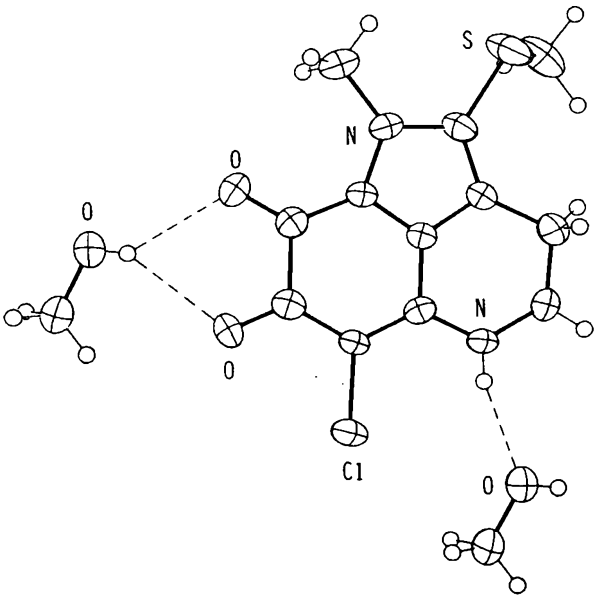
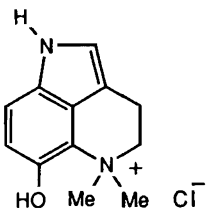


Fig. Perspective drawing of the X-ray structure of 1 showing the hydrogen bonds with methanol

The presence of a free NH group was confirmed by proton exchange with D_2O and by methylation with methyl iodide to give **4**.[2] Catalytic hydrogenation of **1** resulted in reductive debromination and desulfurization furnishing the parent product **5**.[3] Unfortunately, NMR spectral analyses of **1** and **5** were insufficient to reveal their structures without ambiguity. Consequently, an X-ray analysis of a suitable crystal was performed, thereby establishing the structure of **1**[4] (Fig).

The molecular formulas of batzellines B (**2**) and C (**3**) were determined by high resolution EIMS as $C_{11}H_9N_2O_2SCl$ and $C_{11}H_9N_2O_2Cl$ respectively. Their structures were established as **2** and **3** by comparison of their NMR data with that of **1**. The absence of the N-methyl in **2** and the S-methyl group in **3** was evident from their ^1H and ^{13}C NMR spectra (Table). Moreover, **2** on treatment with MeI was converted to the same methylated product **4** as derived from **1**; while **3** on hydrogenation was reduced to **5**.

Examples of 1,3,4,5-tetrahydropyrrolo[4,3,2-e]quinolines have been found both in marine and terrestrial sources. A South American toad *Bufo marinus* contains dehydrobufotenine **6**,[6] and the marine sponges *Latrunculia* and *Prianos* produce discorhabdin C (**7**) and sulfur-containing derivatives.[7] Indeed, batzellines and the sponge metabolite derived from tyrosine **8**[7,8] may well be the biosynthetic precursors of discorhabdins.



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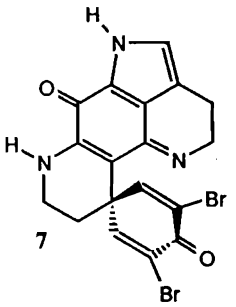

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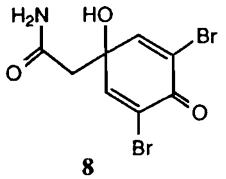

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REFERENCES AND NOTES

1. **1**, HREIMS M^+ 282.0224 (calcd. for $C_{12}H_{11}N_2O_2S^{35}Cl$, Δ 0.6 mmu); LREIMS 284/282 (M^+ 41/100 rel. %), 269 (4), 267 (4), 256 (12), 254 (32), 247 (25), 241 (15), 239 (41), 232 (8), 211 (19), 209 (23), 198 (8), 176 (11), 161 (4), 141 (4), 127 (5), and 88 (4); IR (KBr) 3420, 1667, 1588, 1560, 1525, 1427, 1349, 1309, 1253, 1229, 1192, 1161, 1081, 1000, 976, 838, and 726 cm^{-1}; ^1H and ^{13}C NMR: Table.

2. **4** (9.4 mg), prepared by refluxing **1** (9.5 mg) with CH_3I (5 ml) and K_2CO_3 (50 mg) in $CHCl_3$ (5 ml) at 70°C overnight, followed by HPLC (Hibar NH_2 column, 20:1 $CHCl_3$-MeOH); m.p. 202°C. HREIMS M^+ 296.0386 (calcd. for $C_{13}H_{13}N_2O_2S^{35}Cl$, Δ 0.1 mmu); LREIMS 298/296 (M^+ 47/100 rel. %), 285 (6), 283 (17), 270 (14), 268 (36), 262 (50), 261 (78), 255 (28), 253 (75), 246 (28), 234 (22), 223 (11), 219 (47), 217 (17), 212 (17), 191 (11), 190 (22), 189 (19), 184 (11), 175 (8), 149 (14), 134 (17), 85 (47), and 83 (75); ^1H NMR ($CDCl_3$) δ 3.92 (3H, s, 5-Me), 3.64 (2H, t, J=7.0 Hz, H-4), 3.62 (3H, s, 1-Me), 2.81 (2H, t, J=7.0 Hz, H-3), and 2.33 (3H, s, SMe); ^{13}C NMR ($CDCl_3$) δ 174.1 (s), 167.9 (s), 148.2 (s), 132.2 (s), 125.1 (s), 124.1 (s), 122.5 (s), 100.6 (s), 54.9 (q), 42.8 (t), 33.0 (q), 20.1 (t), and 18.7 (q).

3. **5** (2.3 mg), prepared by hydrogenation of **1** (7.5 mg) with 10% Pd/C (70 mg) in 5 ml of $CHCl_3$ at 50 psi. overnight, followed by HPLC (Hibar NH_2 column, 15:1 $CHCl_3$-MeOH); m.p. (dec.) HREIMS M^+ 202.0742 (calcd. for $C_{11}H_{10}N_2O_2$, Δ 0.1 mmu); LREIMS 202 (M^+ 100 rel. %) 201 (48), 174 (29), 155 (13), 145 (22), 119 (61), 104 (17), 91 (14), 89 (12), 85 (10), 83 (16), 79 (15), 77 (23), 63 (13), 57 (11), 51 (22), and 42 (39); ^1H NMR (DMSO-d_6) δ 8.18 (1H, br s, D_2O exchangeable, H-5), 7.07 (1H, s, H-2), 5.02 (1H, s, H-6), 3.82 (3H, s, 1-Me), 3.47 (2H, dt, J=2.5, 6.9 Hz, H-4), and 2.69 (2H, t, J=6.9 Hz, H-3); ^{13}C NMR (DMSO-d_6) δ 177.7 (s), 171.5 (s), 153.7 (s), 128.6 (d), 124.3 (s), 123.9 (s), 116.0 (s), 92.5 (d), 41.1 (t), 35.4 (q), and 19.0 (t).

4. *Crystal data*: **1**, $C_{12}H_{11}N_2O_2SCl/CH_3OH$, m = 314.8. Triclinic, space group P-1, a = 7.673(3), b = 9.264(2), c = 11.494(4) Å, α = 101.10(3), β = 102.99(2), γ = 112.71(3)°, Z = 2, Dc = 1.50 gr·cm^{-3}, μ = 0.422 mm^{-1}, Fooo = 328. MoKα radiation, R = 0.056, ωR = 0.044 ($\omega = 1/\sigma^2(Fo)$) for 1603 contributing reflections. All coordinates of the H atoms were observed and refined except those of the -SMe group, which were calculated. The molecular packing is fixed by a network of hydrogen bonds involving all potential donors between 1 and the methanol molecule. Data were collected at room temperature on an automatic four-circle Philips PW1100 diffractometer. The structure was solved by direct methods[9]. Calculations were performed with the XTAL program.[10] Observed and calculated structure factors may be obtained on request from G.B.. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England.

5. **2**, m.p. (dec.); HREIMS M^+ 268.0062 (calcd. for $C_{11}H_9N_2O_2S^{35}Cl$, Δ 1.1 mmu); LREIMS 270/268 (M^+ 54/100 rel. %), 257 (8), 253 (25), 242 (14), 240 (37), 235 (11), 233 (19), 227 (32), 225 (86), 199 (16), 197 (37), 170 (10), 161 (12), 149 (7), 134 (14), 58 (40), and 43 (100); UV (MeOH) λ_{max} 211 (ϵ 58,300), 266 (ϵ 120,800), 330 (sh), 387 (ϵ 51,800), and 550 (ϵ 4,000) nm; IR (KBr) 3400, 3315, 2960, 1645, 1620, 1580, 1530, 1410, 1330, 1300, 1145, and 840 cm^{-1}; ^1H and ^{13}C NMR: Table. **3**, m.p. (dec.); HREIMS M^+ 236.0350 (calcd. for $C_{11}H_9N_2O_2{}^{35}Cl$, Δ 0.3 mmu); LREIMS 238/236 (M^+ 10/16 rel. %), 223 (2), 210 (5), 208 (15), 201 (6), 180 (2), 145 (5), 118 (7), 101 (20), 86 (100), and 58 (35); UV (MeOH) λ_{max} 211 (ϵ 7,900), 250 (ϵ 17,300), 331 (ϵ 9,100), 380 (sh), and 540 (ϵ 900) nm; IR (KBr) 3340, 3070, 2950, 1645, 1560, 1530, 1420, 1405, 1350, 1250, 1205, 1130, and 825 cm^{-1}; ^1H and ^{13}C NMR: Table.

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