

Quinolizidines alkaloids: Petrosin and xestospongins from the sponge *Oceanapia* sp.

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Abstract. A bisquinolizidine alkaloid, petrosin (**1**) and a series of *bis*-1-oxaquinolizidine, xestospongins (**2–9**), were obtained from the ethyl acetate extract of the sponge *Oceanapia* sp. Petrosin was obtained along with xestospongins- C, D, E, F, G, H, I and J having di-heteroatom rings, from the ethyl acetate extract of the sponge. The compounds exhibited moderate to high activities against some microorganisms and clinical isolates. The structures of the alkaloids were elucidated by NMR and ESIMS spectroscopic data. The structure of petrosin was confirmed by an X-ray diffraction study.

Keywords. Alkaloid; bisquinolizidine; *Oceanapia*; sponge; crystallography.

1. Introduction

Current interest in marine natural products arises owing to rich in secondary metabolites in marine organisms and larger biodiversity of ocean than in the terrestrial counterpart. Indeed, several secondary metabolites have been reported from marine invertebrates, microbes and their biological activities were investigated.^{1–3} Recently, brominated acetylenic acids isolated from the marine sponges of genera *Xestospongia* and *Oceanapia* were reported to possess antibacterial,⁴ antifungal⁵ and HIV protease inhibitory⁶ activities. Sugar derivatives such as rhizochalin,⁷ oceanalin⁸ and bifunctional sphingolipids⁹ were also reported from the marine sponge belonging to the genus *Oceanapia*. During the course of our investigation on bioactive metabolites from marine organisms,¹⁰ we found petrosin and a series of *bis*-1-oxaquinolizidines alkaloid viz., xestospongins from the sponge *Oceanapia* sp. Previous workers have isolated petrosin¹¹ and xestospongins¹² from other sources and shown to possess anti-HIV¹³ and ichthyotoxicity properties.¹⁴

It is noteworthy that alkaloids reported from *Oceanapia* sp. are mainly peridoacridin,¹⁵ a few reports are available on alkaloids such as peridosamine,¹⁶ isoquinoline glycosides,⁸ bromotyrosin¹⁷ and oceana-pamine.¹⁸ However, to the best of our knowledge alka-

loids having a bisquinolizidine ring have not been reported from the *Oceanapia* sp. As a part of our study on bioactive metabolites from marine organisms,¹⁰ herein, we wish to report the structural elucidation three new (**6**, **7** and **9**) and six known alkaloids (**1–5** and **8**) from the sponge *Oceanapia* sp. We have also investigated the antimicrobial properties of the compounds against some microorganisms and clinical isolates. The structures of the compounds were established on the basis of NMR and ESIMS spectroscopic data. The solid state structure of petrosin was determined by X-ray crystallography.

2. Experimental

2.1 General experimental procedures

Infrared spectra were obtained in a diffused reflection spectroscopy (DRS) assembly on a Shimadzu-8201PC spectrometer with sample prepared in KBr. ¹H and ¹³C NMR spectra were recorded on a Bruker-Avance 300 MHz spectrometer at 300.13 MHz for ¹H and 75.47 MHz for ¹³C, in CDCl₃ with SiMe₄ as internal reference and coupling constants are given in Hertz. ESIMS data were performed on QTOP (MS/MS) Applied Biosystem mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh or 60–120 mesh, Merck, Darmstadt, Germany or SRL, Pvt. Ltd, India) or Shephadex LH-20.

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2.2 Animal materials

Specimens of *Oceanapia* sponge were collected by hand by divers equipped with SCUBA off the coast of southern coast of India at a depth of 5–15 m. Freshly collected sponge specimens were kept in the fridge till work-up. Reference voucher have been deposited at the National Institute of Oceanography, Goa, under the voucher no. NIO-837.

2.3 Extraction and isolation

Dried and minced specimens of *Oceanapia* (dry weight 120 g, dried using lyophilizer) were extracted subsequently with petroleum ether, ethyl acetate and *n*-butanol. The ethyl acetate fraction was subjected to flash column chromatography over silica gel mesh 230–400. Ethyl acetate: Petroleum ether (v/v) solvent system was used as eluant with increasing polarity (0:100 to 100:0 v/v) and finally 200 ml of methanol to remove polar compounds from the column. This leads to collection of fractions 1–52. Fractions 30–32 on re-crystallization with chloroform/methanol gave colourless rectangular crystals of compound **1** (30 mg).

Compound **1** (Petrosin).

FTIR (KBr, cm^{-1}): 2927, 1710 1456.

^1H NMR: 0.634 (q, 2 H, $J_{\text{H-H}} = 9.6$), 0.78 (q, 2 H, $J_{\text{H-H}} = 12.6$), 0.96 (d, 6 H, $J_{\text{H-H}} = 6.3$), 1.10 (m, 2H), 1.26–1.68 (br, 20H), 1.96 (br, 4H), 2.55 (m, 4 H), 2.95 (m, 10H).

^{13}C NMR: 1.16 (s, CH_3), 23.76 (s, CH_2), 24.05 (s, CH_2), 24.17 (s, CH_2), 24.84 (s, CH_2), 27.28 (s, CH_2), 28.71 (s, CH_2), 29.28 (s, CH_2), 36.83 (s, CH), 40.28 (s, CH), 51.70 (s, CH), 56.02 (s, CH_2), 64.59 (s, CH_2), 70.35 (s, CH), 213.80 (s, C=O).

ESIMS: m/z 471.4889 $[\text{M}+\text{H}]^+$ (calculated for $\text{C}_{30}\text{H}_{51}\text{N}_2\text{O}_2$, 471.3950), m/z $[\text{M}/2+\text{H}]^+$ 236.2829.

Isolation of **2** and **3**: Compounds **2** and **3** were obtained by repeated chromatography of fractions 38–44 over silica gel column using $\text{CHCl}_3/\text{MeOH}$ as eluant. This resulted in isolation of a light yellow solid containing a mixture of compounds **2–6**, which after separation over sephadex LH-20 yielded the pure compounds **2** and **3** along with a mixture of compounds **4–6**. Fractions 35–37 on concentration under reduced pressure gave a viscous liquid which changed into light yellow solid when refrigerated contained a mixture of compounds **7–9**.

2.4 Spectroscopic data

2.4a Compound **2** (*Xestospongine-C*): FTIR (KBr, cm^{-1}): 2927, 2850, 1438.

^1H NMR: 4.38 (s, H-10), 3.57 (br t, $J = 12.6$, H-2), 3.36 (br t, $J = 10.8$ Hz, H-2'), 3.11 (br d, H-10', $J = 7.7$), 3.09 (br d, $J = 10.5$, H-4, H-6' α), 2.95 (br d, $J = 10.7$, H-4, 2xH4'), 2.77 (br t, H-6 α $J = 16.8$) 2.50 (br d, H-4), 2.17 (br t, $J = 23.3$, H-6' β), 1.93 (br t, H-6 β), 1.69–1.17 (m, 18 x CH_2 , H-9 and H-9').

^{13}C NMR: 95.83 (d, C-10'), 87.47 (C-10), 75.99 (C-2), 75.35 (C-2'), 54.12 (C-4'), 53.93 (C-6'), 52.09 (C-4), 45.02 (C-6), 40.39 (C-9'), 39.74 (C-9), 35.71, 35.50, 32.03, 31.56, 31.50, 31.45, 31.15, 31.07, 29.61, 29.26, 28.84, 28.77, 25.97, 25.89, 25.11 (d), 24.89, 24.68.

ESIMS: m/z 447.3458 $[\text{M}+\text{H}]^+$ (calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_2$, 447.3950), m/z 224.1390 $[\text{M}/2+\text{H}]^+$ (223).

2.4b Compound **3** (*Xestospongine-D*): ^1H NMR: 4.59 (s, H-10), 3.94 (br t, $J = 10.3$, H-2), 3.52 (br t, $J = 10.5$, H-2'), 3.32 (br d, $J = 6$, H-6' α), 3.07 (br d, $J = 11.1$, 2xH-4', H-10'), 2.79 (br d, $J = 11.1$, H-6 α), 2.25 (br d, $J = 10.8$, H-4), 2.17 (br t, $J = 8.7$, H-6' β), 1.98 (br t, $J = 8.7$, H-6 β), 1.69–1.17 (m, 18 x CH_2 and H-9').

^{13}C NMR: 95.87 (d, C-10'), 90.27 (C-10), 77.21 (C-2), 75.91 (C-2'), 70.14 (C-9), 54.05 (C-4'), 53.93 (C-6'), 52.48 (C-4), 45.09 (C-6), 40.29 (C-9'), 39.42, 34.51, 34.26, 32.35, 31.86, 31.77, 30.35, 30.26, 30.05, 29.68, 29.15, 28.76, 28.63, 26.68, 25.69, 25.22, 25.06, 24.58, 22.26, 18.20.

ESIMS: m/z 463.2311 $[\text{M}+1]^+$ (calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_3$, 463.3899), 445.2782 (M-H $_2\text{O}+1$) (18).

2.5 Mass analysis data of compounds 4–9

2.5a Compound **4** (*Xestospongine-E*): m/z 479.2505 $[\text{M}+1]^+$ (calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_4$, 479.3848); 461.2703 [M-H $_2\text{O}+1]^+$ (18), 443.2659 [M-2H $_2\text{O}+1]^+$ (36), 222.1018 [M-2H $_2\text{O}/2+1]^+$ (221).

2.5b Compound **5** (*Xestospongine-F*): ESI-MS m/z 495.2682 $[\text{M}+1]^+$ (calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_5$, 495.3797).

2.5c Compound **6** (*Xestospongine-G*): ESI-MS m/z 511.2176 $[\text{M}+1]^+$ (calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_6$, 511.3747).

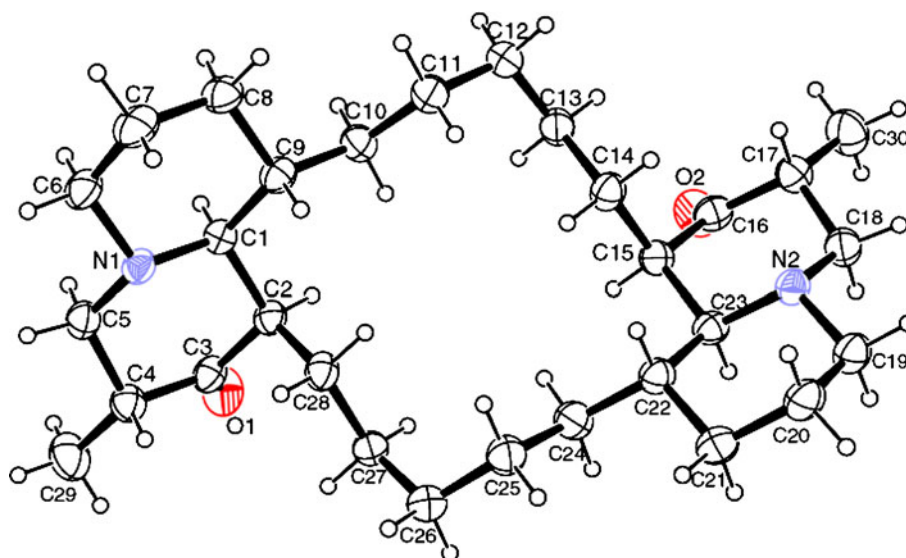


Figure 1. ORTEP diagram of **1** with atoms numbering scheme.

2.5d *Compound 7 (Xestospongina-H)*: ESI-MS m/z 461.2524 $[M+H]^+$ (calculated for $C_{29}H_{53}N_2O_2$, 461.4107).

2.5e *Compound 8 (Xestospongina-I)*: ESI-MS, m/z 477.2544 $[M+H]^+$ (calculated for $C_{29}H_{53}N_2O_3$, 477.4056); 459.2192 $[M-H_2O]^+$ (18), 224.1162, 238.1248.

2.5f *Compound 9 (Xestospongina-J)*: ESI-MS: m/z 493.2458 $[M+H]^+$ (calculated for $C_{29}H_{53}N_2O_4$, 493.4005); 475.2083 $[M-H_2O]^+$ (18), 457.1984 $[M-2H_2O]^+$ (36), 220.1242, 236.1162.

2.6 Antimicrobial assays

Antimicrobial properties of the compounds were tested employing the Disk diffusion technique.¹⁹ Antifungal assay was carried out against the fungus *Aspergillus fumigatus*, *Rhodotorula*, *Candida albicans* and *Aspergillus niger* whereas *Vibrio cholera*,

Escherichia coli and *Staphylococcus aureus* were employed in the antibacterial assay. A 50–200 μ g portion of the compounds were applied onto pre-sterilised disc. The disc was placed on agar plates that had been seeded with respective organisms. Diameters of inhibitory zones were measured after the plates were incubated at 28°C for 24–48 h.

2.7 X-ray crystallography studies

The X-ray intensity data were collected on a Bruker Nonius SMART CCD area detector at 296(2)°K using M_0-K_{α} radiation ($\lambda = 0.71073 \text{ \AA}$) equipped with graphite monochromator. Intensity data were corrected for Lorentz and polarization effects and absorption correction was made using SAINT program.²⁰ The structures were solved by direct methods (SHELXS-97)²¹ and refined by full matrix least squares based on F^2 using (SHELXL-97).²² Non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined using a ‘riding’ model. Refinement converged at a final $R = 0.0608$ and $wR_2 = 0.1279$ for unique data F^2). ORTEP diagram²³ of the compound is shown in figure 1. Selected bond lengths and angles are listed in table 1. Summary of crystal structure determination and refinement are presented in table 2.

Table 1. Bond lengths (\AA) and angles ($^\circ$).

Bond lengths			
C23-N2	1.484 (2)	C1-N1	1.477 (2)
C3-O1	1.213 (2)	C16-O2	1.219 (2)
C18-N2	1.451 (2)	C6-N1	1.474 (2)
C19-N2	1.470 (2)	C5-N1	1.459 (3)
Bond angles			
C18-N2-C19	107.65 (15)	C5-N1-C6	108.08 (16)
C21-C22-C24	111.05 (17)	C2-C1-C9	112.74 (16)
C15-C23-C22	113.56 (15)	C3-C2-C28	110.86 (17)

3. Results and discussion

3.1 Structure elucidation of the compounds

The ethyl acetate extract of sponges of the genus *Oceanapia*, collected from the southern coast of India,

Table 2. Summary of structure determination and refinement for compound **1**.

Empirical formula	C ₃₀ H ₅₀ N ₂ O ₂
Formula weight	470.72
Temperature (K)	296(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	P2(1)/c
Unit cell dimensions	
a (Å)	9.9989(12)
b (Å)	15.8599(12)
c (Å)	17.8402(16)
β (°)	90.392(4)
Volume (Å ³)	2829.1(5)
Z	4
Density (calculated) Mg/m ³	1.105
F(000)	1040
Crystal size (mm ³)	0.45 × 0.28 × 0.16 mm ³
Theta range for data collection (°)	1.72 to 28.25
Index ranges	−13 ≤ h ≤ 11, −20 ≤ k ≤ 20, −23 ≤ l ≤ 22
Reflections collected	28970
Independent reflections	6255 [R(int) = 0.0556]
Completeness to theta (°, %)	28.25, 95.4
Absorption correction	None
Refinement method	Full-matrix least-squares on F ²
Data/restraints/parameters	6255/0/309
Goodness-of-fit on F ²	6255/0/309
Final R indices [I > 2σ(I)]	R ₁ = 0.0608, wR ₂ = 0.1279
R indices (all data)	R ₁ = 0.1284, wR ₂ = 0.1524
Largest diff. peak and hole	0.160 and -0.151 e.Å ⁻³

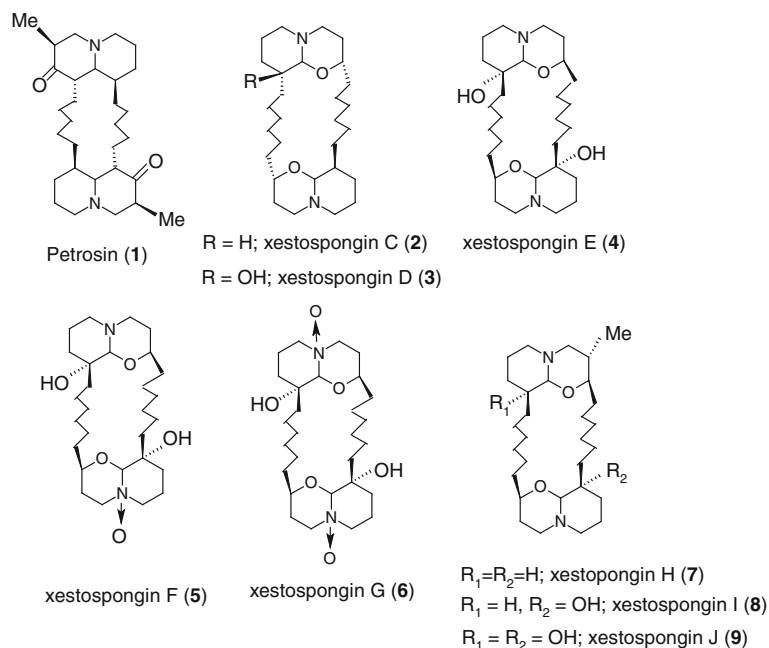
upon chromatography yielded two types of alkaloids having a bisquinolizidine ring, namely petrosin (**1**) and a series of alkaloids containing 1-oxa-bisquinolizidine ring, xestospongins C (**2**), D (**3**), E (**4**), F (**5**), G (**6**), H (**7**), I (**8**) and J (**9**) (scheme 1).

Compound **1** was separated as colourless crystals from the ethyl acetate extract by column chromatography, followed by crystallization from CHCl₃:MeOH, while several xestospongins alkaloids were obtained as a mixture from this same extract. Compound **1** shows IR absorption bands at 2929 cm⁻¹ assignable to Bohlmann bands²⁴ and 1710 cm⁻¹ due to νC=O group. Presence of C=O group was further supported by the appearance of signal at δ 213 in ¹³C NMR spectrum. Molecular formula of **1** was deduced as C₃₀H₅₀N₂O₂ based on ¹³C NMR and ESI-MS data analysis. The presence of two methylene and one methine adjacent to heteroatom nitrogen was confirmed by the appearance of signal at δ 70.35 (CH₂), 64.59 (CH), 56.02 (CH₂) in the ¹³C NMR spectrum. These values are in accordance with the reported values.¹¹ The mass spectrum of the compound showed three distinct peaks at *m/z* 471.4889 [M+H]⁺, 493.5385 [M+Na]⁺ and 509.5316 [M+K]⁺.

The dimeric nature of petrosin was confirmed by the appearance of only fifteen peaks in the ¹³C NMR spectrum against the actual C₃₀ skeleton. This is further supported by mass analysis data which showed peak at *m/z* 236.2829 corresponding to [M/2+H]⁺. The spectroscopic data of **1** suggest that the compound could be petrosin and data are in accordance with the reported values.¹¹ The solid state structure of **1** was finally established by a single crystal X-ray analysis and the chiral centres were assigned as (1S*, 2R*, 4R*, 9S*, 15R*, 17R*, 22S*, 23S*).

Notably, it has been reported that petrosin may exist as six possible stereoisomers, which differ only by configuration at the junctions of the quinolizidines with the sixteen-membered ring. Molecular calculation showed that petrosin is the most stable isomer among the six possible stereoisomers viz., petrosin, petrosin-A, petrosin-B, petrosin-B', petrosin-C, and petrosin-D.²⁵

A series of closely related 1-oxaquinolizidine alkaloids (**2–9**) having di-hetero atom rings were also obtained from the ethyl acetate extract of the same sponge. They have been identified as xestospongins alkaloids by extensive NMR, mass spectral data analyses



Scheme 1. Chemical structure of compounds.

and comparison of their spectroscopic data with those of related compounds (^1H , ^{13}C and mass spectra for selected compounds is available as [supplementary materials](#)). Mass spectral data of the xestospongins containing fraction revealed the presence of at least eight closely related compounds. Among the eight xestospongins alkaloids identified, only xestospongins C (2) and xestospongins-D (3) could be isolated in pure form by chromatography. However, attempt to isolate the other alkaloids in their pure state by chromatography was unsuccessful and as they are found in minor quantity. They were mainly identified by comparison of their ^{13}C NMR and mass data with related compounds. Isolation of xestospongins I (8), C (2) and D (3) from different sources have been reported¹² and a synthesis of petrosin and some of xestospongins alkaloids was described recently.²⁵ The proton NMR spectrum of 2 displayed a singlet at δ 4.38 assignable to CH proton of the heterocyclic ring. The ^{13}C NMR spectrum of these alkaloids showed characteristic signals at around δ 95 attributed to the C–H group attached to two hetero-atoms. The ^{13}C NMR of 2 and 3 are similar, so that initially they were difficult to distinguish. In the case of 2, methine carbon attached to nitrogen appeared at δ 87.47 whereas in 3 the signal is appeared at δ 90.27. In the case of fraction containing compounds 7–9, the ^{13}C NMR spectra showed a signal at δ 14.09 which could be attributed to the methyl groups. It is notable that mass spectrum of the crude fraction containing xestospongins

showed distinct peaks at m/z 447.3458 $[\text{M}+\text{H}]^+$ (2), calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_2$, 447.3950; m/z 463.2311 $[\text{M}+\text{H}]^+$ (3), calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_4$, 463.3899; m/z 479.2765 $[\text{M}+\text{H}]^+$ (4), calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_5$, 479.3848; m/z 495.2682 $[\text{M}+\text{H}]^+$ (5) calculated for 495.3797; m/z 511.2176 $[\text{M}+\text{H}]^+$ (6) calculated for $\text{C}_{28}\text{H}_{51}\text{N}_2\text{O}_6$, 511.3747. This indicates that the fraction contains several xestospongins alkaloids. Mass spectrum also displayed peaks at m/z 461.2524 $[\text{M}+\text{H}]^+$ (7) calculated for $\text{C}_{29}\text{H}_{53}\text{N}_2\text{O}_2$, 461.4107, m/z 477.2544 $[\text{M}+\text{H}]^+$ (8) calculated for $\text{C}_{29}\text{H}_{53}\text{N}_2\text{O}_3$, 477.4056, and m/z 493.2458 $[\text{M}+\text{H}]^+$ (9) calculated for $\text{C}_{29}\text{H}_{53}\text{N}_2\text{O}_4$, 493.4005. The mass of these consecutive alkaloids (2–4) as well as (7–9) differ by 16 mass units corresponding to the substitution of one hydrogen atom with a hydroxyl group (OH) suggesting that alkaloids have a similar skeleton but differ in the number of hydroxyl groups attached to the quinolizidine ring.

The structure of xestospongins-C (2) and xestospongins-D were determined by ESIMS, ^1H and ^{13}C NMR data. The ^1H NMR The ESIMS showed a molecular ion peak at m/z 447.2391 $[\text{M}+\text{H}]^+$ for 2 and 463.2311 for 3. Fragmentation of 3 showed peak at m/z 445.2782 corresponding to loss of water, suggesting the presence of a hydroxyl group in 3. The ^1H NMR spectrum of 3 showed signals at δ 4.82, 4.42 and 4.22 assignable to methine and methylene proton adjacent to nitrogen. These data are identical with those in the literature.¹¹ The structures of the xestospongins-E (4) was deduced by comparing ^{13}C NMR and mass analysis data with

those of the known xestospongine-C whose structure was determined by X-ray analysis.^{12a} The ¹³C NMR of the fraction 38–44 revealed the presence of prominent doublet peak at δ 98.07 in addition to the characteristic signals for xestospongine alkaloids. This value is comparable to the C-10 or C10' of N-oxide xestospongine alkaloids.^{12c} Comparison of ¹³C NMR spectrum and mass analysis data with known N-oxide xestospongine enable us to conclude that the alkaloids present in this fraction were N-oxide xestospongine (5 and 6).

Compounds 7 and 9 were obtained as a mixture along with the known compound 8. The structure of the alkaloids was mainly established by mass spectral data. The mass spectrum of the fraction showed peaks at m/z 461.2524, 477.2544 and 493.2548, thus deduce the molecular formula of 7–9 as C₂₉H₅₂N₂O₃, C₂₉H₅₂N₂O₂ and C₂₉H₅₂N₂O₄, respectively. The spectroscopic data of compound 8 is identical with that of the known xestospongine alkaloid.^{12a} Molecular mass of 7 and 8, as well as 8 and 9, differ by 16 mass units which is equivalent to the substitution of one H by OH group. Moreover, since further fragmentation of 9 gives peaks at m/z 475.2083 (M-18)⁺ and 457.1984 (M-36)⁺ equivalent to successive loss of two water molecules, suggesting the presence of two hydroxyl groups in compound 9.

3.2 Antimicrobial activities of the compounds

The antimicrobial activities of compounds 1–3 were tested using disk diffusion assays.¹⁹ A moderate to high activities was observed against *Escherichia coli* and *Staphylococcus aureus*. Compounds 2 and 3 were also found to be active against other pathogens such as *Rhodotorula*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Rhodotorula*, *Candida albicans* and *Aspergillus niger*. The gram positive bacteria penicillin or streptomycin was used for bacteria test while Nystatin was employed for fungus test.

4. Conclusion

This paper describes the isolation of bisquinolizidine alkaloid, petrosin and bis-1-oxaquinolizidine, xestospongines from the ethyl acetate extract of the sponge *Oceanapia* sp., collected from the southern coast of India. Petrosin, xestospongine-C and xestospongine-D were characterized with the help of NMR and mass spectroscopic data while the structure of xestospongine-E, F, G, H, I and J were estab-

lished mainly by MS/MS analysis data and comparison of their ¹³C NMR data with the known and related compounds.

Supplementary materials

CCDC 716746 contains supplementary crystallographic data for compound 1. This data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif.

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