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# Zyzzyanone A, a novel pyrrolo[3,2-*f*]indole alkaloid from the Australian marine sponge *Zyzzya fuliginosa*

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**Abstract**—A new dipyrroloquinone, zyzzyanone A **1**, having a pyrrolo[3,2-*f*]indole-4,8(1*H*,7*H*)-dione skeleton, was isolated from the Australian marine sponge *Zyzzya fuliginosa*, along with the known pyrroloquinoline alkaloids, makaluvamines C, E, G, H, and L, and damirones A and B. The structure of **1** was determined by spectroscopic data. Zyzzyanone A **1** shows moderate cytotoxic activity against mouse Ehrlich carcinoma cells ($IC_{50}$ 25 μg/mL), inhibits the cell division of fertilized sea urchin eggs at a concentration of 25 μg/mL, and exhibits UV-A and UV-B absorbing activity.


Marine sponges of the genera Latrunculia, Batzella, Prianos, Zyzzya are a rich source of alkaloids bearing a pyrrolo[4,3,2-*de*]quinoline skeleton. This series of alkaloids comprises of about 60 metabolites: discorhabdins, epinardins, batzellines, isobatzellines, makaluvamines, damirones, veiutamine.[1] Several structurally related alkaloids, for example, wakayin, isolated from the ascidian *Clavelina* sp.[2] and tsitsikammamines A and B, isolated from a South African latrunculid sponge,[3] have a dipyrrolo[4,3,2-*de*:2′,3′-*h*]quinoline core. Pyrroloquinoline alkaloids have shown a variety of biological activities such as inhibition of topoisomerase I[4] and II,[5] cytotoxicity against different tumor cell lines,[5,6] inhibition of the phosphatase activity of calcineurin and peptidase activity of CPP32,[7] antifungal,[4] and antimicrobial[8] activities.

In the course of our search for biologically active metabolites from marine sponges we have investigated the aqueous EtOH extract of the Australian marine sponge *Zyzzya fuliginosa* (order Poecilosclerida).[†] We have isolated a new dipyrroloquinone, named zyzzyanone A **1**, together with the known makaluvamines C,[5] E,[5] G **5**,[4] H,[9] and L,[9] and damirones A and B.[10] The known compounds were identified by comparison of their spectral data with published values. In this letter we describe the isolation and structural elucidation of the new compound **1**.

The freeze-dried sponge (125 g) was extracted with 50% EtOH at room temperature. The extract was concentrated under reduced pressure to yield a dark red residue. This residue was subjected to column chromatography on a Polychrome-1 (powder Teflon) column with a solvent elution gradient from $H_2O$ to EtOH. Dark red fractions eluted with 25–40% EtOH gave makaluvamines C,[5] H,[9] and damirones A and B[10] after chromatography on a Sephadex LH-20 column in $CHCl_3$–EtOH–TFA (4:1:0.1%). A brownish-green fraction eluted with 50% EtOH was chromatographed on a Sephadex LH-20 column in $CHCl_3$–EtOH–TFA (3:1:0.1%) to yield makaluvamines E (0.004%),[5] G (0.055%),[4] L (0.008%),[9] and among them compound **1** (0.006% on the dry weight of the sponge).

Zyzzyanone A **1** was obtained as a purple TFA salt, mp 300 °C (decomp.), which gave an orange color as a methanolic solution; UV–vis (MeOH) $\lambda_{max}$ (log ε) 242 (4.26), 286 (3.90), 342 (3.59), 484 (3.44) nm; UV–vis (MeOH/KOH) 243 (4.28), 286 (3.96), 346 (3.60), 505 (3.48) nm; IR (KBr) $\nu_{max}$ 3600–2100 (with peaks at 3400, 3221), 1678, 1633, 1591, 1545, 1519, 1501, 1479, 1457, 1438,

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† Collected at Mid Island, Australian Great Barrier Reef, at a depth of 10 m by hand using scuba.

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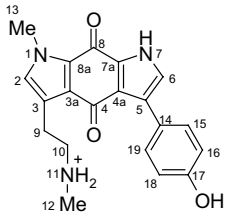
1402, 1382, 1270, 1204, 1182, 1134, 984, 909, 838, 798, 722 $cm^{-1}$; HRFABMS *m/z* 350.1483 ($M^+$+H), $C_{20}H_{20}N_3O_3$ requires 350.1504.



**1**

Compound **1** showed a protonated molecular ion at *m/z* 350.1483 by HRFABMS. The $^{13}C$ NMR and DEPT spectra revealed 20 carbons and indicated the presence of 2 methyls, 2 methylenes, 6 methines, and 10 quaternary carbons (Table 1). On the basis of HRFABMS and $^{13}C$ NMR data of **1**, its molecular formula was established as $C_{20}H_{20}N_3O_3$. The UV and IR [1678, 1633 (C=O), 1591 (C=C)] spectra indicated the presence of a 1,4-benzoquinone moiety in **1**. This was supported by the two quaternary carbon signals at $\delta$ 180.4 and 168.7 in the $^{13}C$ NMR spectrum (Table 1). The IR spectrum of **1** also showed the band of a phenolic hydroxyl group (3400). The presence of a *para*-substituted phenol in **1** was evident from a pair of *ortho*-coupled doublets at $\delta$ 6.75 and $\delta$ 7.56, two protons each, and an exchangeable proton at $\delta$ 9.46 in the $^1H$ NMR spectrum. This was substantiated by COSY, DEPT, HSQC, and HMBC experiments.

The $^1H$ NMR spectrum of **1**, recorded in $CD_3OD$, in part resembled the spectra of pyrroloquinoline alkaloids. Aside from the signals of the *para*-substituted phenol, the $^1H$ NMR spectrum contained two singlets of two *N*-methyl groups, a pair of triplets from two methylene groups, and two one-proton singlets in the aromatic region (Table 1). The values of coupling constants $^1J_{CH}$ (184 Hz) of these aromatic protons at $\delta$ 7.06 and $\delta$ 7.20, measured in gated decoupling experiments, showed that both belong to pyrrole rings. The $^1H$ NMR spectrum of **1**, recorded in DMSO-$d_6$ revealed three additional signals of exchangeable protons. Moreover, one of the signals in the aromatic region ($\delta$ 7.20) was now a doublet coupled with an exchangeable proton at $\delta$ 12.65, and a signal from one *N*-methyl group at 2.56 was a triplet coupled with a broad signal from two exchangeable protons at $\delta$ 8.51, as shown by a $^1H$–$^1H$-COSY spectrum.

The presence of a 2,3,4-trisubstituted *N*-methyl-pyrrole ring in **1** was suggested by $^1H$ NMR [($\delta$ 3.92 (s) and $\delta$ 7.06 (s)] and $^{13}C$ NMR [35.8 (q), 129.5 (s), 125.4 (s), 119.6 (s), 130.3 (d, $^1J_{CH}$ = 184 Hz)] data (Table 1). The $^1H$–$^1H$-COSY spectrum revealed a correlation of protons in a fragment from Me-13 to Me-12. The protons of Me-13 ($\delta$ 3.92) correlated with the proton at C-2 ($\delta$ 7.06), which itself exhibited long range correlation with protons at δ 2.99 of the mutually coupled methylene proton spin system. The protons at $\delta$ 3.10 of the mutually coupled methylene proton spin system correlated to a broad signal from two exchangeable protons at $\delta$ 8.51, which in turn coupled to the protons of Me-12 ($\delta$ 2.56). These correlation data suggested the protonated methylaminoethyl chain in **1**, is attached to the *N*-methylpyrrole ring. This was supported by HMBC data. The HMBC correlations from the proton at $\delta$ 7.06 (H-2) to the carbons at $\delta$ 125.4, $\delta$ 129.5, and $\delta$ 119.6 supported the presence of the pyrrole ring. The carbon at $\delta$ 129.5 correlated to the *N*-methyl protons at $\delta$ 3.92 and to

**Table 1.** $^{13}C$ (125 MHz) and $^1H$ (500 MHz) NMR data of **1**

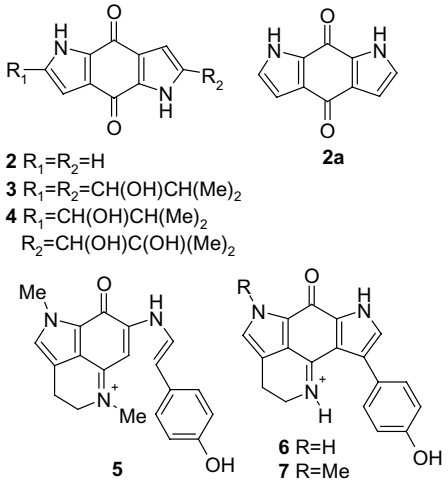
| No. | $CD_3OD$ | | DMSO-$d_6$ | | |
|---|---|---|---|---|---|
| | $\delta$C | $\delta$H (*J*) | $\delta$C | $\delta$H (*J*) | HMBC |
| 2 | 131.9 | 6.80 s | 130.3 (CH) | 7.06 s | 3, 3a, 8a, 9, 13 |
| 3 | 121.4 | | 119.6 (C) | | |
| 3a | 127.9 | | 125.4 (C) | | |
| 4 | 183.3 | | 180.4 (C) | | |
| 4a | 123.6 | | 121.4 (C) | | |
| 5 | 129.4 | | 126.6 (C) | | |
| 6 | 125.2 | 7.01 s | 123.9 (CH) | 7.20 d (2.5) | 4a, 5, 7a, 14 |
| 7a | 135.6 | | 133.3 (C) | | |
| 8 | 170.9 | | 168.7 (C) | | |
| 8a | 132.3 | | 129.5 (C) | | |
| 9 | 24.3 | 3.01 t (7.1) | 22.0 ($CH_2$) | 2.99 t (7.4) | 2, 3, 3a, 10 |
| 10 | 51.2 | 3.19 t (7.1) | 48.1 ($CH_2$) | 3.10 m | 3, 9, 12 |
| 12 | 34.3 | 2.67 s | 32.6 ($CH_3$) | 2.56 t (5.5) | 10 |
| 13 | 37.1 | 3.89 s | 35.8 ($CH_3$) | 3.92 s | 2, 8a |
| 14 | 126.6 | | 124.0 (C) | | |
| 15 | 131.7 | 7.54 d (8.7) | 129.8 (CH) | 7.56 d (8.7) | 5, 17, 19 |
| 16 | 116.2 | 6.77 d (8.7) | 114.6 (CH) | 6.75 d (8.7) | 14, 17, 18 |
| 17 | 158.6 | | 156.7 (C) | | |
| 18 | 116.2 | 6.77 d (8.7) | 114.6 (CH) | 6.75 d (8.7) | 14, 16, 17 |
| 19 | 131.7 | 7.54 d (8.7) | 129.8 (CH) | 7.56 d (8.7) | 5, 15, 17 |
| NH-7 | | | | 12.65 d (2.5) | 4a, 5, 7a |
| $N^+H_2$-11 | | | | 8.51 m | |
| OH | | | | 9.46 b s | |

the proton at $\delta$ 7.06, allowing assignment of this carbon as C-8a. The proton at $\delta$ 7.06 (H-2) and the protons at $\delta$ 2.99 ($H_2$-9) correlated to the carbon at $\delta$ 125.4 indicating it to be C-3a. The HMBC correlations from the proton at $\delta$ 7.06 (H-2) to the carbon at $\delta$ 22.0 (C-9), and from the protons at $\delta$ 2.99 ($H_2$-9) to the carbons at $\delta$ 130.3 (C-2), $\delta$ 119.6 (C-3), and $\delta$ 125.4 (C-3a) pointed to the position of the methylaminoethyl chain being at C-3.

The presence of another 2,3,4-trisubstituted pyrrole ring was suggested by $^1$H NMR [($\delta$ 12.65 (d, $J$ = 2.5 Hz) and $\delta$ 7.20 (d, $J$ = 2.5 Hz)] and $^{13}$C NMR [133.3 (s), 121.4 (s), 126.6 (s), 123.9 (d, $^1J_{CH}$ = 184 Hz)] data (Table 1). The HMBC correlations from the proton at $\delta$ 7.20 (H-6) and from the exchangeable proton at $\delta$ 12.65 (NH) to the carbons at $\delta$ 133.3, $\delta$ 121.4, and $\delta$ 126.6 supported the presence of the pyrrole ring. The position of the attachment of the *para*-substituted phenol to this pyrrole ring and assignment of C-5 were decided on the basis of HMBC data. Key HMBC correlations from the pyrrole proton at $\delta$ 7.20 (H-6) to the carbon atom at $\delta$ 124.0 (C-14) of the phenol ring and from the protons H-15(19) at $\delta$ 7.56 of the phenol ring to the carbon at $\delta$ 126.6 indicated the attachment of the phenol to this carbon ($\delta$ 126.6), and have allowed assignment of this carbon as C-5, as shown in **1**.

The $^1$H and $^{13}$C NMR spectra and the unsaturation requirements of the molecular formula indicated **1** to be tetracyclic. Indeed, the presence of a tetrasubstituted 1,4-benzoquinone unit, two 2,3,4-trisubstituted pyrrole rings, and the *para*-substituted phenol in **1** were substantiated by COSY, DEPT, HSQC, and HMBC experiments. Unfortunately, the connectivity between a tetrasubstituted 1,4-benzoquinone unit and two 2,3,4-trisubstituted pyrrole rings was not fully clarified on the basis of the HMBC correlations. The data obtained allowed for two skeletons, **2** and **2a**, as the possible tricyclic backbone of **1**.

Two dipyrroloquinones, terreusinone **3**, and terreusinol **4**, having a skeleton **2**, have been recently isolated as the products of the marine-derived fungus *Aspergillus terreus*.[11,12] Dipyrroloquinones **2** and **2a** were known previously as the products of isomerization of dipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-5,10-dione during a flash vacuum pyrolysis at 850–900 °C.[13] When we compared the $^{13}$C NMR data of the 1,4-benzoquinone units in compounds with skeletons **2** and **2a** (Table 2), we noted that significant differences in $^{13}$C chemical shifts occurred at C-4, C-4a, C-7a, and C-8. The presence of substituents on pyrrole rings have no significant influence on the chemical shifts of the carbonyl carbons in compounds **1**, **3**, and **4** in comparison with the parent dipyrroloquinones **2** and **2a**. Thus, the chemical shift at $\delta$ 180.4 is a diagnostic C-4 chemical shift for the presence of the skeleton **2a** in compound **1**. The structure of zyzzyanone A was thus determined to be pyrrolo[3,2-*f*]indole-4,8(1*H*,7*H*)-dione.




To confirm the structure of zyzzyanone A **1**, we prepared it from makaluvamine G **5**. Treatment of an EtOH solution of **5** with aqueous $NH_3$ at room temperature for 10 h gave a mixture of products. Chromatography of the reaction mixture on a Sephadex LH-20 column in $CHCl_3$–EtOH–TFA (3:1:0.1%) yielded damirone A[10] (14.5%) and a cyclized product **1** (32%), the spectral and physical data of which were identical with those for zyzzyanone A. Thus the structure **1** was confirmed.

The marine sponge *Z. fuliginosa* has been investigated for makaluvamines and damirones,[5,9] veiutamine,[14] batzellines, and isobatzellines,[15] discrhabdins,[16] makaluvic acids,[17] and modified purines.[18] To our knowledge, compound **1** is the first example of a pyrrolo[3,2-*f*]indole-4,8(1*H*,7*H*)-dione alkaloid isolated from a marine

**Table 2.** $^{13}$C NMR data for 1,4-benzoquinonoe units of **1**–**4** (DMSO-$d_6$)

| No. | $\delta$C | | | | |
|---|---|---|---|---|---|
| | **1** | **2a**[a] | **2**[a] | **3**[b] | **4**[c] |
| 3a | 125.4 | 127.3 | 125.7 | 126.0 | 127.7 |
| 4 | 180.4 | 179.3 | 174.0 | 174.0 | 173.9 |
| 4a | 121.4 | 127.3 | 132.9 | 131.3 | 130.9 |
| 7a | 133.3 | 131.9 | 125.7 | 126.0 | 125.7 |
| 8 | 168.7 | 168.3 | 174.0 | 174.0 | 174.0 |
| 8a | 129.5 | 131.9 | 132.9 | 131.3 | 131.3 |

[a] Data reported by Qiao et al.[13]
[b] Data reported by Lee et al.[11]
[c] Data reported by Li et al.[12]

sponge. The tricyclic dipyrroloquinone skeleton of zyzzyanone A **1** can be considered as a *seco*-derivative of a tetracyclic dipyrroloquinoline core of tsitsikammamines A **6** and B **7**, isolated from the South African latrunculid sponge.[3] Zyzzyanone A could arise from makaluvamine G **5** by intramolecular cyclization at the benzylic position with a concomitant hydrolysis of an imino bond in a hypothetical analogue of tsitsikammamines. Thus, zyzzyanone A can be seen to have a plausible interrelationship with the makaluvamines and with the tsitsikammamines.

Zyzzyanone A **1** showed moderate cytotoxic activity against mouse Ehrlich carcinoma cells ($IC_{50}$ 25 μg/mL), inhibited the cell division of fertilized sea urchin eggs at concentration of 25 μg/mL, and exhibited UV-A and UV-B absorbing activity. An investigation of UV-protecting activity is in progress.

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