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Manzacidin D: An Unprecedented Secondary Metabolite from the "Living Fossil" Sponge *Astrosclera willeyana*[1]

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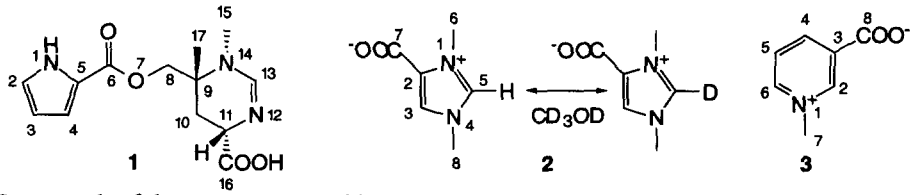
ABSTRACT:-The methanol extract of *Astrosclera willeyana* was found to contain the unprecedented natural product manzacidin D (**1**), norzooanemonin (**2**), and trigonelline (**3**). © 1997 Elsevier Science Ltd.

The coralline demosponge *Astrosclera willeyana* Lister 1900 (Agelasidae, Axinellida) was collected in a small cave at Pearl Reef in the northern part of the Great Barrier Reef, Australia. This animal is a recent representative of late Palaeozoic and Mesozoic reef building sponges and thus termed a "living fossil".

The MeOH extract of the most recent living tissue contained alkaloids, three of which, **1**, **2** and **3**, have been successfully purified.[2]

Compound **1**[3] was shown, by accurate mass measurement, to have the molecular formula $C_{13}H_{17}O_4N_3$. From its ^1H and ^{13}C NMR data it was evident that five of the elements of unsaturation implied by its molecular formula were present as double bonds; two CC, two CO (one as part of a conjugated ester and the other as a free acid), and one CN; the molecule is thus bicyclic. It was also clear from the NMR data that **1** was a pyrrole-5-carboxylic acid or pyrrole-2-carboxylic acid derivative,[4, 5] which accounted for the two CC double bonds, the ester function and one ring. The remaining part of the molecule was thus composed of a tertiary methyl group (1.49 (s), 20.8 q ppm), a N-methyl group (3.28 (s), 36.5 q ppm), two methylene groups, (33.5 t, 66.0 t), an imine moiety (8.00 (br. s), 154.1 d ppm, 1630 cm^{-1}), a methine group (51.4 d ppm), a quaternary carbon (58.7 s ppm), and a carboxyl function (181.4 s ppm, 1680 cm^{-1}). After assigning all NMR resonances *via* an HMQC measurement, it was evident from the COSY spectrum that H-11 coupled with H_2-10, which in turn showed long range coupling to H_3-17. From the COSY spectrum cross peaks were also observed between the resonances for H-13 and H-8 (4.40 (d, *J* 12.6 Hz)), H-11, H_3-15, and H_3-17. From the HMBC spectrum of **1** cross peaks between the resonances for H_2-8 and C-6, indicate C-8 to be an oxymethylene group and the other end of the ester linkage. Further, cross peaks between the resonances for H-11 (adjacent to N) and C-16 suggest the carboxyl function to be located at C-11, while cross peaks between the resonances for H_3-17 and C-8, C-9 and C-10, and between H_3-15 ($-NCH_3$) and C-9 and C-13, and between H-13 and C-15, C-11 and C-9 clearly delineated a 3,4,5,6-tetrahydro-4-oxymethyl-4-methyl-N-methyl-pyrimidine-6-carboxylic acid moiety. With the basic structure of **1** established the relative configurations at C-9, and C-11 remained to be resolved. From the magnitude of the ^1H-^1H couplings between H-11 and H_2-10 (*J* 5.7, 10.5 Hz) it was evident that H-11 must be pseudo-axial. NOE difference measurements made with **1** indicated H_3-17 and H-11 to both be on the same face of the molecule and hence CH_3-17 is also axial. These measurements also indicated CH_3-15 to be pseudo-equatorial and down (α). Thus, the relative configuration of **1**, for which we propose the trivial name manzacidin D, is as shown.



Compounds of the type represented by **1** are extremely rare in nature. The only other report of this class of compound concerns three none N-methylated 3-bromo derivatives, manzacidins A-C, also of sponge origin[6]. The pyrrole moiety found in **1** is somewhat typical for sponges belonging to the family Agelasidae,[7, 8] but also found in other taxonomic groups.[9] It should also be noted that the pyrrole moiety of **1** contained no bromo functions, which is unusual for secondary metabolites from sponges of the family Agelasidae. Two of the very few compounds which have a pyrrole moiety and no bromo function also come from an Agelasidae sponge sample (*Agelas oroides*) collected on the Great Barrier Reef.[4] A sample of *A. willeyana*,[10] from Ponphei, Micronesia, seems to have contained exclusively ageliferins, compounds commonly encountered in

Agelas species. Thus, the same species from two different locations has been found to have a markedly different secondary metabolite chemistry. In this respect it is important to note that *Astrosclera* populations show significant differences in their spicule morphology, giving rise to the suggestion that there could be more than one species. The results presented here tend to support this idea.

Norzooanemonin (**2**)[11] and trigonelline (**3**)[12] have been previously isolated from marine sources.[13-15] It has been suggested that these quaternary ammonium compounds play an important role in cellular osmotic activities of marine invertebrates and their occurrence appears to be quite widespread[16] suggesting them to be primary, not secondary metabolites.

GENERAL EXPERIMENTAL PROCEDURES.- As per (17).

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REFERENCES and NOTES

1. Presented, in part, at the 44th Annual Congress of the Society for Medicinal Plant Research, Prague, Czech Republic, September 3-7, 1996.
2. Specimens of the sponge *Astrosclera willeyana* were collected at a depth of 26 m, from Pearl Reef, Great Barrier Reef, Queensland, Australia. Dried animals were broken into two parts, upper (360.7 g) and lower parts (239.7 g), which were then extracted with dichloromethane (DCM) followed by methanol. From the upper part 0.40 g (0.11 %) of DCM soluble material was obtained, and shown by (^{1}H NMR and TLC) to be predominantly lipids. The methanol extract (1.55 g, 0.43 %), in contrast, by ^{1}H NMR and TLC analysis appeared to contain a number of UV active, low molecular weight, alkaloids. Vacuum liquid chromatography of the MeOH extract over silica gel, using an elution gradient from ethyl acetate to methanol, yielded 10 fractions. To date, from these fractions, three unusual alkaloids **1**, **2** and **3**, have been isolated using a combination of normal and reversed phase (C18) column chromatography and HPLC.
3. Manzacidin D (**1**): (6.2 mg, 0.0017 % of upper mantle), a viscous oil; $[\alpha]_D^{25}$ +14.5° (c, 0.31, $CHCl_3$); IR υ_{max} 3200, 2930, 1705, 1680, 1630, 1410, 1390, 1315 cm^{-1}; UV λ_{max} 270, 225 (ε 7980, 5980) nm; ^{1}H NMR (300 MHz, CD_3OD) 1.49 (3H, s, H_3-17), 2.30 (1H, dd, *J* 10.5, 12.9 Hz, H-10ax), 2.37 (1H, dd, *J* 5.7, 12.9 Hz, H-10eq), 3.28 (3H, s, H_3-15), 4.11 (1H, dd, *J* 5.7, 10.5 Hz, H-11), 4.40 (1H, d, *J* 12.6 Hz, H-8), 4.60 (1H, d, *J* 12.6 Hz, H-8), 6.26 (1H, dd, *J* 2.6, 3.8 Hz, H-3), 6.97 (1H, dd, *J* 1.5, 3.8 Hz, H-4), 7.05 (1H, dd, *J* 1.5, 2.6 Hz, H-2), 8.00 (1H, br. s, H-13) ppm; ^{13}C NMR (75.5 MHz, CD_3OD) 20.8 (q, C-17), 33.5 (t, C-10), 36.5 (q, C-15), 51.4 (d, C-11), 58.7 (s, C-9), 66.0 (t, C-8), 111.0 (d, C-3), 117.6 (d, C-4), 122.4 (s, C-5), 125.6 (d, C-2), 154.1 (d, C-13), 174.2 (s, C-9), 181.4 (s, C-16) ppm; EIMS, m/z (% rel. int.); 280 ([M+1]$^+$, 5), 279 (M$^+$, 12), 261 (8),236 (18), 235 (100), 1555 (60), 141 (38), 125 (50), 109 (86); accurate EIMS obsd. 279.121, $C_{13}H_{17}O_4N_3$ req. 279.121.
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11. Norzooanemonin (**2**): (21.4 mg, 0.0059 % of upper mantle), an optically inactive, crystalline solid; IR υ_{max} 3450, 1635 cm^{-1}; UV λ_{max} 212 (ε 2065) nm; ^{1}H NMR (300 MHz, CD_3OD) 3.94 (3H, s, H_3-8), 4.12 (3H, s, H_3-6), 7.74 (1H, s, H-3), 8.85 (1H, s, H-5) ppm; ^{1}H NMR (300 MHz, DMSO, d_6) 3.77 (3H, s, H_3-8), 3.98 (3H, s, H_3-6), 7.60 (1H, br. s, H-3), 8.84 (iH, br. s, H-5) ppm; ^{13}C NMR (75.5 MHz, CD_3OD) 36.4 (q, C-6), 36.5 (q, C-8), 126.9 (d, C-3), 133.7 (s, C-2), 139.1 (t and 139.4 d, C-5), 162.9 (s, C-7) ppm; ^{13}C NMR (75.5 MHz, DMSO, d_6) 35.0 (q, C-8), 35.3 (q, C-6), 124.1 (d, C-3), 134.1 (s, C-2), 136.6 (d, C-5), 158.4 (s, C-7) ppm; FABMS, m/z (% rel. int.); 163 ([M+Na]$^+$, 30), 141 ([M+H]$^+$, 100); accurate FABMS obsd. 141.0665, $C_6H_9N_2O_2$ req. 141.0664.
12. Trigonelline (**3**): (0.8 mg, 0.0002 % of upper mantle), an optically inactive, crystalline solid; IR υ_{max} 3400, 1645, 1610, 1385 cm^{-1}; UV λ_{max} 270 (sh), 262 (ε 67,000) nm; ^{1}H NMR (300 MHz, CD_3OD) 4.47 (s, 3H, H-7), 8.10 (dd, 1H, H-5, *J* 8.2, 6.0 Hz), 8.89 (d, 1H, H-4, *J* 6.0 Hz), 8.95 (d, 1H, H-6, *J* 8.2 Hz), 9.24 (s, 1H, H-2) ppm; FABMS, m/z (% rel. int.); 139 ([M+2H]$^+$, 80), 138 ([M+H]$^+$, 100), 95 (85), 94 (80); accurate FABMS obsd. 138.0559, $C_7H_8NO_2$ req. 138.0555.
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