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THEONELLADINS A ~ D, NOVEL ANTINEOPLASTIC PYRIDINE ALKALOIDS FROM THE OKINAWAN MARINE SPONGE THEONELLA SWINHOEI

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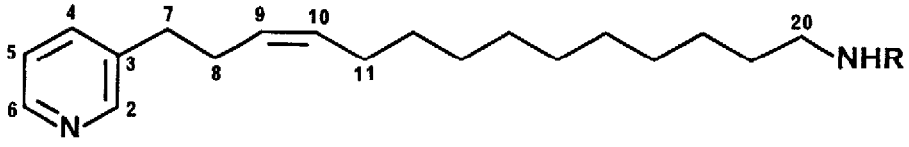
SUMMARY: Novel pyridine alkaloids, theonelladins A ~ D, with potent antileukemic activity have been isolated from the Okinawan marine sponge Theonella swinhoei and the structures elucidated on the basis of spectroscopic data.

Several pyridine-derived alkaloids including navenones[1], pulo'upone[2], anabaseine[3], halitoxins[4] and niphatynes[5] have been isolated from marine organisms such as molluscs, a nemertean and sponges. During our survey of bioactive metabolites from Okinawan marine organisms[6], methanol extracts of a sponge were found to exhibit potent antileukemic activity. In this communication, we describe the isolation and the structure elucidation of novel pyridine alkaloids, named theonelladins A ~ D (1 ~ 4), with potent antineoplastic activity from the Okinawan marine sponge Theonella swinhoei.

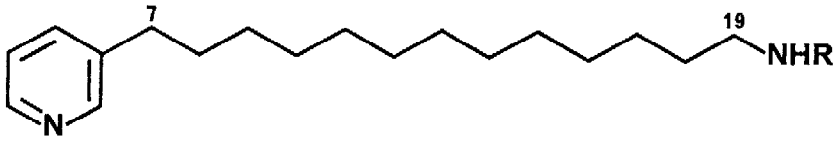
The sponge collected at Unten Bay (-70 ~ -80 m), Motobu Peninsula, Okinawa, was kept frozen until used. The methanol extract was partitioned between ethyl acetate and water. The ethyl acetate-soluble material, showing antileukemic activity, was subjected repeatedly to silica gel column chromatographies ($CHCl_3$/MeOH, 80:20 and $CHCl_3$/n-BuOH/AcOH/H_2O, 15:60:10:10) followed by reversed phase HPLC (ODS, MeOH/H_2O/AcOH, 60:40:0.5) to give theonelladins A (1, 0.0028% wet weight), B (2, 0.0029%), C (3, 0.0035%) and D (4, 0.0015%).

The molecular formula $C_{21}H_{34}N_2$ of 2 was determined by HRFABMS (m/z 303.2766, M + H^+, Δ -3.4 mmu). The presence of a 3-alkyl substituted pyridine ring was suggested by UV maxima[7] at 210 (ε 12300), 257 (3300), 263 (3600) and 269 (2700) nm in MeOH; ^{13}C NMR[8] ($CDCl_3$) signals at δ 150.3 (d, C-2), 147.5 (d, C-6), 139.6 (s, C-3), 138.5 (d, C-4) and 125.1 (d, C-5); ^1H NMR[8] (MeOH-d_4) signals for four aromatic protons at δ 8.36 (brs, H-2), 8.34 (d, J = 5.0 Hz, H-6), 7.69 (d, J = 7.9 Hz, H-4) and 7.34 (q, J = 5.0 and 7.9 Hz, H-5); and an EIMS[9] fragment ion at m/z 210 (M^+ - C_6H_6N). The five degrees of unsaturation were account for by the pyridine ring and



1 R = H

2 R = CH_3



3 R = H

4 R = CH_3

a disubstituted olefin at δ 128.9 (d, C-9) and 132.4 (d, C-10). The configuration of the double bond was assigned as Z by the coupling (11 Hz) between H-9 and H-10 (both δ 5.38) obtained by the J-resolution experiment. The CH_2NHCH_3 terminus was detected by characteristic NMR signals for C-20/H-20 (δ 52.2, t/δ 2.60, brt) and C-22/H-22 (δ 35.5, q/δ 2.41, s), and by an EIMS fragment ion at m/z 272 ($M^+ - CH_4N$). The partial structure $CH_2CH_2CH{=}CHCH_2CH_2$ (C-7 ~ C-11) was deduced from cross peaks for H_2-7 (δ 2.71, t)/H_2-8 (δ 2.38, q), H_2-8/H-9, and H-10/H-11 (δ 1.90, m) in ^1H-^1H COSY spectrum. One end (C-7, δ 33.8) of the alkyl chain was attached to the pyridine ring, since the HMBC[10] spectrum revealed cross peaks for H-7 to C-2, C-3 and C-4. The ten methylenes between the olefin and $NHCH_3$ were evident from the EIMS fragments (M-CH_4N - n x CH_2; n = 1~10). Thus the structure of theonelladin B was assigned to be **2**.

Theonelladin A (**1**) was shown to have the molecular formula $C_{20}H_{32}N_2$ (HRFABMS, m/z 289.2575, M^+ + H, Δ -6.9 mmu). The ^1H and ^{13}C NMR[11] spectra were very similar to those of **2**. However, the decrease in the molecular weight by 14 (CH_2) and the loss of a methyl signal resonance (δ 2.41/δ 35.5) indicated that **1** is the N-demethyl form of **2**. The CH_2NH_2 terminus was also supported by the EIMS fragment ion at m/z 272 ($M^+ - NH_2$) and the CH_2 chemical shifts (δ_C 41.0, t/δ_H 2.83, t).

Theonelladins C (**3**) and D (**4**)[12] were the saturated analogs of **1** and **2** with loss of a methylene unit, respectively . The HRFABMS spectra of **3** and **4** afforded the molecular formulae $C_{18}H_{32}N_2$ (m/z 277.2633, M + H^+, Δ -1.1 mmu) and $C_{19}H_{34}N_2$ (m/z

291.2810, M + H^+, Δ +1.0 mmu), respectively. In each ^1H NMR spectrum of **3** and **4** no olefin proton signal at δ 5.0 ~ 5.5 was observed but a multiplet signal newly appeared at δ 1.59 (H-8) as compared with those of **1** and **2**. The only difference between **3** and **4** was the presence of a singlet at δ 2.42 (NCH_3) in **4**. The saturated alkyl side-chain with a NH_2 or a $NHCH_3$ terminus was also evident from the EIMS[13,14] fragmentation patterns in **3** or **4**.

Pyridine-derived compounds from marine origins are very few[15] and theonelladins A ~ D (**1** ~ **4**) are the first pyridine alkaloids from the sponge of the Theonella genus. The origin of the pyridine ring might be resolved by more examples of pyridine alkaloids from marine sources[16]. Theonelladins A ~ D (**1** ~ **4**) exhibited potent antineoplastic activity[17] against murine lymphomas L1210 (IC_{50} = 4.7 (**1**), 1.0 (**2**), 3.6 (**3**), and 1.6 (**4**) μg/ml) cells and human epidermoid carcinoma KB (IC_{50} = 10 (**1**), 3.6 (**2**), 10 (**3**), and 5.2 (**4**) μg/ml) cells in vitro. These compounds all also showed powerful Ca^{2+}-releasing activity from sarcoplasmic reticulum[18], being twenty times more potent than caffeine, a well-known Ca^{2+} inducer.

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Products": Pergamon Press: New York, 1964, p.179.

8. **2**: NMR signals were assigned from COSY, DEPT, HMQC[19] and HMBC data, ^{13}C NMR ($CDCl_3$) δ 150.3 (d, C-2), 139.6 (s, C-3), 138.5 (d, C-4), 125.1 (d, C-5), 147.5 (d, C-6), 33.8 (t, C-7), 29.8 (t, C-8), 128.9 (d, C-9), 132.4 (d, C-10), 28.2 (t, C-11), 30.3 (t, C-12), 29.8 ~ 30.6 (t, 5C, C-13 ~ C-17), 28.1 (t, C-18), 29.6 (t, C-19), 52.2 (t, C-20), and 35.5 (q, C-22); ^1H NMR (CD_3OD) δ 8.36 (brs, H-2), 7.69 (d, J = 7.9 Hz, H-4), 7.34 (q, J = 5.0 and 7.9 Hz, H-5), 8.34 (d, J = 5.0 Hz, H-6), 2.71 (t, J = 7.4 Hz, H-7), 2.38 (q, J = 7.4 and 13.6 Hz, H-8), 5.38 (m, 2H, H-9 and H-10), 1.90 (brt, H-11), 1.20 (m, H-12), 1.17 ~ 1.37 (m, 1H, H-13 ~ H-17), 1.33 (m, H-18), 1.52 (m, H-19), 2.60 (t, J = 7.4 Hz, H-20), and 2.41 (s, H-22).
9. EIMS of **2**, m/z (%); 302 (M^+, 33), 287 (56), 272 (17), 259 (58), 258 (18), 244 (18), 230 (18), 216 (23), 210 (78), 202 (10), 188 (7), 174 (10), 160 (11), 156 (12), 146 (28), 132 (22), 106 (42), 92 (48), and 93 (100).
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11. **1**: Spectral data were analogous to those of **2** excepting ^{13}C NMR C-19 (t, δ 29.6) and C-20 (t, δ 52.2) and EIMS m/z (%) 288 (M^+, 38).
12. Theonelladins C (**3**) and D (**4**) were contaminated with a trace of analogs with methyl branching alkyl chains which were not separated by HPLC under various conditions.
13. EIMS of **3**: m/z (%) 276 (M^+, 12), 260 (9), 247 (15), 246 (14), 232 (29), 218 (19), 204 (13), 190 (16), 176 (12), 162 (10), 148 (10), 134 (12), 120 (19), 106 (100), 93 (89) and 93 (42).
14. **4**: Spectral data were analogous to those of **1** excepting ^{13}C NMR C-21 (s, δ 35.1) and EIMS m/z (%) 290 (M^+, 15).
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