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Three Novel Anti-microfouling Nitroalkyl Pyridine Alkaloids from the Okinawan Marine Sponge *Callyspongia* sp.

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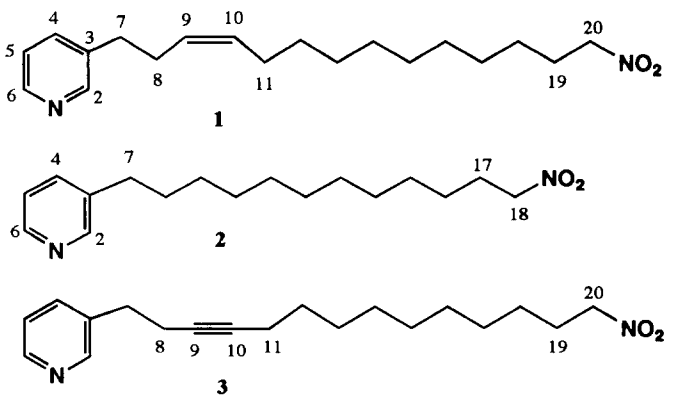
Abstract: Three novel nitroalkyl pyridine alkaloids, untenines A, B and C, with anti-microfouling activity, were first isolated from the Okinawan marine sponge *Callyspongia* sp. Their structures were elucidated on the basis of spectroscopic methods. The synthesis of untenine A was also carried out.

Macrofouling, which is the settlement of large organisms such as barnacles, mollusks and other forms of sea life on ships' hulls, may slow ships by several knots, increase fuel use as much as 40% and cause corrosion. The gram-negative bacteria which first stick to a hull, fishing net, or harbor construction, to create biofouling (microfouling), trigger macrofouling by larger marine organisms. The search for anti-microfouling substances is a subject of great interest.[1] In the course of our search for anti-microfouling compounds from marine organisms, three novel 3-pyridine derivatives with a nitroalkyl group, untenines A , B and C, were isolated from the Okinawan marine sponge *Callyspongia* sp. This is the first isolation of nitroalkyl metabolites from marine organisms, although 3-pyridine alkaloids, such as navenones,[2] halitoxins,[3] aplysinopsins,[4] niphatynes,[5] theonelladins,[6] ikimins,[7] xestamines,[8] niphatoxins,[9] niphatesines,[10] and haminols[11] have been isolated by several groups. We report here the isolation and structure determination of these three novel nitroalkyl pyridines.



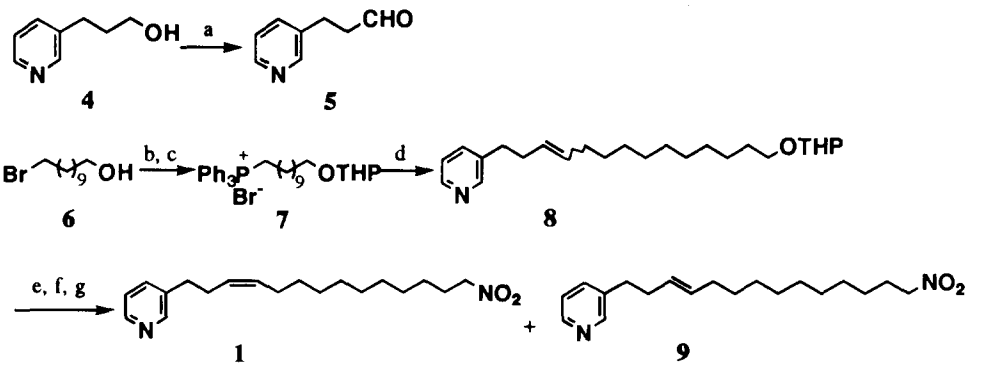
The sponge *Callyspongia* sp. was collected off the coast of Unten, Okinawa in December 1994 and immediately frozen at -20°C until use. The methanolic extract of this sponge (350 g, wet weight) was partitioned between ethyl acetate and water. The organic layer was chromatographed on silica gel (AcOEt/*n*-C_6H_{14}), with monitoring of anti-microfouling activity,[12] and the bioactive 40% and 60% AcOEt/*n*-C_6H_{14} eluates were purified repeatedly by ODS HPLC using 70% MeCN-H_2O as an eluent to yield untenine A (1.0 mg), untenine B (0.6 mg) and untenine C (0.6 mg).

The molecular formula of untenine A (**1**) was determined to be $C_{19}H_{30}N_2O_2$ by HR-EIMS (*m/z* 318.2286, Δ -1.9 mmu). The ^{13}C NMR spectrum and DEPT showed that there were 7 unsaturated carbons, consisting of 6 tertiary carbons and one quaternary carbon, 12 saturated secondary carbons, and no primary or other tertiary carbons. The ^1H NMR and ^{13}C NMR data for untenine A[13] indicated the presence of 3-substituted pyridine and one disubstituted olefin: ^1H NMR (CD_3OD): δ8.35 (1H, bs, H-2), 7.68 (1H, d, J= 8.0 Hz, H-4), 7.33 (1H, dd, J= 4.8, 8.0 Hz, H-5), 8.33 (1H, d, J= 4.8 Hz, H-6), and 5.37 (2H, m, H-9 and H-10); ^{13}C NMR (CD_3OD): δ151.0 (d, C-2), 140.2 (s, C-3), 139.2 (d, C-4), 125.8 (d, C-5), 148.3 (d, C-6), 129.6 (d, C-9) and 133.2 (d, C-10).[6] The correlation between H-7/H-8, H-8/H-9, H-10/H-11 and H-11/H-12 in the ^1H-^1H COSY spectrum suggested the partial structure of C-7~C-12; -CH_2CH_2CH=$CHCH_2CH_2$-. The double bond was determined to be in the *Z*-configuration based on the coupling constant of H-9/H-10 (11 Hz) by the J-resolution spectrum and stronger shielding of the α carbons (C-8 and C-11) than in the *E*-isomer **9** described later.[14] The signals at δ_H 4.42 ppm (2H, t, J= 7.1 Hz, H-20) and δ_C 77.4 ppm (t, C-20) were particularly interesting because of their gradual disappearance when **1** was allowed to stand in CD_3OD for more than one week. The acidity of H-20 led us to assign the partial structure of -CH_2(20)NO_2. The connection of C-7 to C-3 in the pyridine ring was suggested by the correlation of H-7/C-3 in the HMBC spectrum. Thus, the structure of untenine A was clarified to be **1**.

Nitroalkyl compounds were first isolated from marine organisms and showed potent anti-microfouling activity. To confirm the structure of untenine A and investigate its practical use as an anti-foulant, we synthesized **1** and its *E*-isomer **9**, as shown in scheme 1. 3-Pyridinepropanal (**5**) was obtained by Dess-Martin oxidation[15] of 3-pyridinepropanol (**4**).[16] The phosphonium salt (**7**) was obtained from 11-bromo-1-undecanol (**6**)[16] by protecting the hydroxyl group of **6** with tetrahydropyranyl and reacting this mixture with triphenylphosphine in acetonitrile at 85°C for 24 hours.[17] Wittig reaction of aldehyde **5** with the ylide formed by the reaction of **7** and NaH in anhydrous DMSO afforded **8** (*Z*:*E* = 5:2).[18] **1** and **9** were obtained from **8** by (1) removal of the tetrahydropyranyl protecting group in AcOH-THF-H_2O solution; (2) bromination with carbon tetrabromide and triphenylphosphine,[19] followed by treatment with cold 10% sodium bicarbonate solution for 5 minutes; and (3) reaction with silver nitrite stirred in ether in the dark.[20] The NMR spectra, EIMS and HPLC charateristics of synthetic **1** were completely consistent with those of untenine A.

The molecular formula of $C_{17}H_{28}N_2O_2$ for **2** was established from HR-EIMS (*m/z* 292.2152, Δ +0.3 mmu). The five degrees of unsaturation were accounted for by the 3-alkyl substituted pyridine ring and one nitro group. The saturated alkyl chain without branch points terminated by CH_2NO_2 (δ_C 77.4 ppm, δ_H 4.42 ppm) was assigned according to NMR data[21] and the EIMS fragments [*m/z* 246-14n (M-NO_2-nCH_2)$^+$ and 92+14n ($C_5H_4NCH_2$+nCH_2)$^+$]. ^1H-^1H COSY showed correlation peaks of H-7 (2.65 ppm, t, J= 7.2 Hz)/H-8 (1.63 ppm, m) and the long-range correlation of H-7/H-2 and H-4. Therefore, the complete structure of untenine B was **2**.

Scheme 1. Synthesis of **1** and its *E*-isomer **9**



(a) Dess-Martin Oxidation, 96%; (b) DHP, *p*-TsOH, CH_2Cl_2; (c) Ph_3P, MeCN, 80°C, 24hs, 98%; (d) NaH, DMSO, then **5** in DMSO, 45%; (e) AcOH, THF, H_2O; (f) CBr_4, Ph_3P, CH_2Cl_2; (g) $AgNO_2$, ether, 48hs, 58%

The MS spectra of **3** gave the molecular formula $C_{19}H_{28}N_2O_2$ [HR-EIMS: M^+ 316.2153, Δ +0.4 mmu; CIMS: *m/z* 317 $(M+H)^+$]. Except for the presence of the 3-alkyl substituted pyridine ring and the NO_2 group, the NMR spectra[22] also showed that there was an alkynyl bond [δc 80.4 ppm (s) and 83.5 ppm (s)] and no olefinic bonds. The cross peaks of H-7 (2.80 ppm, t, J= 7.3 Hz)/H-2 and H-4, H-7/H-8 (2.46 ppm, m), H-11 (2.07 ppm, m)/H-12 (1.44 ppm, m) and the long-range correlation of H-8/H-11 were observed in the ^1H-^1H COSY spectrum. This finding enabled us to deduce the structure of untenine C as **3**.

Untenines A, B and C exhibited potent anti-microfouling activity with IC_{100} values of 3.0, 6.1 and 5.8 mg/cm², respectively.[12] Field examination of our synthetic untenine A is proceeding successfully. The biogenesis of nitroalkyl compounds is an interesting process, since nitroalkyl compounds are extremely rare metabolites from marine organisms and show a high oxidative state for nitrogen derivatives. These nitroalkyl metabolites are believed to be precursors of nitroso, hydroxyamine and amine derivatives,[23] which may be transient intermediates from nitro groups reduced by reductase in the presence of NADPH. We are continuing to look for these transient compounds in this sponge.

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13. NMR data of **1** in CD_3OD (400 MHz, ppm, J_{HH} in Hz): ^1H NMR: 8.35 (1H, bs, H-2), 7.68 (1H, d, J= 8.0, H-4), 7.33 (1H, dd, J= 8.0, 4.8, H-5), 8.33 (1H, d, J= 4.8, H-6), 2.71 (2H, t, J= 7.3, H-7), 2.38 (2H, m, H-8), 5.37 (2H, m, H-9 and 10), 1.90 (2H, m, H-11), 1.15~1.38 (14H, m, H-12~18), 1.96 (2H, m, H-19), 4.42 (2H, t, J= 7.1, H-20); ^{13}C NMR: 151.0 (d, C-2), 140.2 (s, C-3), 139.2 (d, C-4), 125.8 (d, C-5), 148.3 (d, C-6), 34.6 (t, C-7), 30.5 (t, C-8), 129.6 (d, C-9), 133.2 (d, C-10), 28.9 (t, C-11), 31.4 (t, C-12); 31.3, 31.3, 31.2, 30.8, 30.7 and 28.1 (t for each, C-13~18); 29.2 (t, C-19) and 77.4 (t, C-20).
14. NMR data of **9** in CD_3OD (400 MHz, ppm): the different signals from **1** were only at ^1H NMR: 2.32 (2H, m, H-8) and 1.96 (4H, m, H-11 and H-19). ^{13}C NMR: 35.7 (t, C-8), 130.5 (d, C-9), 134.0 (d, C-10) and 34.3 (t, C-11).
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20. Kornblum, N.; Ungnade, H. E. *Org. Syn.* **1963**, Coll. Vol. 4, 724. **1** and **9** were separated readily by reversed-phase ODS HPLC (YMC-ODS-AQ AQ303 250x4.6 mm I.D., 80% MeOH-H_2O, flow rate 1.0 ml/min, detector wavelength 210 nm, retention time: **1**, 14.0 minutes; **9**, 16.2 minutes)
21. NMR data of **2** in CD_3OD (400 MHz, ppm, J_{HH} in Hz): ^1H NMR: 8.35 (1H, bs, H-2), 7.68 (1H, d, J= 8.0, H-4), 7.33 (1H, dd, J= 4.8, 8.0, H-5), 8.32 (1H, d, J= 4.8, H-6), 2.65 (2H, t, J= 7.2, H-7), 1.63 (2H, m, H-8), 1.20~1.40 (16H, m, H- 9~16), 1.96 (2H, m, H-17), 4.42 (2H, t, J= 7.1, H-18); ^{13}C NMR: 151.1 (d, C-2), 140.5 (s, C-3), 139.1 (d, C-4), 125.6 (d, C-5), 148.4 (d, C-6), 34.1 (t, C-7), 30.0~31.5 and 28.1 (t, C-8~16); 29.1 (t, C-17) and 77.4 (t, C-18).
22. NMR data of **3** in CD_3OD (400 MHz, ppm, J_{HH} in Hz): ^1H NMR: 8.42 (1H, bs, H-2), 7.74 (1H, d, J= 8.0, H-4), 7.34 (1H, dd, J= 8.0, 4.8, H-5), 8.37 (1H, d, J= 4.8, H-6), 2.80 (2H, t, J= 7.3, H-7), 2.46 (2H, m, H-8), 2.07 (2H, m, H-11), 1.20~1.45 (14H, m, H-12~18), 1.96 (2H, m, H-19), 4.44 (2H, t, J= 7.1, H-20); ^{13}C NMR: 151.2 (d, C-2), 140.6 (s, C-3), 139.1 (d, C-4), 125.6 (d, C-5), 148.6 (d, C-6), 34.2 (t, C-7), 22.0 (t, C-8), 80.4 (s, C-9), 83.5 (s, C-10), 20.0 (t, C-11), 30.5~31.5 and 28.1 (t, C-13~18); 29.1 (t, C-19) and 77.4 (t, C-20).
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