Niphatesines A–D, New Antineoplastic Pyridine Alkaloids from the Okinawan Marine Sponge *Niphates* sp.

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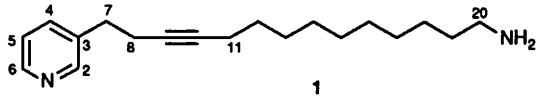
Four new pyridine alkaloids, niphatesines A–D (**1–4**), with potent antineoplastic activity were isolated from the Okinawan marine sponge *Niphates* sp. and their structures were elucidated on the basis of spectroscopic data.

Recently several pyridine alkaloids have been isolated from marine organisms[1,2] and we have also reported four new pyridine alkaloids, theonelladins A–D, that exhibit potent antineoplastic activity.[3] During our studies on bioactive substances from Okinawan marine organisms,[4] extracts of the Okinawan sponge belonging to the genus *Niphates* were investigated and four new monosubstituted pyridine derivatives, which we have named niphatesines A–D (**1–4**), were isolated, and shown to be antineoplastic compounds. Here we describe the isolation and structure elucidation of **1–4**.



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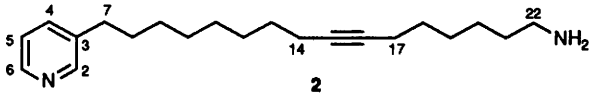

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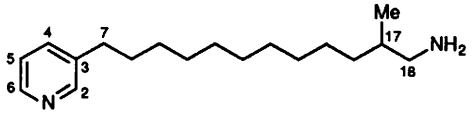

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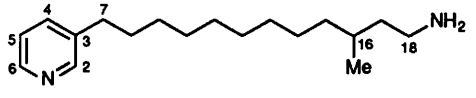

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Results and Discussion

The sponge *Niphates* sp. was collected at Kerama Islands, Okinawa, and kept frozen until used. The methanol extract was partitioned between ethyl acetate and water. The ethyl acetate-soluble material was subjected to silica gel flash column chromatography and eluted with increasing amounts of MeOH starting from 0% and finishing at 50% in $CHCl_3$, followed by reversed-phase HPLC [ODS; MeOH–water–CF_3CO_2H (TFA) 55:45:0.1] to give niphatesines A **1** (0.000 36% yield, wet weight), B **2** (0.0011%), C **3** (0.000 25%) and D **4** (0.000 15%).

The molecular formula of niphatesine A **1** was determined to be $C_{19}H_{30}N_2$ by high-resolution FABMS (*m/z* 287.2481, $M + H^+$, Δ −0.7 mmu). The ^1H and ^{13}C NMR signals for the aromatic region [in CD_3OD: δ_H 8.80 (br s, 2-H), 8.75 (br d, *J* 5.4 Hz, 6-H), 8.53 (d, *J* 8.1 Hz, 4-H) and 8.01 (dd, *J* 5.4 and 8.1 Hz, 5-H); δ_C 148.6 (d, C-4), 143.0 (s, C-3), 142.5 (d, C-2), 140.7 (d, C-6) and 128.3 (d, C-5)] as well as the UV absorption maxima [λ_{max}(MeOH) 207 (ε 5900), 258 (2800), 263 (3200) and 268 nm (2500)] suggested the presence of a mono-3-alkyl-substituted pyridine ring, which was deduced by comparison with spectral data of theonelladins.[3] The six degrees of unsaturation were accounted for by the pyridine ring (C-2–C-6) and a disubstituted alkyne [δ_C 78.5 (s, C-9) and 83.9 (s, C-10)]. Since all other carbons were shown to be CH_2s by the DEPT spectrum, no alkyl-chain branch points exist and the remaining nitrogen atom was assigned to an NH_2 group at the terminus of the aliphatic chain. The NMR spectra showed characteristic signals for the amino-bearing position (δ_H 2.95, 2 H, t, *J* 7.6 Hz, 20-H_2; δ_C 40.9, t, C-20). The ^1H and ^{13}C NMR signals were firmly assigned by ^1H–^1H COSY, ^1H–^{13}C HMQC (heteronuclear multiple quantum coherence),[5] and ^1H–^{13}C HMBC (heteronuclear multiple-bond correlation)[6] experiments and the HMBC spectrum unambiguously verified the position of the alkyne group to be at C-9 and C-10 [correlations (H/C): 7-H_2/C-2, 7-H_2/C-3, 7-H_2/C-4, 7-H_2/C-8, 7-H_2/C-9, 8-H_2/C-3, 8-H_2/C-7 and 8-H_2/C-9]. The ^1H–^1H COSY spectrum revealed a long-range coupling between 8-H_2 and 11-H_2 of 2.3 Hz. This assignment was supported by the observation of EIMS fragmentations at *m/z* 130 ($M^+ - [CH_2]_{10}NH_2$) and 106 ($M^+ - [CH_2]_{10}NH_2 - 2C$). Thus the structure of niphatesine A was concluded to be **1**.

Niphatesine B **2** was shown to have the molecular formula $C_{21}H_{34}N_2$ by high-resolution FABMS (*m/z* 315.2786, $M + H^+$, Δ −1.4 mmu). The spectroscopic properties of compound **2** closely paralleled those of **1** except for the increase in the molecular weight by 28 ($CH_2 \times 2$). This increase suggested that compound **2** possesses two more methylene units in the side-chain. The ^1H and ^{13}C NMR signals were also assigned on the basis of the HMQC and HMBC spectra. The position of the alkyne group was clearly revealed to be at C-15 and C-16 by the EIMS fragmentation pattern [*m/z* 176 (fission of C-13/C-14 bond), 190, 214, 229 and 242]. This pattern was quite similar to that of niphatyne A.[2] The structure of niphatesine B was, therefore, assigned to be **2**.

Niphatesines C **3** and D **4** were also mono-3-alkyl-substituted

pyridine derivatives with an alkyl side-chain terminated by an amino group. The common molecular formula, $C_{18}H_{32}N_2$, of compounds **3** and **4** was established by high-resolution FABMS (**3**: *m/z* 277.2669, M + H$^+$, Δ +2.5 mmu; **4**: *m/z* 277.2635, M + H$^+$, Δ −0.9 mmu). Since the unsaturation number (4) was accounted for by the pyridine ring, both compounds **3** and **4** possess a saturated alkyl side-chain, which was also suggested by the ^{1}H and ^{13}C NMR spectra: no sp^2 signal except those for the pyridine ring was observed. It was characteristic that the ^{1}H and ^{13}C NMR spectra of both compounds **3** and **4** showed the presence of a secondary methyl group [**3**: δ_H 1.01 (3 H, d, *J* 6.5 Hz); δ_C 17.3 (q, C-19) and 32.9 (d, C-17); **4**: δ_H 0.97 (3 H, d, *J* 6.5 Hz); δ_C 19.6 (q, C-19) and 31.7 (d, C-16)]. The ^{1}H–^{1}H COSY spectrum of compound **3** revealed that the position of the secondary methyl group was on C-17. One of the protons attached to the amino-bearing carbon (18-H^a, δ_H 2.88) showed a cross-peak with the signal at δ_H 1.75 (17-H), which was in turn coupled to the methyl protons (δ_H 1.01, 19-H$_3$). This assignment was coincident with the EIMS fragmentation pattern, in which intense peaks were observed at *m/z* 246 [M$^+$ − CH_2NH_2] and *m/z* 218 [M$^+$ − $CH(CH_3)CH_2NH_2$]. The ^{1}H–^{1}H COSY spectrum of compound **4** did not provide direct evidence for the position of the secondary methyl group due to heavily overlapped signals. However, the secondary methyl position of compound **4** was deduced from the EIMS fragmentation pattern on comparison with that of compound **3**. The EIMS of compound **4** showed intense ion peaks at *m/z* 232 and 204 due to (M$^+$ − $[CH_2]_2NH_2$) and {M$^+$ − $CH(CH_3)[CH_2]_2NH_2$}, respectively, thus locating the secondary methyl on C-16.

Known pyridine alkaloids from marine organisms are still few.[7] Niphatesines C **3** and D **4** bearing a side-chain with a branched methyl group seem to be biogenetically related to halitoxin, a polymeric pyridinium salts complex, previously isolated from marine sponges from the genus *Halichlona*.[8] Niphatesines A–D (**1**–**4**) exhibited potent antileukemic activity against L1210 murine leukemia cells *in vitro*, with IC_{50}-values of 3.0, 0.72, 4.5 and 0.95 μg/cm^3, respectively.

Experimental

Optical rotations were determined on a JASCO DIP-360 polarimeter. IR spectra were obtained on a Hitachi 260-50 IR spectrometer, and UV spectra on a JASCO 660 UV/VIS spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on JEOL GX-500 and Bruker AM-500 spectrometers. EI and FAB mass spectra were obtained on a Shimadzu GCMS-QP1000A and a JEOL HX-100 spectrometer, respectively. Wako C-300 silica gel (Wako Pure Chemical) was used for glass column chromatography. TLC was carried out on Merck silica gel GF_{254}.

Isolation.—The sponge, *Niphates* sp. (0.52 kg wet weight) collected by SCUBA at Kerama Islands, Okinawa, was kept frozen until used. The methanol extract of the sponge was evaporated under reduced pressure to afford the residue (100 g), which was dissolved in a mixed solvent of ethyl acetate (600 cm^3) and water (600 cm^3) and then partitioned between ethyl acetate (600 cm^3 × 3) and water (600 cm^3). The ethyl acetate-soluble material (8.43 g) was partly (0.8 g) subjected to silica gel flash column chromatography with gradient elution of methanol in chloroform (0–50%) to give an active fraction (25.5 mg), which was further purified by HPLC [YMC-Pack AM-324 ODS, Yamamura Chemical, 10 × 250 cm^3; eluant, methanol–water–TFA (55:45:0.1)] to yield niphatesines A **1** (1.9 mg, t_R 13.9 min), B **2** (5.8 mg, t_R 27.2 min), C **3** (1.3 mg, t_R 16.2 min), and D **4** (0.8 mg, t_R 13.9 min).

Niphatesine A **1**.—An oil; λ_{max}(MeOH) 207 (ε 5900), 258 (2800), 263 (3200) and 268 nm (2500); ν_{max}(KBr)/cm^{-1} 3450, 1590 and 1450; δ_H(CD_3OD) 8.80 (1 H, br s, 2-H), 8.75 (1 H, br d, *J* 5.4 Hz, 6-H), 8.53 (1 H, d, *J* 8.1 Hz, 4-H), 8.01 (1 H, dd, *J* 5.4 and 8.1 Hz, 5-H), 3.05 (2 H, t, *J* 6.9 Hz, 7-H$_2$), 2.95 (2 H, t, *J* 7.6 Hz, 20-H$_2$), 2.63 (2 H, tt, *J* 2.3 and 6.9 Hz, 8-H$_2$), 2.14 (2 H, tt, *J* 2.3 and 7.0 Hz, 11-H$_2$), 1.69 (2 H, tt, *J* 7.3 and 6.9 Hz, 19-H$_2$) and 1.46–1.30 (14 H, m, 12–18-H$_2$); δ_C(CD_3OD) 148.6 (d, C-4), 143.0 (s, C-3), 142.5 (d, C-2), 140.7 (d, C-6), 128.3 (d, C-5), 83.9 (s, C-10), 78.5 (s, C-9), 40.9 (t, C-20), 32.8 (t, C-7), 30.6 (t), 30.5 (t), 30.2 (t) (2 C), 30.1 (t) and 30.0 (t) (C-12–17), 28.6 (t, C-19), 27.5 (t, C-18), 20.4 (t, C-8) and 19.3 (t, C-11); EIMS *m/z* (relative intensity %) 286 (8, M$^+$), 270 (5), 256 (12), 242 (10), 228 (6), 214 (7), 200 (10), 186 (12), 172 (10), 158 (42), 144 (15), 130 (11), 106 (25) and 93 (100).

Niphatesine B **2**.—An oil; λ_{max}(MeOH)/nm 206 (ε 7800), 228 (3300), 257 (3100), 264 (3400), 269 (2500) and 313 (300); ν_{max}(KBr)/cm^{-1} 3450, 1570 and 1415; δ_H(CD_3OD) 8.41 (1 H, br s, 2-H), 8.34 (1 H, br s, 6-H), 7.72 (1 H, d, *J* 7.0 Hz, 4-H), 7.39 (1 H, m, 5-H), 2.92 (2 H, t, *J* 7.0 Hz, 22-H$_2$), 2.70 (2 H, t, *J* 7.5 Hz, 7-H$_2$), 2.16 (4 H, m, 14- and 17-H$_2$), 1.67 (4 H, m, 8- and 21-H$_2$) and 1.50–1.30 (16 H, m, 9–13-H$_2$ and 18–20-H$_2$); δ_C(CD_3OD) 148.1 (d, C-4), 145.5 (d, C-6), 138.3 (s, C-3), 136.2 (d, C-4), 123.2 (d, C-5), 79.1 (s) and 78.6 (s) (C-15 and -16), 38.7 (t, C-22), 31.8 (t, C-7), 30.2 (t, C-8 and -21), 28.4 (t), 28.2 (t), 28.1 (t), 28.0 (t), 27.8 (t), 27.3 (t) and 26.7 (t) (C-9–13 and C-18 and -19) and 17.3 (t) and 17.2 (t) (C-14 and -17); EIMS *m/z* (relative intensity %) 314 (3, M$^+$), 298 (4), 285 (8), 271 (6), 256 (4), 244 (6), 242 (25), 229 (12), 214 (2), 190 (3), 176 (15), 162 (9), 148 (4), 134 (5), 120 (18), 107 (85), 106 (83) and 93 (100).

Niphatesine C **3**.—An oil; $[\alpha]_D^{25}$ +9.4° (*c* 0.053, MeOH); λ_{max}(MeOH)/nm 206 (ε 2600), 258 (2400), 264 (2700) and 269 (2200); ν_{max}(KBr)/cm^{-1} 3400, 1610 and 1450; δ_H(CD_3OD) 8.76 (1 H, br s, 2-H), 8.71 (1 H, br s, 6-H), 8.54 (1 H, d, *J* 7.5 Hz, 4-H), 8.04 (1 H, br s, 5-H), 2.90 (2 H, t, *J* 7.6 Hz, 7-H$_2$), 2.88 (1 H, m, 18-H^a), 2.71 (1 H, m, 18-H^b), 1.75 (1 H, m, 17-H), 1.70 (2 H, m, 8-H$_2$), 1.50–1.20 (16 H, m, 9–16-H$_2$) and 1.10 (3 H, d, *J* 6.5 Hz, 19-H$_3$); δ_C(CD_3OD) 148.2 (d, C-4), 145.1 (s, C-3), 142.1 (d, C-6), 140.3 (d, C-4), 128.4 (d, C-5), 46.5 (t), 35.1 (t), 33.5 (t), 32.9 (d, C-17), 31.6 (t), 30.8 (t), 30.7 (t) (3 C), 30.5 (t), 30.2 (t), 27.8 (t) and 17.3 (q, C-19); EIMS *m/z* (relative intensity %) 276 (4, M$^+$), 260 (6), 246 (68), 232 (6), 218 (21), 204 (19), 190 (13), 176 (13), 162 (17), 148 (11), 134 (6), 120 (36), 107 (100) and 93 (85).

Niphatesine D **4**.—An oil; $[\alpha]_D^{25}$ +4.4° (*c* 0.045, MeOH); λ_{max}(MeOH)/nm 207 (ε 4300), 259 (2800), 264 (3200) and 270 (2400); ν_{max}(KBr)/cm^{-1} 3420, 1620 and 1460; δ_H(CD_3OD) 8.74 (1 H, s, 2-H), 8.72 (1 H, d, *J* 5.5 Hz, 6-H), 8.53 (1 H, d, *J* 8.0 Hz, 4-H), 8.06 (1 H, dd, *J* 5.5 and 8.0 Hz, 5-H), 2.92 (4 H, m, 7- and 18-H$_2$), 1.74 (2 H, m, 8-H$_2$) 1.71 (1 H, m, 17-H^a), 1.54 (1 H, m, 16-H), 1.50 (1 H, m, 17-H^b), 1.50–1.20 (14 H, m, 9–15-H$_2$) and 0.97 (3 H, d, *J* 6.5 Hz, 19-H$_3$); δ_C(CD_3OD) 148.2 (d, C-4), 145.1 (s, C-3), 142.1 (d, C-6), 140.3 (d, C-4), 128.4 (d, C-5), 39.1 (t), 37.9 (t), 35.7 (t), 33.5 (t), 31.7 (d, C-16), 31.6 (t), 31.0 (t), 30.8 (t), 30.7 (t), 30.5 (t), 30.2 (t), 28.0 (t) and 19.6 (q, C-19); EIMS *m/z* (relative intensity %) 276 (9, M$^+$), 260 (9), 246 (17), 232 (41), 217 (5), 204 (21), 190 (16), 176 (10), 162 (10), 148 (10), 134 (9), 120 (13), 106 (100) and 93 (83).

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