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FOUR BETA-ALKYLPYRIDINES FROM A SPONGE

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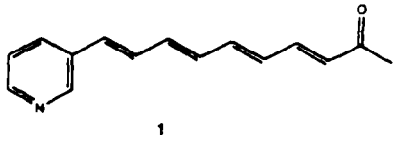
The structures of four sponge metabolites (**7**, **9**, **11**, **12**), β-alkylpyridines terminating in oximino or amino methyl ethers, were determined by NMR measurements. Their cytotoxicity against KB cells (IC_{50}) ranged from 5 to 10 μg/mL.

Long-chain hydrocarbons functionalized by pyridine at one end have been isolated from opisthobranch mollusks of the suborder Philinacea, e.g. navanone A (**1**)[1], pulo'upone (**2**)[2], and more recently also from sponges.[3,4] Biosynthetic studies on the navanones[5] indicated that these compounds are produced by *Navanax inermis* and used as alarm pheromones. The niphatynes (**3** and **4**) from the Fijian sponge *Niphates* sp.[3] and the theonelladins (**5** and **6**) from the Okinawan sponge Theonella swinhoei[4] differ structurally by having nitrogen functions at the hydrocarbon terminus. We report here further examples of sponge-derived alkylpyridines which terminate in methoxyimino or methoxyamino functions and which we have named ikimines.[6]



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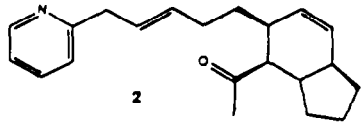


2

	x	y
3	5	3
4	7	1

5 R = H
6 R = CH_3

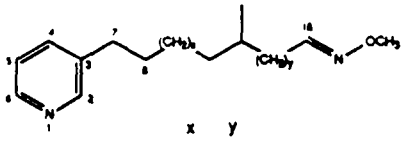
A red spotted orange sponge not yet identified[7] was collected by SCUBA from a steep drop-off (-40 m) on the southwest side of Ant Atoll, Micronesia. The crude lipophilic extract resulting from partitioning the aqueous ethanolic extract with chloroform showed significant cytotoxicity against KB cells.[8] Purification of this extract by vacuum liquid chromatography (VLC)[9] on silica (elution with a solvent gradient: hexane-ethyl acetate-methanol) followed by repeated HPLC on silica and an amino bonded phase eventually

led to the isolation of four major cytotoxic components ikimine A (35 mg), ikimine B (25 mg), ikimine C (35 mg) and ikimine D (8 mg) and two minor artifacts, *syn*-ikimine A (12 mg) and *syn*-ikimine B (4 mg).

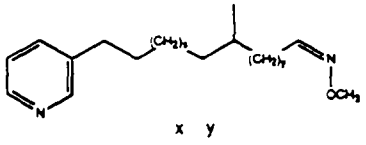
Ikimine A (**7**) which was obtained as a colorless oil had a molecular formula of $C_{19}H_{32}N_2O$ established by HREIMS. An extremely small molecular ion peak and a base peak at *m/z* 273 (M^+-31) in the EIMS indicated facile loss of a methoxy group from the molecule. Further loss of C_5H_6N from the base peak followed by eight incremental losses of CH_2 was in accord with the presence of a long hydrocarbon chain terminating in a $C_5H_6NOCH_3$ group. Further fragments at *m/z* 106 and 93 could be assigned to ethylpyridine (minus H) and methylpyridine respectively.

The ^1H NMR (Table I) and ^{13}C NMR (Table II) spectra for **7** confirmed the presence of a pyridine moiety in the molecule. The proton chemical shifts, multiplicities and coupling constants for this aromatic spin system were characteristic of a β-substituted pyridine ring. Homonuclear decoupling experiments allowed the $C_5H_6NOCH_3$ terminus to be assigned to a methoxy imino group: $-CH(CH_3)CH{=}NOCH_3$. The $-CH{=}NOCH_3$ portion was characterized by a hetero-substituted olefinic ^{13}C NMR signal at δ155.43 and a methoxy signal at $^{13}C/^1H$:δ61.11/δ3.77. The presence of only one oxygenated carbon resonance necessitated that the methoxy group was bonded to the second nitrogen atom. This methoxy imino group was the only hetero moiety of the chain, since the remaining aliphatic signals in the ^1H and ^{13}C NMR spectra could be assigned to eight methylene groups terminating at the pyridine end in a CH_2(δ 32.94/δ 2.62, t, J=7.8 Hz) and at the other end in a $-CH(CH_3)CH{=}NOCH_3$ group.

The oximino ether group was assigned *anti* stereochemistry on the basis of the chemical shifts of H17 and H18 in analogy with oxime chemical shifts.[10] This assignment was corroborated by a large NOE between the methoxy and H18.



	x	y
7	7	0
9	6	1



	x	y
8	7	0
10	6	1

Analysis of the ^1H NMR spectrum of *syn*-ikimine A (**8**) (Table I) indicated that it was the *syn* isomer of **7** since all signals apart from 17-Me, H17 and H18 were superimposable. Of the three non-superimposable signals, H18 was shifted upfield by 0.79 ppm, H17 was shifted downfield by 0.65 ppm and 17-Me was shifted upfield by 0.05 ppm as compared with the corresponding resonances of **7**.

Both **7** and **8** isomerized upon standing in chloroform solution for 24 h at room temperature to produce a 3:1 mixtures of the *anti*(**7**)/*syn*(**8**) oximino ethers, as seen from the intensities of the H18 NMR signals.

Ikimine B (**9**) differed from ikimine A only in the position of the methyl group on the hydrocarbon chain. Ikimine B analyzed for $C_{19}H_{32}N_2O$ by HREIMS and identically with ikimine A, showed a base peak at *m/z* 273 indicating facile loss of methoxy from the molecule. The LREIMS of ikimine B differed from that of ikimine A since cleavage of the bond β to the olefinic oxime carbon led to a fragment at *m/z* 232 ($M^+-C_2H_3NOCH_3$), which was heavier by 14 dalton than the fragment derived from the parallel cleavage in **7** (*m/z* 218:$M^+-C_3H_6NOCH_3$). The remainder of the LREIMS of **9** was identical with that of **7**, thereby suggesting that the methyl group was situated one carbon farther removed from the oxime ether, at C16. That the olefinic oxime carbon was adjacent to a methylene was indicated by the multiplicity of the attached proton resonance H18, δ 7.34, t, *J*=6.6 Hz in the ^1H NMR spectrum of **9** (Table I). Other salient features of the ^1H NMR spectrum of **9** were a methylene envelope at δ 1.59-1.23 integrating for seventeen protons, a β-substituted pyridine spin system, a methylene triplet, δ 2.60, J = 7.8 Hz, and a methoxy singlet at δ 3.78. Homonuclear decoupling experiments allowed the remaining protons to be assigned to $-CH(CH_3)CH_2CH=N-$.

The stereochemistry of the oxime ether portion of ikimine B was also determined to be *anti* based on the chemical shifts of H17α, H17β and H18. NOE studies confirmed the assignment.

A second minor component, *syn*-ikimine B, which had spectroscopic properties very similar to ikimine B was assigned structure **10** the *syn* isomer of **9**. In particular, the shift to lower field of H17α by 0.12 ppm and H17β by 0.14 ppm as compared with H17α and H17β in **9** could be explained by deshielding produced by the *syn* methoxy group in this configuration. Conversely, the 0.72 ppm upfield shift of H18 could be explained by the lack of a deshielding methoxyl group.

Chloroform solutions of **9** and **10** were observed to isomerize on standing to produce 3:1 mixtures of **9** and **10** in both cases. From an examination of the ^1H NMR spectrum of the original crude chloroform extract it was clear that both *syn*-ikimine A and *syn*-ikimine B were artifacts produced during extraction.

The ^1H NMR spectrum of ikimine C (Table I) suggested that it was similar to the other four compounds, since it contained resonances for a β-substituted pyridine ring, a long hydrocarbon chain and a methoxy group. It differed from the other compounds since it did not contain an olefinic oxime proton resonance nor an aliphatic methyl resonance. It did however contain an exchangeable proton at δ 4.58 and a methylene bearing a nitrogen ($^1H/^{13}C$:δ 2.67/δ 54.84). A molecular ion was not observed in the EI mass spectrum but a facile loss of methoxy was assumed since the most abundant mass fragment, the base peak at *m/z* 275 ($C_{18}H_{31}N_2$ from HREIMS), did not contain oxygen. The molecular formula was therefore assumed to be $C_{19}H_{34}N_2O$. This was in agreement with the observation of 19 carbon resonances in the ^{13}C NMR spectrum and 34 proton resonances in the ^1H NMR spectrum of ikimine C. An amino group was placed at the terminus of the hydrocarbon chain since irradiation of the methylene envelope caused the methylene triplet H_2-19 at δ 2.67 to collapse to a singlet. As there were no branch points in the hydrocarbon chain and only

Table 1 ^{1}H NMR Data for ikimine A-D and B[a]
($CDCl_3$)

proton	ikimine A(7)	syn-ikimine A(8)	ikimine B(9)	syn-ikimine B(10)	ikimine C(11)	ikimine D(12)
2	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)
4	7.58(bd,7.8,1h)	7.58(bd,7.8,1H)	7.55(bd,7.8,1H)	7.55(bd,7.8,1H)	7.55(bd,7.8,1H)	7.55(bd,7.8,1H)
5	7.28(dd,7.8,5.0,1H)	7.28(dd,7.8,5.0,1H)	7.25(dd,7.8,4.5,1H)	7.25(dd,7.8,4.5,1H)	7.25(dd,7.8,4.8,1H)	7.25(dd,7.8,4.5,1H)
6	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)	8.42(bs,1H)
7	2.62(t,7.8,2H)	2.62(t,7.8,2H)	2.60(t,7.8,2H)	2.65(t,7.8,2H)	2.62(t,7.5,2H)	2.61(t,7.8,2H)
8	1.61(tt,7.8,7.1,2H)	1.61(tt,7.8,7.1,2H)	1.59(tt,7.8,7.5,2H)	1.59(tt,7.8,7.5,2H)	1.59(tt,7.5,7.5,2H)	1.58(tt,7.8,7.5,2H)
9	1.27(m,2H)	1 27(m,2H)	1.27(m,2H)	1.28(m,2H)	1.27(m,2H)	1.27(m,2H)
10	1.22(m,2H)	1.22(m,2H)	1.23(m,2H)	1.23(m,2H)	1.27(m,2H)	1.27(m,2H)
11	1.22(m,2H)	1.22(m,2H)	1.23(m,2H)	1.23(m,2H)	1.27(m,2H)	1.27(m,2H)
12	1.22(m,2H)	1.22(m,2H)	1.23(m,2H)	1.23(m,2H)	1.27(m,2H)	1.27(m,2H)
13	1.22(m,2H)	1.22(m,2H)	1.23(m,2H)	1.23(m,2H)	1.27(m,2H)	1.27(m,2H)
14	1.22(m,2H)	1.22(m,2H)	1.23(m,2H)	1.23(m,2H)	1.27(m,2H)	1.27(m,2H)
15	1.22(m,2H)	1.22(m,2H)	1.23(m,2H)	1.23(m,2H)	1.27(m,2H)	1.27(m,2H)
16	1.22(m,2H)	1.22(m,2H)	1 59(m,1H)	1.59(m,1H)	1.27(m,2H)	2.09(bt,6.9,2H)
16-Me	-	-	0 88(d,6.9,3H)	0.89(d,6.9,3H)	-	-
17Hα	2.32(ddq,7.5,7.5,6.8,1H)	2.97(ddq,7.5,7.5,6 8,1H)	2.15(ddd,14.1,7.5,6.6,1H)	2.27(ddd,14.1,7.5,6.6,1H)	1.27(m,2H)	-
17Hβ			1.98(ddd,14.1,7.5,6.6,1H)	2.12(ddd,14.1,7.5,6.6,1H)	-	-
17-Me	1.04(d,6.8,1H)	0.99(d,6.8,1H)	-	--	-	-
18	7.17(d,7.5,1H)	6.38(d,7.5,1H)	7.34(t,6.6,1H)	6.62(t,6.6,1H)	1.27(m,2H)	-
19	-	-	-	-	2.67(bt,6.9,2H)	2.45(bt,6.9,2H)
20	-	-	-	-	-	2.79(t,6.9,2H)
NH	-	-	-	-	4.58(bs,1H)	3.98(bs,1H)
OCH_3	3.77(s,3H)	3.80(s,3H)	3.78(s,3H)	3.83(s,3H)	3.56(s,3H)	3.57(s,3H)

[a] ^{1}H NMR spectra were recorded at 300 MHz. Assignments were aided by spin decoupling experiments. J values are reported in Hertz and chemical shifts are in δ units (downfield of TMS).

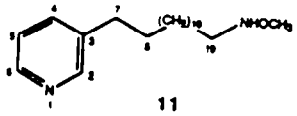
Table II. ^{13}C NMR Data for Ikimine A, B, C and D[a] ($CDCl_3$)

carbon	ikimine A(7)	ikimine B(9)	ikimine C(11)	ikimine D(12)
2	149.10	149.41	149.57	148.01
3	138.50	138.31	138.08	138.08
4	136.56	136.17	136.01	136.57
5	123.49	124.73	123.27	123.48
6	146.30	146.85	146.79	146.24
7	32.94	32.95	32.97	32.98
8	31.04	30.75	31.07	31.03
9	29.34[b]	29.73[b]	29.97[b]	29.41[b]
10	29.45[b]	29.48[b]	29.60[b]	29.60[b]
11	29.45	29.48[?]	29.60[b]	29.60[b]
12	29.45	29.48[b]	29.60[b]	29.55[b]
13	29.34[b]	29.48[b]	29.55[b]	29.14[b]
14	29.52[b]	29.35[b]	29.55[b]	29.10[b]
15	29.08[b]	29.10[b]	29.40[b]	27.10
16	26.98	29.02[b]	29.40[b]	18.63
16-Me	-	19.55	-	-
17	34.76	26.89	29.10[b]	81.19
17-Me	18.22	-	-	-
18	155.43	155.31	27.21	81.19
19	-	-	54.84	20.59
20	-	-	-	56.86
OCH_3	61.11	61.11	61.34	61.36

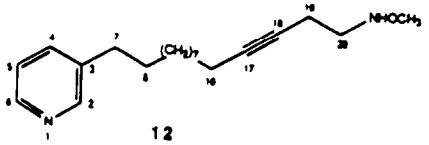
[a] ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts are in δ units (downfield of TMS).

[b-e] Assignments may be interchanged within the column.

one oxygenated carbon resonance in the ^{13}C NMR spectrum of ikimine C, the methoxy group could logically only be a substituent of the nitrogen atom N20. The LREIMS fragmentation pattern confirmed that 13 CH_2's separated the pyridine and the methoxy amine units. Combination of the above spectroscopic data allowed structure **11** to be assigned to ikimine C.



11



12

Ikimine D was obtained as part of an inseparable mixture of ikimine C and ikimine D. The ^1H NMR spectrum of ikimine D (Table I) differed from that of ikimine C only by two additional methylene resonances downfield from the methylene envelope of δ 2.50 and 2.10 and by three fewer methylene resonances in the methylene envelope. As was the case of ikimine C, a molecular ion was not observed in the EI mass spectrum of ikimine D. However the most abundant mass fragment, the base peak *m/z* 285 ($C_{19}H_{29}N_2$) was again assumed to lack only a methoxy group. The molecular formula $C_{20}H_{32}N_2O$ was therefore deduced, containing six elements of unsaturation. Four elements of unsaturation were accounted for by the pyridine ring, leaving two unsaturation elements unaccounted for. A quarternary carbon resonance at δ 81.19 and two upfield methylene resonances (δ 18.63, H16 and δ 20.59, H19) in the ^{13}C NMR spectrum of ikimine D (Table II) provided the only major differences between the ^{13}C NMR spectra of ikimine C and ikimine D. The downfield resonance at δ 81.19 was assigned to two coincident alkyne carbon resonances, thus accounting for the two remaining elements of unsaturation, while the two resonances upfield from 21 ppm could be assigned to the two progargylic carbons. The position of the acetylenic group on the hydrocarbon chain was established by homonuclear decoupling experiments. The two methylene signals at δ 2.09 and 2.45 obviously arose from protons at either end of the acetylene group, since irradiation of one of these signals produced sharpening of the other signal. Irradiation of the methylene attached to the nitrogen H_2-19 at δ 2.79 indicated that it was vicinal to one of the propargylic methylenes at δ 2.45, while irradiation of the methylene envelope at δ 1.27 caused the other propargylic methylene triplet at δ 2.09 to collapse to a broad singlet. Ikimine D was therefore assigned structure **12**.

While superficially similar, marine alkylpyridines exhibit considerable structural diversity. The molluskan metabolites, navanone A (**1**)[1] and pulo'upone (**2**)[2] terminate in methyl ketone functions, but pulo'upone is the only α-alkylpyridine encountered so far. The sponge metabolites, the niphatynes (**3** and **4**), the theonelladins (**5** and **6**), and the ikimines (**7**, **9**, **11** and **12**) which terminate in methoxyamino functions might be derived from chains with aldehydic termini. No pattern is apparent for the positions of the acetylenic functions or the methyl substituents.

Experimental Part

General Procedures. Infrared spectra were recorded on a Perkin-Elmer Model 1600 spectrometer and ultraviolet spectra on a Hewlett-Packard Model 8452A diode array spectrophotometer. Mass spectra were measured on a VG-70SE instrument and NMR spectra on a General Electric QE-300 instrument at 300 MHz(^{1}H) and 75 MHz (^{13}C) respectively. Solvents were freshly distilled before use.

The sponge (120g wet weight) was extracted with ethanol followed by chloroform. The aqueous ethanol extract was reduced to half its volume and partitioned against chloroform. The two chloroform extracts were combined yielding a dark green brown viscous oil (2.3g, 2%). This residue was fractionated by vacuum liquid chromatography (VLC) on silica with a hexane/ethyl acetate/methanol gradient, yielding the ikimine mixture concentrated in the non-polar fractions. HPLC on silica (hexane/ethyl acetate, 93:7) yielded pure ikimine A (35 mg, 0.029%), *syn*-ikimine A (12 mg, 0.012%), ikimine B (25 mg, 0.02%), *syn*-ikimine B (4 mg, 0.0003%), and a crude mixture of ikimine C and ikimine D (120 mg). Repeated chromatography of this crude fraction on both an amino bonded phase (Brownlee Labs Lichrosorb NH_2, 10μm) with ethyl acetate/hexane, 12:88) and silica (elution with ethyl acetate/hexane, 20:80) yielded pure ikimine C (35 mg, 0.029%), and a second fraction containing a 2:1 mixture of ikimine D and ikimine C (8 mg).

Ikimine A (7) - Colorless oil. UV (MeOH):λ_{max}260(log ∈ 3.32), 265 (3.89), 272 nm (3.22); IR 2950, 2850, 1477, 1465, 1438, 1422, 1376, 1186, 1056, 1026, 884, 797, 714 cm^{-1}; HREIMS *m/z* 304.2515 ($C_{19}H_{32}N_2O$ requires 304.2515), 273.2329 ($C_{18}H_{29}N_2$ requires 273.2327), 218.1910 ($C_{15}H_{24}N$ requires 218.1909), 106.0661 (C_7H_8N requires 106.0657), 93.0578 (C_6H_7N requires 93.0577); EIMS *m/z* 304 (1.3%), 273 (100), 246 (7), 218 (49), 204 (8), 190 (7), 176 (7), 162 (9), 148 (8), 134 (4), 120 (11), 106 (88), 93 (62), 92 (36).

***syn*-Ikimine A** (8) - Colorless oil. UV (MeOH): λ_{max} 262 (log ∈ 3.34), 265 (3.95), 272 nm (3.22); IR 2950, 2851, 1480, 1465, 1435, 1422, 1376, 1196, 1050, 1020, 890, 800, 720 cm^{-1}; HREIMS *m/z* 304.2515 ($C_{19}H_{32}H_2O$ requires 304.2515); EIMS *m/z* 304 (1.3%), 273 (100), 246 (7), 218 (49), 204 (8), 190 (7), 176 (7), 162 (9), 148 (8), 134 (4), 120 (11), 106 (88), 93 (62), 92 (36).

Ikimine B (9)- Colorless oil. UV (MeOH):λ_{max} 260 (log ∈ 3.30), 264 (4.01), 274 nm (3.23); IR 3000, 2800, 1560, 1465, 1445, 1390, 1200, 1000 cm^{-1}; HREIMS *m/z* 304.2528 ($C_{19}H_{32}N_2O$ requires 304.2515), 273.2324 ($C_{18}H_{29}N_2$ requires 273.2327), 232.2070 ($C_{16}H_{26}N$ requires 232.2075); EIMS *m/z* 304 (5%), 273 (100), 259 (20), 232 (78), 218 (12), 204 (22), 190 (13), 176 (10), 162 (13), 148 (15), 134 (10), 120 (15), 106 (100), 93 (70), 92 (40).

***syn*-Ikimine B** (10) - Colorless oil. UV (MeOH): λ_{max} 263 (log ∈ 3.00), 267(3.89), 276 nm (3.46); IR 3000, 2850, 1547, 1460, 1430, 1380, 1180 cm^{-1}; HREIMS *m/z* 304.2528 ($C_{19}H_{32}N_2O$ requires 304.2515); EIMS *m/z* 304 (5%), 273 (100), 259 (20), 232 (78), 218 (12), 204 (22), 190 (13), 176 (10), 162 (13), 148 (15), 134 (10), 120 (15), 106 (100), 93 (70), 92 (40).

Ikimine C (11)- Colorless oil. UV (MeOH): λ_{max} 260 (log ϵ 3.20), 264 (3.79), 272 nm (3.56); IR 3250, 2950, 2850, 1580, 1477, 1465, 1438, 1422 cm^{-1}; HREIMS *m/z* 275.2494 ($C_{18}H_{31}N_2$ requires 275.2500), 261.2404 ($C_{18}H_{31}N$ requires 261.2352), 246.2222 ($C_{17}H_{28}N$ requires 246.2223), 232.2066 ($C_{16}H_{26}N$ requires 232.2067); EIMS *m/z* 275 (23%), 261 (10), 246 (20), 232 (18), 218 (12), 204 (18), 190 (15), 176 (10), 162 (10), 148 (13), 120 (16), 106 (100), 93 (87), 92 (38).

Ikimine D (12)- Colorless oil. UV (MeOH): λ_{max} 260 (log ϵ 3.30), 265 (3.82), 270 nm (3.46); IR 3200, 3000, 2850, 2300, 1580, 1442 cm^{-1}; EIMS *m/z* 285 (100%), 271 (10), 270 (5), 256 (15), 242 (40), 218 (50), 204 (40), 190 (15), 176 (10), 162 (10), 148 (13), 120 (17), 106 (80), 93 (60).

References and Notes

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6. The Hawaiian word *iki* meaning small was chosen since the animal was collected on a small atoll (Ant) in Micronesia.
7. Pending identification a voucher specimen is available at the University of Hawaii.
8. Cytotoxicity against KB cells for the crude extract and pure compounds are as follows: crude chloroform extract IC_{50}=10 μg/mL; ikimine A IC_{50}=5 μg/mL; ikimine B IC_{50}= 7 μg/mL; ikimine C IC_{50}=5 μg/mL.
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