Montipyridine, a New Pyridinium Alkaloid from the Stony Coral *Montipora* Species

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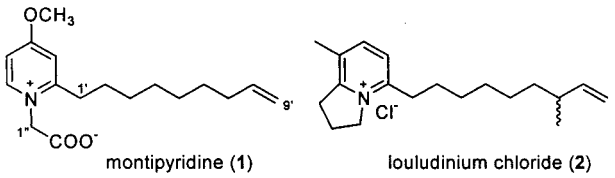
A new pyridinium alkaloid, montipyridine (**1**), has been isolated from the stony coral *Montipora* sp. The structure was established from spectroscopic data.

Stony corals have been reported to contain nitrogenous compounds such as the mycalolides,[1] aplysinopsin[2] and related derivatives,[3] tubastrine,[4] and tubastraine.[5] Encouraged by the isolation of a lactam from *Montipora* sp.,[6] we further investigated the coral for nitrogenous compounds and isolated a new *N*-alkyl pyridinium alkaloid, montipyridine (**1**), present in a trace amount. To date, only a few *N*-alkyl pyridinium alkaloids have been isolated from marine organisms, and they have been isolated mostly from marine sponges.[7−20] There was a single report of the incidence of *N*-alkylpyridinium alkaloids each in a sea anemone,[21] a tunicate,[22] and cyanobacteria.[23] To the best of our knowledge, this is the first report of an *N*-alkylpyridinium alkaloid from a stony coral. In common with louludinium chloride (**2**)[23] and spongidine C,[7] montipyridine is also unique for the alkyl substitution at C-2 in contrast to the prevailing C-3 substitutions as observed in halitoxin,[20] sulcatin,[22] amphikuemin,[21] niphatoxins,[18] xestamines F−H,[19] cyclostellettamines,[14] amphitoxin,[13] and the alkaloid from *Callyspongia fibrosa*.[16] Structural similarity of montipyridine with the cyanobacterial metabolite, louludinium chloride, is interesting since most hermatypic corals are known to contain symbiotic zooxanthellae (mostly dinoflagellates).



The LRFABMS of montipyridine (**1**) showed a pseudomolecular ion $[M + H]^+$ at *m/z* 292. The HRFABMS provided the exact value of *m/z* 292.1913 for $[M + H]^+$, which corresponded well with the formula $C_{17}H_{25}NO_3$. The formula suggested six degrees of unsaturation. The ^{1}H NMR spectrum of **1** exhibited signals at δ 8.50 (d, $J = 7.5$ Hz), 7. 37 (d, $J = 3.0$ Hz), and 7.34 (dd, $J = 7.5$, 3.0 Hz), whose chemical shifts and coupling constants were reminiscent of a 2,4-disubstituted pyridine ring. These values were assigned to H-6, H-3, and H-5 protons, respectively, and their connectivities to the respective carbons (δ_C 149.1, 113.8, and 112.2) were determined from an HMQC experiment. The HMBC spectrum revealed that the two substituted carbons of the ring, C-2 and C-4, resonated at δ 161.5 and 172.2, respectively.

Table 1. NMR Data of **1** in CD_3OD[a]

position	δ_H (mult., *J* in Hz)	δ_C	HMBC
2		161.5	
3	7.37 (d, 3.0)	113.8	C-4, C-5
4		172.2	
5	7.34 (dd, 7.5, 3.0)	112.2	C-3, C-6, C-4
6	8.50 (d, 7.5)	149.1	C-2, C-4, C-5, C-1″
1′	2.91 (t, 7.2)	33.7	C-2, C-3, C-2′
2′	1.75 (quint., 7.5)	28.9	C-1′, C-3′
3′-6′	1.29−1.42 (m)	30.0−30.1	C-7′, C-2′
7′	2.05 (quart., 7.3)	34.8	C-6′, C-8′, C-9′
8′	5.81 (ddt, 17.0, 10.3, 2.0)	140.1	
9′	4.97 (dd, 17.0, 2.0)	114.8	
	4.91 (dd, 10.3, 2.0)		
1″	4.91 (s)	60.0	C-2, C-6, C-2″
2″		170.0	
OCH_3	4.11 (s)	58.3	C-4

[a] ^{1}H NMR, ^{13}C NMR, and HMBC were measured at 500, 150, and 600 MHz, respectively.

The presence of an aliphatic side chain with a terminal olefin was evident from the ^{1}H NMR spectrum (Table 1). The signals at δ 5.81 (ddt, $J = 17.0$, 10.3, 2.0 Hz), 4.97 (dd, $J = 17.0$, 2.0 Hz), 4.91 (dd, 10.3, 2.0 Hz), and 2.05 (quart., $J = 7.0$ Hz), which were mutually coupled, were assigned to the terminal olefinic and the corresponding allylic protons, respectively. A two-proton triplet at δ 2.91, which was coupled to a carbon at δ_C 33.7 in the HMQC spectrum and showed strong correlations with C-2 and C-3 in the HMBC spectrum, was assigned to the H-1′ protons. The ^{1}H NMR spectrum also exhibited a two-proton singlet at δ 4.91 (δ_C 60.0), which showed HMBC correlations with C-2 (δ_C 161.5) and C-6 (δ_C 149.1) and a carbonyl carbon at δ_C 171.0. This evidence indicated the presence of a carboxymethyl group on N-1, similar to 1-carboxymethylnicotinic acid reported earlier.[24] A three-proton singlet at δ 4.11, correlated to a carbon at δ_C 58.3 in the HMQC spectrum, was assigned to the methoxy protons at C-4. This assignment was further corroborated by HMBC cross-peaks between H-3/H-5/H-6 and C-4 (δ_C 172.2) and a cross-peak between the methoxy protons and C-4 (Figure 1). NOESY correlations between H-1″ and H-6/H-1′, H-1′ and H-3, and between the methoxy protons and H-3/H-5 further supported this structure (Figure 1). Thus the structure was

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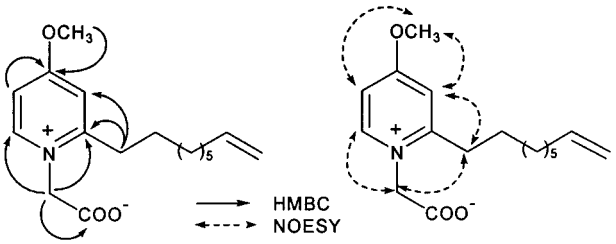

Figure 1. Key HMBC and NOESY correlations of **1**.

established as 2-(8-nonenyl)-4-methoxypyridinio-1-methylcarboxylate.

The compound showed ED_{50} values higher than 30 μg/mL against a small panel of human solid tumor cells: A549 (human lung cancer), SK-OV-3 (human ovarian cancer), SK-MEL-2 (human skin cancer), XF498 (human CNS cancer), and HCT15 (human colon cancer).

Experimental Section

General Experimental Procedures. ^{1}H NMR spectra were recorded on a Varian Inova 500 spectrometer, while ^{13}C NMR and 2D experiments were performed on a Bruker DMX 600 spectrometer. Chemical shifts were reported in reference to the respective residual solvent peaks (δ_H 3.3 and δ_C 49.0 for CD_3OD). LRFABMS and HRFABMS data were obtained using a JEOL JMS-HX110/110A. HPLC was performed on a Gilson 370 pump with a YMC ODS-H80 (250 × 10 mm i.d., S-4 μm, 80 Å) column using a Shodex RI-71 detector.

Animal Material. The animals were collected by hand using scuba at a depth of 8 m on November 4, 1996, along the shore of Mundo, Cheju Island, Korea. The morphology of the coral was described in the previous report.[6] A voucher specimen was deposited in the Natural History Museum, Ewha Womans University (voucher no. EWUA. Ant. 961104).

Extraction and Isolation. The frozen coral (2.5 kg, wet wt) was extracted with MeOH at room temperature. Guided by the brine shrimp lethality assay,[25] the MeOH extract was partitioned between water and EtOAc. The EtOAc layer was further partitioned between H_2O and $CHCl_3$ to afford 8.8 g of the $CHCl_3$ layer (LD_{50} 30−86 μg/mL), which was subjected to a reversed-phase MPLC (YMC gel ODS-A, 60 Å 500/400 mesh) eluting with a step gradient solvent system of 25 → 0% H_2O/MeOH to obtain 14 fractions (1−14). Fraction 2 (0.3 g) showed significant activity in the brine shrimp assay and was further purified by repeated HPLC (YMC ODS-H80, 250 × 10 mm i.d., S-4 μm, 80 Å) using 60% MeOH/H_2O as the solvent system to give montipyridine (**1**) (0.8 mg) as a minor component.

Montipyridine (1): light brown solid; ^{1}H NMR and ^{13}C NMR, see Table 1; LRFABMS *m/z* $[M + H]^+$ 292 (100), 278 (1), 264 (1), 250 (5), 236 (1), 222 (1) 208 (4), 194 (15), 181 (9); HRFABMS *m/z* $[M + H]^+$ 292.1913 (calcd for $C_{17}H_{26}NO_3$, 292.1913).

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