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Monamphilectine A, a Potent Antimalarial β-Lactam from Marine Sponge *Hymeniacidon* sp: Isolation, Structure, Semisynthesis, and Bioactivity

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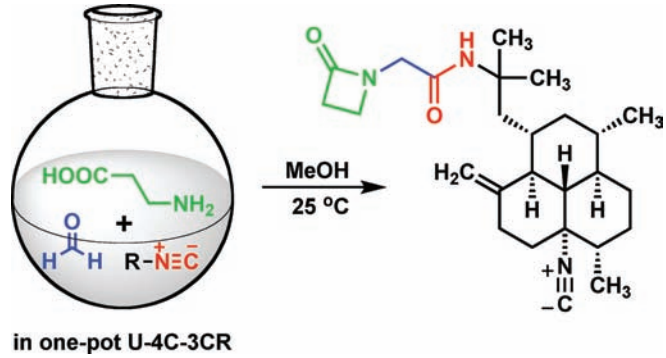
ABSTRACT



in one-pot U-4C-3CR

Monamphilectine A (1), a new diterpenoid β-lactam alkaloid showing potent antimalarial activity, was isolated in milligram quantities following bioassay-directed extraction of a Puerto Rican marine sponge *Hymeniacidon* sp. Its structure, established by interpretation of spectral data, was confirmed unequivocally by chemical interconversion and comparison of physical, chemical, and bioactivity data with the natural product. The one-step semisynthesis of monamphilectine A was based on a multicomponent Ugi reaction that also established its absolute stereostructure.

For decades, malaria has been a serious public health threat mainly due to the development of resistance by the principal lethal causative parasitic species, *Plasmodium falciparum*, to the booster drugs like quinine, artemisinin, and chloroquine.[1] Recent statistics show that nearly two billion people live in areas at risk from the disease, and each year up to one million people die from malaria infection.[2] Thus, new drugs with unique structures and mechanism of action are urgently required to treat sensitive and drug-resistant strains of malaria.[3] Historically, naturally occurring compounds that are based on novel structures represent a major source for the discovery and development of new drugs, but only a small number of these compounds offer novel scaffolds for development as antimalarials.[4]

As part of our continuing research into the discovery of new antimalarial leads, in 2006 we undertook a summer underwater expedition to Mona Island, the third largest island of the archipelago of Puerto Rico (18° 5′ 12″ N, 67° 53′

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22″ W). Extracts of some marine sponges from this location exhibited both antiplasmodial and antituberculosis activities. Among the 39 marine sponge extracts that were tested 4 (10.2%) inhibited *Plasmodium falciparum* W2 strain by more than 50% at both 250 and 50 μg/mL concentrations. Generally, moderate polar extracts were more active against *Plasmodium falciparum* W2 strain than polar and nonpolar extracts. Among 32 marine sponge extracts that were tested for antituberculosis activity 15 (46.9%) showed activity ranging from moderate to strong on an arbitrary criterion. Among the most noteworthy were the crude extracts of *Plakortis halichondrioides*, *Neopetrosia proxima*, *Cribrochalina vasculum*, *Agelas conifera*, and *Agelas sceptrum*, all of which exhibited ≥90% growth inhibition at 128, 64, and 32 μg/mL concentrations against *Mycobacterium tuberculosis* H_{37}Rv. In all, the best antimalarial profile (IC_{50} < 0.08 μg/mL) was exhibited by the crude extract of *Hymeniacidon* sp. (Demospongiae). From the bioscreening data, we subsequently identified one fraction derived from this sponge that showed potent parasitic growth inhibition in our antimalarial assay. Chemical analysis of the active fraction from the crude sponge extract distinctively identified two compounds with pseudomolecular ion clusters in the (+)-ESIMS spectrum at *m*/*z* 426 and 325. Mass-directed fractionation on the large-scale organic extract of *Hymeniacidon* sp. resulted in the purification of a new amphilectane-type metabolite, monamphilectine A (**1**), along with the previously isolated compound 8,15-diisocyano-11(20)-amphilectene (**2**).[5] Herein we report the isolation, structure elucidation, and semisynthesis of monamphilectine A (**1**), and antiinfective properties for compounds **1** and **2**. A detailed scheme for the extraction of the dry sponge specimen has been provided as Supporting Information.

The sponge *Hymeniacidon* sp. was collected by scuba diving off the coasts of Mona Island during the summer of 2006 and stored frozen until February 2007. After lyophilization, the dried sponge (200 g) was blended with a 1:1 mixture of $CHCl_3$−MeOH and the combined extracts were filtered and concentrated in vacuo to yield an orange thick paste (28 g). The $CHCl_3$−MeOH crude extract was suspended in water, partitioned against hexane, and rotoevaporated to give 7.6 g of a yellowish oil that was fractionated on silica gel (170 g) by stepwise elution with hexane−EtOAc mixtures (2−50%) to yield 528 mg (0.27%) of 8,15-diisocyano-11(20)-amphilectene (**2**). Further purification of a more polar fraction (13.1 mg) by silica gel (1.0 g) column chromatography with 20% EtOAc in hexane afforded 3.0 mg (0.002%) of pure monamphilectine A (**1**) as a yellowish oil.[6]

The molecular formula of monamphilectine A (**1**) was determined to be $C_{26}H_{39}N_3O_2$ by accurate mass measurement. From the IR and ^{13}C NMR spectra of **1**, it became evident that the molecule contained an isonitrile function attached to a quaternary carbon [2125 cm^{-1}, 156.2 (C), 66.9 (C) ppm], thus accounting for two of the nine degrees of unsaturation indicated by the molecular formula.[7] As there were only four further resonances in the ^{13}C NMR spectrum of **1** for carbon atoms associated with multiple bonds [105.8 (CH_2), 150.4 (C), 166.3 (C), 168.3 (C)], it was evident that the molecule was tetracyclic. Additional IR absorptions indicated the presence of −NH (3323 cm^{-1}), olefin (3082 cm^{-1}), and carbonyl (1747 and 1666 cm^{-1}) groups. ^{1}H and ^{13}C NMR data (Table 1) disclosed the presence of one exchangeable proton, two amide carbonyls, a 1,1-disubstituted olefin, three nitrogen-bearing carbons, all of which were sp^3, six sp^3 methines, nine sp^3 methylenes, and four methyl groups. Although the majority of the signals were overlapped in the region between δ 2.30 and 0.80, 2D NMR studies allowed all resonances to be assigned (Table 1). After all proton and carbon resonances had been associated from the results of a ^{1}H−^{13}C shift correlated 2D NMR measurement (HSQC), it was possible to deduce the planar structure of **1** from the results contained in its ^{1}H−^{1}H COSY and HMBC spectra.

Interpretation of the ^{1}H−^{1}H COSY spectrum revealed the proton connectivities of partial structure **A** from H-1 through H-7, including vicinal couplings from H-1 to both H-12 and H_2-14, H-3 to H_3-18, H-4 to H-13, H-7 to H_3-19, and H-12 to H-13 (Figure 1). Two of the remaining partial structures, **B** and **D**, were easily characterized from the ^{1}H−^{1}H COSY spectrum of **1** as two isolated ethylene bridges. Confirmation of the proton connectivity networks already established from ^{1}H−^{1}H COSY experiments was obtained directly from long-range ^{1}H−^{13}C couplings (Table 1), which also allowed substructure **C** to be elucidated as a sp^3 quaternary carbon bearing two geminal methyl groups and a nitrogen atom.

Connections among unit **A** and the remaining five carbons (C-8 through C-11, and C-20) encompassing substructure **B** were assigned on the basis of ^{1}H−^{13}C long- range couplings observed in the HMBC spectrum as follows (Table 1). The ^2J HMBC correlations from H-7 (δ_H 0.84), H-9αβ (δ_H 1.29 and 2.26), and H-13 (δ_H 0.99) to the low-field quaternary carbon at δ_C 66.9 (C-8) suggested multiple connectivities between units **A** and **B**, thereby partially constructing an amphilectane ring system (Figure 1).[8] This was subsequently confirmed by complementary HMBC correlations between C-11 and H-10αβ (δ_H 2.29), H-12 (δ_H 1.83), and H-20αβ (δ_H 4.85 and 4.60). Moreover, ^3J HMBC correlations from H-4, H-6αβ, H-10αβ, and H-12 to the isonitrile-bearing quaternary carbon at δ_C 66.9 (C-8), and from H-1, H-9αβ, and H-13 to the quaternary olefin carbon at δ_C 150.4 (C-11) suggested the connectivity between units **A** and **B** through C-8 and C-11 in accordance with the relative low-field ^{13}C resonances of these carbons.

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(6) Monamphilectine A (**1**): yellowish oil; $[\alpha]^{20}_D$ −105.9 (*c* 0.34, $CHCl_3$); IR (neat) ν_{max} 3323, 3082, 2961, 2924, 2872, 2125, 1747, 1666, 1549, 1454, 1410, 1265, 1184, 1122, 1057, 895, 754 cm^{-1}; EIMS *m*/*z* $[M]^+$ 425 (3), 398 (5), 341 (5), 314 (10), 270 (34), 255 (29), 215 (29), 199 (19), 169 (100), 159 (29), 129 (33), 99 (78); HREIMS *m*/*z* $[M]^+$ 425.3039 (calcd for $C_{26}H_{39}N_3O_2$ 425.3042).

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Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, HMBC, and NOESY Spectral Data for Monamphilectine A (**1**)[a]

position	δ_H, mult, intgt (J in Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	HMBC[c]	NOESY
1	1.84 br m, 1H	32.9 (CH)	H2αβ, H12, H14αβ	H-3, H-13	H-2β, H-3, H-13, NH
2α	1.98 br m, 1H	41.0 (CH_2)	H-1, H-2β, H-3	H-4, H-12, H-14αβ, H_3-18	H-2β, H-4, H-12, H_3-18
2β	1.35 br m, 1H		H-1, H-2α, H-3		H-1, H-2α, H-3
3	1.04, br m, 1H	35.6 (CH)	H-2αβ, H-4, H_3-18	H-1, H-5αβ, H-13	H-1, H-2β, H-13, H_3-18
4	1.08, br m, 1H	42.4 (CH)	H-3, H-5αβ, H-13	H-2αβ, H-6αβ, H-12, H_3-18	H-12, H_3-18
5α	0.79, br m, 1H	29.7 (CH_2)	H-4, H-5β, H-6αβ	H-3, H-7, H-13	H-5β, H-6α
5β	1.43, br m, 1H		H-4, H-5α, H-6αβ		H-5α, H-6β
6α	1.95, br m, 1H	29.8 (CH_2)	H-5αβ, H-6β, H-7	H-4, H_3-19	H-5α, H-6β
6β	1.52, br m, 1H		H-5αβ, H-6α, H-7		H-5β, H-6α
7	0.84, br m, 1H	40.8 (CH)	H-6αβ	H-5αβ, H-9αβ, H-13, H_3-19	H-13, H_3-19
8		66.9 (C)		H-4, H-6αβ, H-7, H-9αβ, H-10αβ, H-12, H-13, H_3-19	
9α	1.29, br m, 1H	39.6 (CH_2)	H-9β, H-10αβ	H-7, H-13	H-9β, H-10α
9β	2.26, br m, 1H		H-9α, H-10αβ		H-9α, H-10β
10αβ	2.29, br m, 2H	33.6 (CH_2)	H-9αβ	H-12, H-20αβ	H-9αβ
11		150.4 (C)		H-1, H-9αβ, H-12, H-13, H-20αβ	
12	1.83, br m, 1H	46.1 (CH)	H-1, H-13	H-2αβ, H-4, H-10αβ, H-14αβ, H-20αβ	H-4
13	0.99, br m, 1H	55.6 (CH)	H-4, H-12	H-1, H-3, H-5αβ, H-7	H-1, H-3, H-7
14α	1.98, br m, 1H	44.5 (CH_2)	H-1, H-14β	H-2αβ, H-12, H_3-16, H_3-17, NH	H-14β
14β	1.54, br m, 1H		H-1, H-14α		H-12, H-14α
15		54.4 (C)		H-1, H-14αβ, H_3-16, H_3-17, NH	
16	1.39, s, 3H	28.7 (CH_3)		H-14αβ, H_3-17, NH	H_3-17
17	1.38, s, 3H	27.2 (CH_3)		H-14αβ, H_3-16, NH	H-20β
18	0.89, d, 3H (6.1 Hz)	20.0 (CH_3)	H-3	H-2αβ, H-3, H-4	H-2α, H-3, H-4
19	0.98, d, 3H (6.3 Hz)	15.7 (CH_3)	H-7	H-6αβ, H-7	H-7
20α	4.85, br s, 1H	105.8 (CH_2)	H-20β	H-10αβ, H-12	H-10β, H-20β
20β	4.60, br s, 1H		H-20α		H-1, H-14α, H_3-17, H-20α
21		166.3 (C)		H-22αβ, NH	
22α	3.78, d, 1H (16.4)	47.4 (CH_2)	H-22β	H-25αβ	H-25αβ, NH
22β	3.74, d, 1H (16.4)		H-22α		H-25αβ, NH
23		168.3 (C)		H-22αβ, H-24αβ, H-25αβ	
24αβ	3.04, br t, 2H (4.2)	37.5 (CH_2)	H-25αβ	H-25αβ	H-25αβ
25αβ	3.40, br m, 2H	40.7 (CH_2)	H-24αβ	H-22αβ, H-24αβ	H-22αβ, H-24αβ, NH
26		156.2 (C)			
NH	5.81, br s, exchangeable				H-1, H-22αβ, H-25αβ

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in ppm relative to the residual $CHCl_3$ (7.25 ppm) or $CDCl_3$ (77.0 ppm) signals. Assignments were aided by 2D NMR experiments, spin-splitting patterns, number of attached protons, and chemical shift values. [b] ^{13}C NMR multiplicities were obtained from a DEPTQ experiment. [c] Protons correlated to carbon resonances in the ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz.

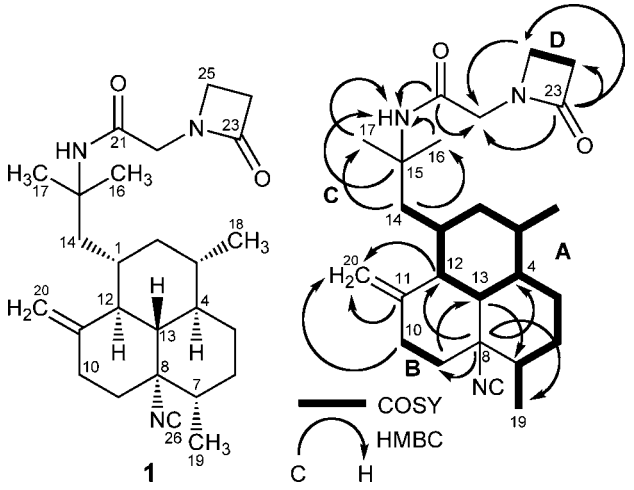
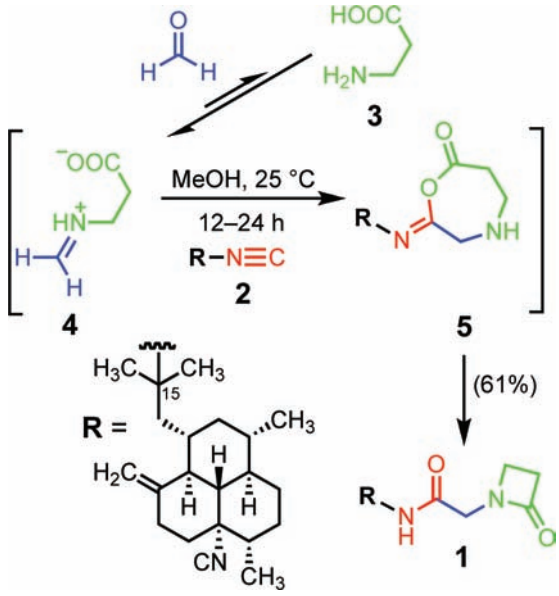


Figure 1. Monamphilectine A (left) and partial structures for **1** generated from ^{1}H−^{1}H COSY, HSQC, and HMBC correlations.

Units **A** and **C** were linked through C-15 by the observation of strong HMBC correlations between H-1 (δ_H 1.84), H-14αβ (δ_H 1.98 and 1.54), H_3-16 (δ_H 1.39), and H_3-17 (δ_H 1.38) with C-15 (δ_C 54.4) in a manner consistent with an amphilectane-based structure. The aforementioned correlations, together with the remarkable similarities of their ^{1}H and ^{13}C NMR data (recorded in $CDCl_3$), showed unequivocally that **1** must have the same amphilectane-base carbon skeleton as compound **2** (compound **1** differed somewhat from **2** only by the shifts of the isobutyl side chain from C-14 to C-17).

The molecular formula of monamphilectine A (**1**) differed from that of **2** by the addition of $C_4H_7NO_2$. Major differences in the ^{1}H NMR of **1** compared with **2** were the appearance of an exchangeable amide proton at δ 5.81 (br s, NH), two mutually coupled methylenes at δ 3.40 (br m, C-25) and 3.04 (br t, C-24) ascribable to unit **D**, and one isolated methylene near δ 3.76 (AB doubled-doublet, C-22). The ^{13}C NMR spectrum of **1** was missing one of the isonitrile carbons present in the spectrum of **2**, but it contained two new amide carbonyl resonances at δ 168.3 (C-23) and 166.3 (C-21), two *N*-methylene carbons at δ 47.4 (C-22) and 40.7 (C-25), and an additional upfield methylene at δ 37.5 (C-24). Analysis of the COSY, HSQC, HMBC, and HREIMS data obtained for **1** showed that the differences in the NMR data described above (including strong upfield shifts observed for C-14 through C-17) could only be consistent with the

Scheme 1. Semisynthesis of Monamphilectine A (**1**) from **2**



presence of a novel *N*-2-(2-oxoazetidin-1-yl)ethanamide moiety in **1** in place of the C-15 isonitrile in **2**.[9]

Near identical chemical shifts, ^{1}H−^{1}H coupling constants, and diagnostic NOESY correlations (Table 1) between **1** and **2** strongly suggested the retention of relative configuration. As monamphilectine A (**1**) was isolated in sparse quantity, and to confirm relative and absolute configuration, as well as to investigate its bioactivity, we formulated plans for a semisynthesis of **1** from **2**, a likely biogenetic precursor of known absolute configuration.[10] Our semisynthesis of monamphilectine A is shown in Scheme 1. Strategic bond disconnections about the β-lactam moiety led us to envision an Ugi four-center three-component reaction (U-4C-3CR), by combining β-amino acid **3**, formaldehyde, and the C-15 isonitrile functionality of **2**.[11] In the first step, reaction of β-alanine (**3**), which supplies the carboxylic acid and the amino function, with formaldehyde affords protonated Schiff base **4**, which reacts with **2** regioselectively affording oxazepinone **5**. The latter undergoes in situ O,N-acyl migration, resulting in monamphilectine A (**1**). The one-pot solution-phase reaction was conducted in MeOH by stirring the mixture at room temperature within 12−24 h, resulting in the β-lactam derivative **1** in 61% yield after separation.[12] The optical rotation, IR, 1D and 2D NMR, and bioactivity data for our synthetic material were in complete agreement with those of naturally occurring monamphilectine A.

When tested against a chloroquine-resistant (CQ-R) *Plasmodium falciparum* W2 strain compounds **1** and **2** showed IC_{50} values of 0.60 and 0.04 μM, respectively.[13] In vitro anti-TB screening against *M. tuberculosis* H_{37}Rv revealed MIC values of 15.3 and 3.2 μg/mL. Preliminary KB assays against *E. coli* revealed that a concentration of 150 nM monamphilectine A (**1**) possesses at 43% and 38% the bactericidal strength of β-lactam antibiotics carbenicillin and amphicillin, respectively.

Acknowledgment. The Institute for Tuberculosis Research at the University of Illinois at Chicago and the Institute for Tropical Medicine and Health Sciences (Panama) provided in vitro antituberculosis and antimalarial activity data, respectively. Mass spectral determinations were provided by the Mass Spectrometry Laboratory of the University of Illinois at Urbana−Champaign. This work was supported by NIH Grant 1SC1GM086271-01A1. Financial support to E. Avilés was provided by the UPR-RISE Fellowship Program.

Supporting Information Available: Experimental details and spectroscopic data for monamphilectine A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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