Tetrahedron 71 (2015) 487–494

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Structures, semisyntheses, and absolute configurations of the antiplasmodial α-substituted β-lactam monamphilectines B and C from the sponge *Svenzea flava*

Edward Avilés [a], Jacques Prudhomme [b], Karine G. Le Roch [b], Abimael D. Rodríguez [a,*]

[a] Department of Chemistry, University of Puerto Rico, PO Box 23346, U.P.R. Station, San Juan 00931-3346, Puerto Rico
[b] Department of Cell Biology and Neuroscience, University of California at Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 20 November 2014
Accepted 24 November 2014
Available online 2 December 2014

Keywords:
β-Lactams
Svenzea flava
Monamphilectines
Malaria
Ugi multicomponent reaction
Drug discovery
Isocyanide

ABSTRACT

Bioassay-guided fractionation of the Caribbean sponge *Svenzea flava* collected near Mona Island, off the west coast of Puerto Rico, led to the isolation of two isocyanide amphilectane-type diterpenes named monamphilectines B and C (**2** and **3**). Attached to the backbone of each of these compounds is the first α-substituted monocyclic β-lactam ring to be isolated from a marine organism. The molecular structures of **2** and **3** were established by spectroscopic methods and then confirmed unequivocally by chemical correlation and comparison of physical and chemical data with the natural products. The new β-lactams were successfully synthesized in one step, starting from the known diisocyanide **4**, via parallel Ugi four-center three-component reactions (U-4C-3CR) that also established their absolute stereostructures. Interestingly, compounds **2** and **3** exhibited activities in the low nanomolar range against the human malaria parasite *Plasmodium falciparum*.

1. Introduction

Only a handful of natural products bearing the β-lactam functionality have been isolated from marine organisms.[1] The most recent is the antiplasmodial monamphilectine A (**1**), which we isolated in 2010 as a minor component from the marine sponge *Svenzea flava* (previously classified as *Hymeniacidon* sp.).[2,3] This metabolite represented the first monocyclic conjugate β-lactam isolated from a marine source. The term 'conjugate' implies that the β-lactam nucleus is N-linked to a terpenoid.[4] These types of compounds are rarely observed in nature, and only a few examples, including those from non-marine sources, have been reported in the literature.[5] Monocyclic β-lactams have been associated with various biological activities, such as plant-specific toxins[6] and cytotoxins[7] as well as anti-bacterial[8] and antiplasmodial[2] effects. As part of our efforts to identify novel structures and bioactive metabolites from Caribbean marine sponges, we further screened the organic extracts of *S. flava*, which led to the identification of two α-substituted β-lactam alkaloids designated as monamphilectines B and C (**2** and **3**). Their structures were elucidated on the basis of extensive spectroscopic data analysis and chemical transformations. Subsequent stereo assignment around the *N*-α-disubstituted azetidin-2-one moiety was accomplished from a combination of simple one-pot syntheses of β-lactam-ring products using an Ugi four-center three-component reaction (U-4C-3CR)[9] and Kishi's method.[10] Herein, we report the isolation, structure elucidation, and antiplasmodial activity of two novel β-lactam alkaloids, adding further evidence that this Caribbean sponge is an abundant source of chemically diverse and biologically active natural products.[11]

2. Results and discussion

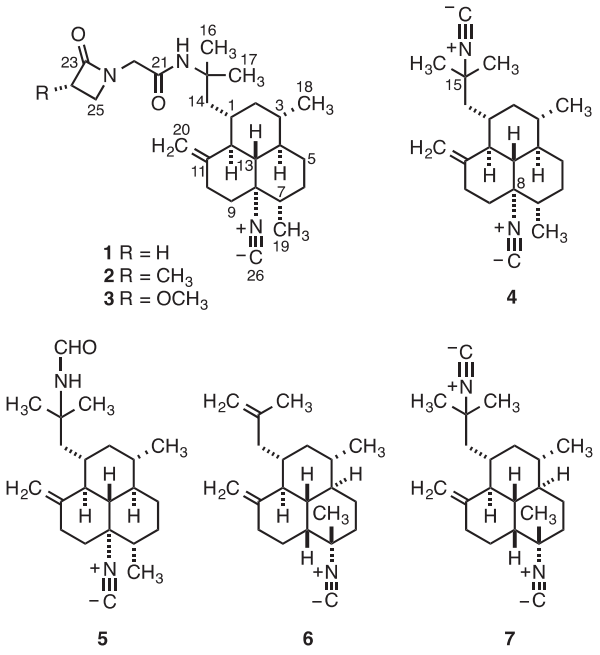
2.1. Extraction and isolation of natural products

The sponge *S. flava* was collected in July 2006 at a depth of approximately 27 m by scuba from Mona Island, Puerto Rico (18° 5′ 12″ N, 67° 53′ 22″ W). A freeze-dried sample was repeatedly extracted with $CHCl_3$/MeOH (1:1). The extracts were combined, concentrated in vacuo, and partitioned between H_2O and *n*-hexane. The resulting *n*-hexane extract was quickly concentrated to produce a residue, which upon biological screening against the human malaria parasite *Plasmodium falciparum* W2, exhibited significant antiplasmodial activity (IC_{50}<0.08 μM). Thus, the latter residue was subjected to normal-phase silica gel column chromatography using a mixture of *n*-hexane and EtOAc in a stepwise elution, leading to

* Corresponding author. E-mail address: abimael.rodriguez1@upr.edu (A.D. Rodríguez).

http://dx.doi.org/10.1016/j.tet.2014.11.060
0040-4020/

the isolation of two new metabolites, monamphilectines B (**2**) and C (**3**), and the following known compounds: 8,15-diisocyano-11(20)-amphilectene [(−)-DINCA] (**4**),[12] 8-isocyano-11(20)-ene-15-amphilectaformamide (**5**),[12] 7-isocyano-11(20)-15(16)-amphilectadiene (**6**),[13] 7,15-diisocyano-11(20)-amphilectene (**7**),[13] and monamphilectine A (**1**).[2] All of the known isolates were characterized unambiguously by spectroscopic analysis including ESI-MS, UV, IR, $[\alpha]_D$, and NMR analysis, and by comparisons to characterization data provided for the natural products.



2.2. Structure elucidation, semisynthesis, and absolute configuration of monamphilectines B (2) and C (3)

A minor constituent from the organic extract, monamphilectine B (**2**), was determined to have the molecular formula $C_{27}H_{41}O_2N_3$ on the basis of high-resolution ESI-MS analysis of the pseudomolecular $[M+Na]^+$ ion peak at m/z 462.3076. Thus, 9° of unsaturation was calculated for this molecule. The IR spectrum revealed the presence of amide (3315 cm^{-1}), alkene (3080 cm^{-1}), isocyanide (2125 cm^{-1}), and carbonyl (1741 and 1670 cm^{-1}) groups, which accounted for all of the multiple bonds within **2**; the molecule was therefore tetracyclic. This observation was supported by ^{13}C and DEPT NMR data that showed signals characteristic of carbonyl amides at δ_C 166.3 (C-21) and 171.9 (C-23), two alkene carbons at δ_C 150.4 (C-11) and 105.9 (C-20), and one isocyanide function at δ_C 156.2 (C-26) and 67.0 (C-8). On the basis of the DEPTQ NMR data, we also established that **2** contained five CH_3, nine CH_2 and seven CH groups, and six quaternary C atoms (Table 1).

Inspection of the ^1H NMR spectroscopic data for **2** also revealed five methyl groups. Three of these methyl groups were doublets displaced at δ_H 0.89 (d, 3H, J=6.1 Hz, H-18), 0.98 (d, 3H, J=6.3 Hz, H-19), and 1.34 (d, 3H, J=7.4 Hz, H-27) and two were singlets at δ_H 1.39 (br s, 3H, H-16) and 1.37 (br s, 3H, H-17). Compound **2** also contained a 1,1-disubstituted olefin at δ_H 4.85 (br s, 1H, H-20α) and 4.60 (br s, 1H, H-20β) (Table 1). Compound **2** was thus easily recognized as a member of the amphilectane skeletal class of diterpenes.[12] Because portions of the ^1H and ^{13}C NMR spectra of monamphilectine B (**2**) were very similar to those previously reported for monamphilectine A (**1**), we assumed that their structures were closely related. Additional relevant signals in the ^1H NMR spectrum included a sharp two-proton singlet at δ_H 3.75 (s, 2H, H-22), two

Table 1
^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for the naturally occurring monamphilectines B and C (**2** and **3**)[a]

Position	Monamphilectine B (**2**)		Monamphilectine C (**3**)	
	δ_H, mult, integration (J in Hz)	δ_C, type[b]	δ_H, mult, integration (J in Hz)	δ_C, type[b]
1	1.83, br m, 1H	33.0, CH	1.84, br m, 1H	32.9, CH
2α	1.99, br m, 1H	41.0, CH_2	1.98, br m, 1H	41.0, CH_2
2β	0.84, br m, 1H		0.85, br m, 1H	
3	1.04, br m, 1H	35.6, CH	1.03, br m, 1H	35.6, CH
4	1.08, br m, 1H	42.4, CH	1.07, br m, 1H	42.4, CH
5α	1.95, br m, 1H	29.8, CH_2	1.96, br m, 1H	29.7, CH_2
5β	0.81, br m, 1H		0.81, br m, 1H	
6α	1.51, br m, 1H	29.9, CH_2	1.51, br m, 1H	29.8, CH_2
6β	1.42, br m, 1H		1.25, br m, 1H	
7	1.35, br m, 1H	40.9, CH	1.32, br m, 1H	40.8, CH
8		67.0, C		66.9, C
9α	2.28, br m, 1H	39.7, CH_2	2.28, br m, 1H	39.6, CH_2
9β	1.29, br m, 1H		1.32, br m, 1H	
10αβ	2.27, br m, 2H	33.6, CH_2	2.28, br m, 2H	33.6, CH_2
11		150.4, C		150.4, C
12	1.84, br m, 1H	46.2, CH	1.84, br m, 1H	46.2, CH
13	1.01, br m, 1H	55.6, CH	1.00, br m, 1H	55.6, CH
14α	1.98, br m, 1H	44.4, CH_2	1.99, br m, 1H	44.5, CH_2
14β	1.56, br m, 1H		1.53, br m, 1H	
15		54.4, C		54.6, C
16	1.39, br s, 3H	28.8, CH_3	1.38, br s, 3H	28.7, CH_3
17	1.37, br s, 3H	27.3, CH_3	1.38, br s, 3H	27.2, CH_3
18	0.89, d, 3H (6.1)	20.0, CH_3	0.90, d, 3H (5.7)	20.0, CH_3
19	0.98, d, 3H (6.3)	15.7, CH_3	0.98, d, 3H (6.1)	15.7, CH_3
20α	4.85, br s, 1H	105.9, CH_2	4.85, br s, 1H	105.9, CH_2
20β	4.60, br s, 1H		4.60, br s, 1H	
21		166.3, C		165.7, C
22	3.75, s, 2H	47.2, CH_2	3.79, s, 2H	46.5, CH_2
23		171.9, C		167.8, C
24	3.30, br m, 1H	45.2, CH	4.70, dd, 1H (4.7, 1.9)	83.4, CH
25α	3.55, t, 1H (5.4)	48.8, CH_2	3.62, dd, 1H (5.8, 4.8)	49.1, CH_2
25β	3.03, dd, 1H (5.4, 2.4)		3.36, dd, 1H (5.9, 1.9)	
26		156.2, C		156.2, C
27	1.34, d, 3H (7.4)	13.6, CH_3	3.53, s, 3H	57.7, CH_3
N–H	5.81, br s, 1H, exchangeable		5.73, br s, exchangeable	

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in parts per million (ppm) relative to the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. Assignments were aided by 2D NMR experiments, spin-splitting patterns, the number of attached protons, and chemical shift values.
[b] ^{13}C NMR types were obtained from DEPTQ NMR experiments.

mutually coupled proton resonances at δ_H 3.55 (t, 1H, J=5.4 Hz, H-25α) and 3.03 (dd, 1H, J=5.4, 2.4 Hz, H-25β), a multiplet methine at δ_H 3.30 (br m, 1H, H-24), and a broad singlet (D_2O exchangeable) at δ_H 5.81 (br s, 1H, N–H). After the association of all of the ^1H and ^{13}C NMR resonances resulting from C–H one-bond interactions observed in the HSQC NMR spectra, ^1H–^1H COSY and HMBC NMR experiments were performed to establish the main connectivities that allowed the assembly of the molecular planar framework (summarized in Fig. 1).

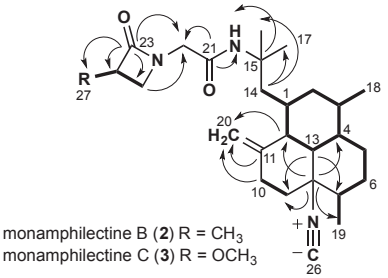


Fig. 1. Key HMBC (C→H) and COSY (—) correlations of **2** and **3**.

As with the previously described monamphilectine A (**1**), the strong absorption peak at 1741 cm^{-1} in the IR spectrum strongly