Tetrahedron 71 (2015) 5013–5018

Contents lists available at ScienceDirect

Tetrahedron

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

Two cell differentiation inducing pyridoacridines from a marine sponge *Biemna* sp. and their chemical conversions

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ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form 18 May 2015
Accepted 19 May 2015
Available online 28 May 2015

Keywords:
Sponge
Cell differentiation
K562
Pyridoacridine

ABSTRACT

Two pyridoacridines, *N*-hydroxymethylisocystodamine (**1**) and neolabuanine A (**2**), together with the known ecionine A (**3**), ecionine B (**4**), isocystodamine (**5**), *N*-methylisocystodamine (**6**), 9-hydroxyisoascididemin (**7**), and biemnadin (**8**), were isolated from a marine sponge *Biemna* sp. Several of these compounds were shown to induce cell differentiation of K562 leukemia cells into erythrocytes. Following inspection of the NMR data, and comparison of these data with literature values, we demonstrated that neolabuanine A (**2**) had the structure previously reported as labuanine A (**2**), and that the compound initially reported under the name of labuanine A possessed the structure assigned to ecionine A (**3**). We found that both neolabuanine A (**2**) and ecionine A (**3**) were gradually converted to 9-hydroxyisoascididemin (**7**), indicating that **2** and **3** can be considered both as precursors of **7**.

1. Introduction

Induction of differentiation from acute myelogenous leukemia tumor cells into mature normal cells by chemical inducers has been considered as a strategy for cancer chemotherapy, because early hematopoietic progenitors fail to give birth to cell lineage restricted phenotype in leukemias due to blockade of differentiation.[1] Against this background we searched for metabolites that exhibit induction of cell differentiation in K562, human myelogenous leukemia cells. We found activity in the organic extract of the marine sponge *Biemna* sp. In this paper we report the isolation, structure elucidation and cell differentiation activity of the isolated constituents.

2. Results and discussion

2.1. Isolation of cell differentiation inducers

The sponge (500 g) was extracted with MeOH and the extract was partitioned between $CHCl_3$ and H_2O. The organic fraction was further partitioned between *n*-hexane and 90% MeOH. The bioassay-guided fractionation of the 90% MeOH fraction by ODS flash chromatography and RP-HPLC afforded two new pyridoacridines, *N*-hydroxymethyl isocystodamine (**1**) and neolabuanine A (**2**), as well as the previously reported natural products, ecionine A (**3**),[2] ecionine B (**4**),[2] isocystodamine (**5**),[3] *N*-methylisocystodamine (**6**),[4] 9-hydroxyisoascididemin (**7**),[5] and biemnadin (**8**).[6]

2.2. Structure elucidation of *N*-hydroxymethyl isocystodamine (1)

Compound **1** was isolated as an yellow amorphous solid with a molecular formula of $C_{19}H_{12}N_4O_2$ as determined by HRESIMS [*m*/*z* 329.1013, $(M+H)^+$]. ^{1}H NMR and HSQC spectra of **1** showed eight aromatic CH (δ_H 9.27/δ_C 149.5, 9.05/120.2, 8.96/124.1, 8.59/152.6, 8.38/131.1, 8.03/131.9, 7.92/129.3, and 7.21/109.5), one hydroxymethyl group (δ_H 4.94/δ_C 65.5), and an exchangeable amino proton at δ_H 10.31 (Table 1). The ^{1}H–^{1}H coupling constants, characteristic carbon chemical shifts, and HMBC correlations suggested the

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http://dx.doi.org/10.1016/j.tet.2015.05.070
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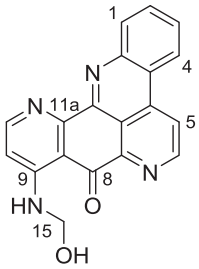
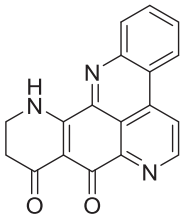
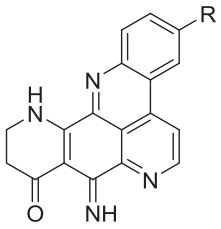


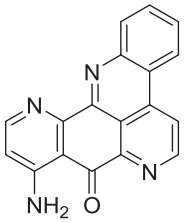
1: *N*-hydroxymethyl isocystodamine



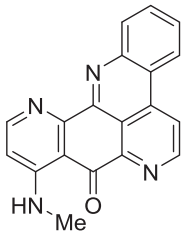
2: neolabuanine A



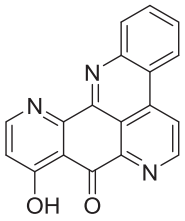
3: R=H ecionine A
4: R=OH ecionine B



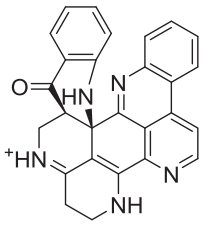
5: isocystodamine



6: *N*-methyl isocystodamine



7: 9-hydroxyisoacididemin



8: biemnadin

presence of one disubstituted benzene ring (partial structure A) and two trisubsituted pyridine rings (partial structures B and C) (Fig. 1). These partial structures and the molecular formula implied that **1** was closely related to isocystodamine.[3] Further HMBC correlation from H_2-15 (δ_H 4.94) to C-9 (δ_C 155.1) indicated that aromatic primary amino group found in the structure of isocystodamine was substituted by a hydroxymethyl group, which was supported by a COSY correlation between H_2-15 and NH-14 (δ_H 10.31). LCMS analysis revealed that **1** gradually converted to isocystodamine (**5**) in solution (Fig. S1), further supporting our structural assignment.

assigned by HRESIMS. Interpretation of the ^{1}H NMR and 2D NMR spectra of **2** suggested the presence of partial structures A and C. There were two methylene protons (δ_H 3.86 and 2.58), an exchangeable proton (δ_H 9.72), and one ketone (δ_C 188.9), belonging to a 2,3-dihydropyridin-4(1H)-one moiety as determined by COSY and HMBC correlations (Table 1). These three partial structures and the three unassigned carbons (δ_C 174.8, 145.0, and 143.8) implied that the structure of compound **2** could be represented by the tautomeric form of the previously proposed structure of labuanine A. Labuanine A was first isolated from *Biemna fortis* by Aoki et al.[3] and while the molecular formula of this compound was determined by

Table 1
NMR Spectroscopic data (600 MHz, DMSO-d_6) for **1** and **2**

Position	1			Position	2		
	^{13}C	^{1}H (*J* in Hz)	HMBC		^{13}C	^{1}H (*J* in Hz)	HMBC
1	131.1, CH	8.38, d (7.4)	3, 4a	1	130.5, CH	8.33, d (7.6)	3, 4a
2	131.9, CH	8.03, t (7.4)	4, 13a	2	132.0, CH	8.08, t (7.6)	4,13a
3	129.3, CH	7.92, t (7.4)	1, 4a	3	130.1, CH	7.99, t (7.6)	1, 4a
4	124.1, CH	8.96, d (7.4)	2,4b,13a	4	124.3, CH	8.97, d (7.6)	2,4b,13a
4a	121.7, C			4a	123.0, C		
4b	136.9, C			4b	136.1, C		
5	120.2, CH	9.05, d (5.3)		5	118.4, CH	8.91, d (5.5)	4a,6,12c
6	149.5, CH	9.27, d (5.3)	4b, 7a	6	150.1, CH	9.18, d (5.5)	4b,5,7a
7a	146.6, C			7a	147.3, C		
8	ND			8	174.8, C		
8a	ND			8a	107.7, C		
9	155.1, C			9	188.9, C		
10	109.5, CH	7.21, d (5.9)		10	36.1, CH_2	2.58, t (7.6)	9
11	152.6, CH	8.59, d (5.9)	9,12a	11	40.4, CH_2	3.86, t (7.6)	9,10,12a
12a	153.2, C			12-NH		9.72, s	
12b	ND			12a	156.6, C		
12c	ND			12b	145.0, C		
13a	145.4, C			12c	116.8, C		
14		10.31, t (5.8)		13a	143.8, C		
15	65.5, CH_2	4.94, d (5.8)	9				

ND: not determined.

2.3. Structure elucidation of neolabuanine A (2)

Compound **2** was isolated as a yellowish amorphous solid with a molecular formula of $C_{18}H_{11}N_3O_2$ [*m*/*z* 324.0747 $(M+Na)^+$] as

HR-FABMS analysis, the coincidence of the ^{1}H and ^{13}C chemical shifts between ecionine A (**3**) and labuanine A suggested that the structure of one of these molecules had been mis-assigned. Careful analysis of the NMR data for both ecionine A and labuanine A