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Polycitorols A and B, New Tricyclic Alkaloids from an Ascidian

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Abstract: Two new tricyclic alkaloids, polycitorols A (**1**) and B (**2**) have been isolated along with the known lepadiformine (**3**) from a marine ascidian of the family Polycitoridae. The structures of the new compounds were elucidated by analysis of NMR data and comparison with those of **3** and other related compounds [1-5]. Compounds **1** and **2** are closely related to cylindricines A and B, lacking C-4 oxygenation found in cylindricines and having a butyl instead of a hexyl appendage. NOE experiments on compounds **1** and **2** suggested the A/B ring fusion to be *cis*.

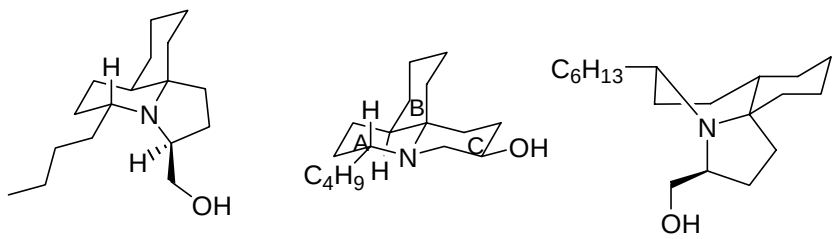
Keywords: polycitorols, Polycitoridae, tricyclic alkaloids, ascidian, polycyclic alkaloids.

Introduction

Tricyclic alkaloids represented by cylindricines [1], lepadiformine (**3**) [2] and fasicularin [3] are unique metabolites of ascidians. Cylindricines A and B isolated from *Clavelina cylindrical* [1] were

the first members of the family based on the perhydropyrrolo[2,1-*j*]quinoline and the perhydropyrido[2,1-*j*]quinoline ring systems, respectively. The structures and relative stereochemistries were unambiguously established by X-ray crystallography of their corresponding picrates. Moreover, these two compounds were reported to interconvert through aziridinium intermediate forming 3:2 equilibrium mixture upon standing in a solution. These compounds were reported toxic to brine shrimps [1]. Initially reported [2] structure for lepadiformine was revised to **3** [4], and its absolute configuration was recently determined [5]. Compound **3** exhibited cytotoxicity against several tumor cell lines [2] and in vivo and in vitro cardiovascular activity [6]. More recently, fasicularin, isolated from *Nephteis fasicularis*, was reported as a cytotoxin to Vero cells and was found to act as a DNA-damaging agent in the assay using a DNA repair-deficient yeast strain [3].

As a part of collaborative investigation of biologically active compounds from Indonesian marine organisms [7], we herein report the isolation and structures of two new tricyclic alkaloids polycitorols A (**1**) and B (**2**) together with known lepadiformine (**3**) from a marine ascidian.



1 **2** **3**

Results and Discussion

Specimens (65.0 g, wet) of the ascidian of the family Polycitoridae were collected by SCUBA (-25 m) off Flores, Indonesia in August 2001 and were stored in ethanol after its collection. The sample was exhaustively extracted with MeOH and the resulting extract was chromatographed on a silica gel column followed by reversed-phase HPLC to give polycitorol A (**1**), B (**2**), and lepadiformine (**3**).

Polycitorol A (**1**), colorless oil, $[\alpha]_D^{25}$ +15º (*c* 0.2, $CHCl_3$), was shown to have a molecular formula of $C_{17}H_{31}NO$ by HREIMS (*m/z* 265.2410, M^+, Δ +0.4 mmu). Strong absorption bands at 3384 and 1671 cm^{-1} in the IR spectrum suggested that **1** contain hydroxyl and amine functionalities. The ^1H NMR spectrum contained a number of unresolved multiplets in the high field region (δ 1.21-2.24), while no olefinic signals were observed. The ^{13}C NMR spectrum revealed the presence of a quaternary carbon (δ 77.9), two heteroatom bearing methines (δ 67.0, 71.9), one methine (δ 44.2), one CH_2OH (δ 65.0), one methyl, and eleven methylenes. Therefore, alkaloid **1** is tricyclic, and the nitrogen atom was suggested as a tertiary amine. The ^{13}C NMR spectrum of **1** displayed 17 carbon signals indicating that **1** has two less carbons than **3**. A mass fragment ion at *m/z* 208 was formed by the cleavage of C_4H_9, thus, differing in the length of the alkyl side chain from **3**. COSY correlations revealed that a methine proton at δ 2.97 (δ_C 67.0) correlated with two methylene protons at δ 1.58 and 2.05 confirming the C2/C15 linkage. Further 2D NMR analysis (Table 1) allowed us to elucidate the planar structure of **1**. The relative stereochemistry of **1** was determined through NOE experiments.

Enhancement of H-2 at δ 2.97 and H-4ax at δ 1.57 upon irradiation of H-9ax at δ 2.15 suggested a *cis*-fusion between rings A and B. The alkyl side chain at C-2 occupied *β* equatorial position on the basis of mutual NOE enhancements between H-2 and H-5. NOE between one of H-12 protons and H-13 may indicate the primary hydroxyl group as shown. Thus, the structure of compound **1** was elucidated.

Table 1. ^{1}H and ^{13}C NMR data for **1** and **2** (500 MHz)

	1[a]			**2**[b]	
#	δ_C	δ_H	HMBC	δ_C	δ_H
2	67.0 d	2.97 brt, *J* = 9.4 Hz	C-15	58.3 d	3.39 brs
3	30.9 t	2.10 m, 1.49 m	C-2, C-4, C-5	28.9 t	1.71 m, 2.08 m
4	24.0 t	1.57 m, 1.74 m	C-2, C-3, C-5, C-10	24.9 t	1.37 m, 1.47 m
5	44.2 d	1.81 m	C-10	43.3 d	1.98 m
6	32.1 t	1.25 m, 1.60 m	C-5, C-10, C-7	29.5 t	1.26 m, 1.53 m
7	26.4 t	1.36 m, 1.70 m	C-6	24.6 t	1.70 m
8	25.4 t	1.47 m, 1.43 m	C-9	22.3 t	1.41 m, 1.78 m
9	32.7 t	1.47 m, 2.15 m	C-8	31.2 t	1.61 m, 2.60 brd, *J* = 12.5 Hz
10	77.9 s			66.5 s	
11	24.4 t	2.09 m, 2.17 m	C-9, C-10, C-12	17.6 t	1.97 m
12	24.6 t	1.81 m, 2.24 dt, *J* = 13.4, 8.2 Hz	C-10, C-11	26.3 t	1.78 m, 2.11 m
13	71.9 d	3.76 m	C-12, C-14	60.5 d	4.06 brs
14	65.0 t	3.72 m	C-13, C-12	45.5 d	3.36 brs 3.53 m
15	34.7 t	1.58 m, 2.05 m	C-2, C-3	30.6 t	1.60 m, 1.94 m
16	29.5 t	1.21 m, 1.36 m	C-15, C-17, C-18	27.4 t	1.24 m, 1.38 m
17	23.6 t	1.31 m, 1.39 m	C-16, C-18	22.4 t	1.28 m
18	14.2 q	0.90, t, *J* = 7.0 Hz	C-16, C-17	13.7 q	0.90 t, *J* = 7.0 Hz

[a]Measured in CD_3OD, [b]measured in $CDCl_3$.

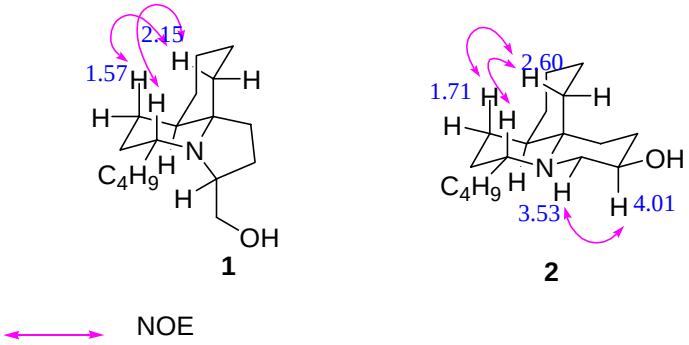


Figure 1. Key NOE correlations

Polycitorol B (**2**), $[\alpha]_D^{25}$ –10.3° (*c* 0.6, $CHCl_3$), was isolated as a colorless oil. The molecular formula $C_{17}H_{31}NO$ was determined by HREIMS, indicating that it was isomeric to **1**. The IR spectrum of **2** was similar to that of **1**. Comparison of NMR data of **1** and **2** revealed that considerable higher field shifts were observed for a methine at δ 58.3 and a quaternary carbon at δ 66.5 in the ^{13}C spectrum of **2**. Furthermore, additional methine signal at δ 4.06 (δ_C 60.5) suggested that **2** had a secondary rather than a primary alcohol. The COSY spectrum indicated that this proton coupled to two nitrogen-attached methylene protons (δ 3.53, 3.36) and two other protons (δ 1.78, 2.11). Thus, the aforementioned data indicated that the pyrrolidine ring was rearranged to a piperidine ring. The relative stereochemistry of A/B rings was shown to be identical to that in **1** with a C-2α butyl side chain based on similar NOE data, particularly a set of mutual NOE enhancements between H-2, H-4ax and H-9ax. A *trans* fusion between rings A and C was suggested by NOE between H-13/H-2, H-14ax/H-2 and H-5/H-2. Thus, rings A and C of **2** adopted a much more rigid *trans* 1-azadecalin system and the C-13 hydroxyl group occupied *β* equatorial position.

Conclusion

Two new tricyclic alkaloids, polycitorols A (**1**) and B (**2**), have been isolated along with the known lepadiformine (**3**) from a marine ascidian. The new compounds contain a butyl side chain instead of a hexyl group observed more commonly in known related compounds.

Experimental

General

The optical rotations were recorded with a Jasco DIP-1000 Digital polarimeter. IR spectra were measured on a Jasco FT IR-300 spectrometer. NMR spectra were taken on a Jeol α 500 FT NMR spectrometer and referenced to $CHCl_3$ solvent signal at δ 7.26 for ^1H NMR and δ 77.0 for ^{13}C NMR spectra in $CDCl_3$ as a solvent and also referenced to TMS signal at δ 0.00 for ^1H NMR and δ 49.0 for ^{13}C NMR spectra in CD_3OD as a solvent. Multiplicities of ^{13}C spectra were assigned by DEPT

experiments. EIMS mass spectra were measured on a Hitachi M-2500 instrument. HPLC separations were carried out on a Hitachi L-6000 or Shimadzu LC 9A pumps equipped with Waters 486 or Hitachi L-4000 UV detector and Waters R401 Differential Refractometer. Columns used for HPLC were reverse phase Nacalai $5C_{18}$-AR II (10 × 250 mm). Kieselgel 60 (230-400 mesh) Si gel was used for column chromatography. TLC was carried out on precoated silica 60_{F254} plates and visualized with vanillin-EtOH-1% H_2SO_4.

Biological material

The specimens (65.0 g, wet wt.) of the ascidian (family Polycitoridae) were collected by hand using SCUBA (-25 m) at Misa Is., Labuhanbajo, Flores, Indonesia in August 2001 and were stored in ethanol after its collection. The ascidian was tentatively identified by Dr. Adrian Gittenberger, National Museum of Natural History *Naturalis*, Leiden, The Netherlands. A voucher specimen (0117J78) is deposited at Department of Chemistry, Biology, and Marine Science, University of the Ryukyus. A photo of the specimen can be viewed at http://www.ascidians.com/families/polycitoridae/polycitoridae_blue/polycitoridaeblue.htm

Extraction and Isolation

A sample of tunicate (65.0 g wet weight) was cut into small pieces and soaked in acetone (300 mL) for 7 hr. After decantation, fresh solvent was added, and the procedure was repeated three times. The combined extracts were concentrated and partitioned between EtOAc and H_2O. The organic layer was concentrated to give an oil (0.39 g). The oil was chromatographed on silica gel by eluting with step gradient of hexane/CH_2Cl_2/EtOAc/MeOH. Seven fractions were obtained. Fraction V was selected for further purification using HPLC (RP-18, MeOH/H_2O/TFA) to afford compounds **1**, **2** and **3**.

Polycitorol A (**1**): Colorless oil; $[\alpha]_D^{25}$ +15.3° (*c* 0.2, $CHCl_3$); IR (film) 3384, 2937, 2867, 1671, 1455, 1201, 1132 cm^{-1}; ^1H and ^{13}C NMR see Table 1; EIMS *m/z* 265 (25, M^+), 234 (100), 222 (97), 208 (45), 194 (5), 178 (5); HREIMS found *m/z* 265.2410 (calcd for $C_{17}H_{31}NO$, 265.2406).

Polycitorol B (**2**): Colorless oil; $[\alpha]_D^{25}$ –10.3° (*c* 0.6, $CHCl_3$); IR (film) 3384, 2937, 2869, 1670, 1457, 1201, 1132 cm^{-1}; ^1H and ^{13}C NMR see Table 1; EIMS *m/z* 265 (6, M^+), 222 (29), 208 (100), 193 (7), 150 (5); HREIMS found *m/z* 265.2406 (calcd for $C_{17}H_{31}NO$, 265.2406).

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Sample availability:

The authentic sample of both polycitorol A and B are deposited at Department of Chemistry, Biology, and Marine Science, University of the Ryukyus.