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 *Nephtea fasicularis*, was reported as a cytotoxic 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.

![Chemical structures of 1, 2, and 3](image_url)

**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, \([\alpha]_D^{25} +15^\circ (c 0.2, \text{CHCl}_3)\), was shown to have a molecular formula of C_{17}H_{31}NO by HREIMS (m/z 265.2410, M^+, \Delta +0.4 mnu). 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 (\(\delta 1.21-2.24\)), while no olefinic signals were observed. The \(^13C\) NMR spectrum revealed the presence of a quaternary carbon (\(\delta 77.9\)), two heteroatom bearing methines (\(\delta 67.0, 71.9\)), one methine (\(\delta 44.2\)), one CH₂OH (\(\delta 65.0\)), one methyl, and eleven methylenes. Therefore, alkaloid 1 is tricyclic, and the nitrogen atom was suggested as a tertiary amine. The \(^13C\) 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 C₄H₉, thus, differing in the length of the alkyl side chain from 3. COSY correlations revealed that a methine proton at \(\delta 2.97 (\delta_C 67.0)\) correlated with two methylene protons at \(\delta 1.58\) 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 $\delta$ 2.97 and H-4ax at $\delta$ 1.57 upon irradiation of H-9ax at $\delta$ 2.15 suggested a cis-fusion between rings A and B. The alkyl side chain at C-2 occupied $\beta$ 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)

|   | $^1$H          | $^{13}$C       | HMBC       | $^1$H          | $^{13}$C       |
|---|---------------|---------------|------------|---------------|---------------|
| 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 | C-12, C-14 | 60.5 d, 4.06 brs |
| 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 |

*aMeasured in CD$_3$OD, bmeasured in CDCl$_3$. 
Polycitorol B (2), \([\alpha]_D^{25} -10.3^\circ \, (c\ 0.6, \text{CHCl}_3)\), 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 \(\delta\ 58.3\) and a quaternary carbon at \(\delta\ 66.5\) in the \(^{13}\text{C}\) spectrum of 2. Furthermore, additional methine signal at \(\delta\ 4.06\ (\delta_C\ 60.5)\) 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 (\(\delta\ 3.53, 3.36\)) and two other protons (\(\delta\ 1.78, 2.11\)). 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-2\(\alpha\) 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 \(\beta\) 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 \(\alpha\) 500 FT NMR spectrometer and referenced to CHCl\(_3\) solvent signal at \(\delta\ 7.26\) for \(^1\text{H}\) NMR and \(\delta\ 77.0\) for \(^{13}\text{C}\) NMR spectra in CDCl\(_3\) as a solvent and also referenced to TMS signal at \(\delta\ 0.00\) for \(^1\text{H}\) NMR and \(\delta\ 49.0\) for \(^{13}\text{C}\) NMR spectra in CD\(_3\)OD as a solvent. Multiplicities of \(^{13}\text{C}\) 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 5C18-AR II (10 × 250 mm). Kieselgel 60 (230-400 mesh) Si gel was used for column chromatography. TLC was carried out on precoated silica 60F254 plates and visualized with vanillin-EtOH-1% H2SO4.

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 (0117378) is deposited at Department of Chemistry, Biology, and Marine Science, University of the Ryukus. 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 H2O. 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/CH2Cl2/EtOAc/MeOH. Seven fractions were obtained. Fraction V was selected for further purification using HPLC (RP-18, MeOH/H2O/TFA) to afford compounds 1, 2 and 3.

Polycitorol A (1): Colorless oil; [α]D25 +15.3° (c 0.2, CHCl3); IR (film) 3384, 2937, 2867, 1671, 1455, 1201, 1132 cm⁻¹; 1H and 13C 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 C17H31NO, 265.2406).

Polycitorol B (2): Colorless oil; [α]D25 −10.3° (c 0.6, CHCl3); IR (film) 3384, 2937, 2869, 1670, 1457, 1201, 1132 cm⁻¹; 1H and 13C 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 C17H31NO, 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.

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