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Amaminols A and B, new bicyclic amino alcohols from an unidentified tunicate of the family Polyclinidae[1]

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Abstract

Two new cytotoxic amino alcohols, amaminols A and B, have been isolated from an unidentified tunicate of the family Polyclinidae. Their structures including stereochemistry were elucidated by spectroscopic and chemical methods. Both compounds were cytotoxic against P388 murine leukemia cells.

Keywords: marine metabolites; amino alcohols; cytotoxins; stereochemistry.

More than ten years ago, Gulavita and Scheuer isolated the first marine aliphatic amino alcohols from a marine sponge *Xestospongia* sp.[2] Subsequently, several related compounds were reported from marine sponges[3,4] and tunicates.[5,6] In the course of our search for potential drugs from Japanese marine invertebrates, we collected an unidentified tunicate of the family Polyclinidae off Ukeshima Island of the Amami Islands whose methanol extract showed cytotoxicity against P388 murine leukemia cells. Bioassay-guided fractionation afforded two new amino alcohols, amaminols A and B. This paper describes isolation, structure elucidation, and cytotoxicity of these compounds.

The frozen tunicate (1.25 kg) was extracted with EtOH; the combined extracts were partitioned between ether and water. The aqueous layer was extracted with *n*-BuOH, while the ether layer was partitioned between hexane and 90% aq. MeOH. The *n*-BuOH and aqueous MeOH layers were combined and separated by ODS flash chromatography and gel-filtration on Sephadex LH-20, followed by repeated HPLC on ODS with 80% aq. MeOH/H_2O containing 100 mM NH_4OAc and with 78% aq. MeCN containing 0.05% TFA to afford amaminol A (**1**) and a mixture of **1** and amaminol B (**2**). The mixture was converted to *N*-Boc derivatives which were separated by reversed-phase HPLC with MeOH/MeCN/H_2O (4:3:1) to furnish *N*-Boc derivatives **1a** and **2a**. Deprotection with TFA afforded amaminol A (**1**; total

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yield, 81.3 mg, 6.5×10^{-3}% based on wet weight) and amaminol B (**2**; 30.5 mg, 2.4×10^{-3}%) as a pale-yellow oil.

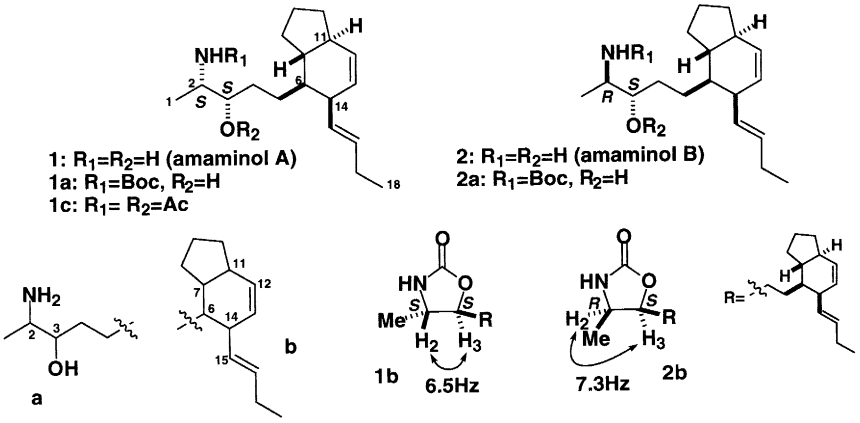

Fig. 1. Coupling constants of oxazolidinone derivatives

Amaminol A (**1**)[7] had a molecular formula of $C_{18}H_{31}NO$ as determined by HRFABMS [*m/z* 278.2479 $(M+H)^+$, Δ −0.5 mmu]. The ^{1}H NMR spectrum displayed four olefinic signals and two methyl signals, in addition to methine and methylene signals resonating between 3.5 and 1.0 ppm. The ^{13}C NMR spectrum together with a DEPT experiment revealed that **1** contained $2\times CH_3$, $6\times CH_2$, and $10\times CH$ segments. Interpretation of the COSY and HMQC spectra led to partial structure **a** which contained a vicinal amino alcohol portion (δ_H/δ_C 3.08/53.1 and 3.45/73.0). NMR data revealed the presence of two vinyl units (δ_H/δ_C 5.79/130.2, 5.49/134.9, 5.38/134.9, and 5.34/130.0) which accounted for two of four degrees of unsaturation, thus indicating the presence of two ring systems in the molecule. Coupling constants ($J_{12,13}$=9.6 Hz and $J_{15,16}$=15.4 Hz) implied *Z* and *E* geometry for the Δ^{12} and Δ^{15} double bonds, respectively. Partial structure **b** resulted from interpretation of the COSY spectrum starting from a triplet methyl signal at δ 0.97 (*t*, *J*=7.5 Hz; H18). Although ^{1}H NMR signals between 1.8 and 1.1 ppm overlapped, connectivities from C6 to C14 could be elucidated by HMBC cross peaks between H7 (δ 1.30)/C8 (29.0), C11 (47.8), C6 (44.0); H12 (5.79)/C11 (47.8), C10 (30.5). HMBC cross peaks, H6/C4, C5, and C7 connected C5 and C6, thereby revealing the gross structure of **1**.

Amaminol B (**2**)[8] had the same molecular formula as **1** as determined by HRFABMS and exhibited NMR data almost superimposable on those of **1** except for the coupling constant between H2 and H3 ($J_{2,3}$=3.1 Hz versus 7.3 Hz for **1**). Detailed analysis of 2D NMR data disclosed that **2** had the identical structure as **1** except for the stereochemistry at C2.

To determine relative stereochemistry of the vicinal amino alcohol of **1** and **2**, they were converted to the corresponding oxazolidinone derivatives (Fig. 1). Treatment of **1** with *N*,*N*′-carbonyldiimidazole afforded the oxazolidinone **1b**. Not only the coupling constant (*J*=6.5 Hz)[9] between H2 and H3 derived by irradiation of CH_3-1 but also difference NOE experiments[10] disclosed that CH_3-1 and H3 were on the same face of the oxazolidinone ring. Similarly, *cis* relationship of H2 and H3 in **2b** was secured by coupling constants (*J*=7.3 Hz) and NOE experiments. 3*S* Stereochemistry was assigned from NMR analysis of MTPA esters **1d** and **2d**[11] of *N*-Boc derivatives **1a** and **2a** (Fig. 2). Thus, the absolute stereochemistry of the amino alcohol moiety of **1** and **2** was 2*S*, 3*S* and 2*R*, 3*S*, respectively.

The relative stereochemistry of the bicyclic ring system of **1** was elucidated by NOESY cross peaks: H7/H15, H8α/H11, H8α/H6, H11/H6, H10β/H12, and H14/H16, thus indicating a *trans*-fused ring system. To determine the absolute configuration, **1** was converted to diacetate **1c** which was oxidized

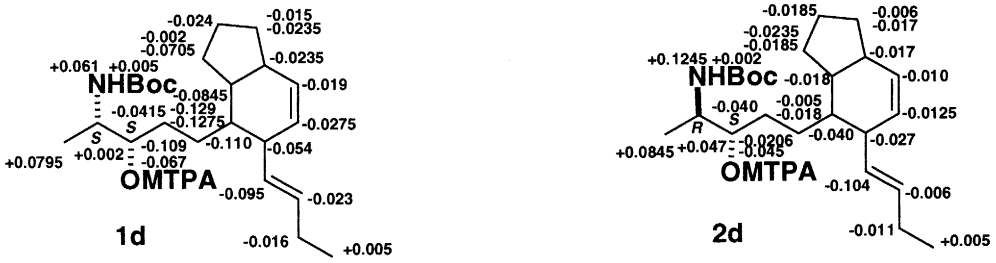

Fig. 2. $\Delta\delta$ values obtained for MTPA esters of **1** and **2**

Table 1
NMR data for amaminol A (**1**) in CD_3OD at 300 K

No.	^{13}Cmult	^{1}Hmult	*J*, Hz	HMBC	No.	^{13}Cmult	^{1}Hmult	*J*, Hz	HMBC
1	16.0q	1.25d	6.9	C2,C3	9	23.0t	1.71m		C7,C10,C11
2	53.1d	3.08dq	7.3,6.9	C1,C3,C4	10α	30.5t	1.83m		C8,C11
3	73.0d	3.45ddd	7.7,7.3,3.1	C1,C2,C4,C5	β		1.16m		C11,C13
4a	31.9t	1.49m		C2,C3,C5	11	47.8d	1.82m		C9,C10,C13
b		1.57m		C2,C3,C5	12	130.2d	5.79d	9.6	C10,C11,C14
5a	27.0t	1.52m		C4,C6	13	132.1d	5.38ddd	9.6,4.2,2.7	C11,C14
b		1.42m			14	44.2d	2.89ddd	8.9,5.8,4.2	C6,C7,C13,C15
6	44.0d	1.62dddd	13.5,10.4,5.8,3.5	C4,C5,C7,C14	15	130.0d	5.34ddd	15.4,8.9,1.2	C13,C14,C16
7	46.0d	1.30m		C6,C8,C11	16	134.9d	5.49dt	15.4,6.2	C15,C17,C18
8α	29.0t	1.18m		C6,C9	17	26.9t	2.03ddq	6.2,1.2,7.5	C15,C16,C18
β		1.75m		C7,C9,C10,C11	18	14.4q	0.97t	7.5	C16,C17

with OsO_4, followed by *p*-bromobenzoylation to furnish di-*p*-bromobenzoate **3** as a major product. A coupling constant of 2.9 Hz between H12 and H13 indicated their *syn* relationship, while the relative configuration of the six membered ring was established by NOESY cross peaks between H12/H7, H10β and H11/H6, H8α (Fig. 3) as well as by coupling constants ($J_{11,12}$=11.7 Hz; $J_{13,14}$=2.9 Hz). The CD spectrum[12] exhibited a negative exciton split [CD (MeOH) 254 nm ($\Delta\varepsilon$-14.5), 235 (+21.1)], which indicated 12*S*,13*R* stereochemistriy. Thus, absolute stereochemistry of the *trans*-fused bicyclic system in **1** was 6*S*, 7*S*, 11*R*, and 14*R*. Similarly, the stereochemistry of **2** was deduced to be the same as **1**.

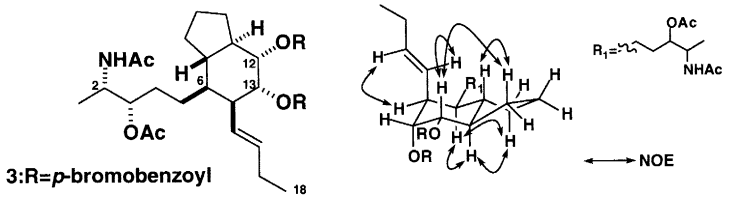

Fig. 3. NOEs observed for **3**

Amaminols A and B were cytotoxic against P388 murine leukemia cells with an IC_{50} value of 2.1 μg/mL. They are closely related to aliphatic amino alcohols isolated from marine sponges, *Xestospongia* sp.[2,3] and *Leucetta microraphis*[4] as well as from tunicates, *Didemnum* sp.[5] and *Pseudodistoma crucigaster*.[6] Some of these metabolites were antifungal. Biogenetically, amaminols may be derived by a Diels–Alder type cyclization of a triene.

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References

1. *Bioactive Marine Metabolites*; Part 99. Part 98; Fusetani, N.; Fujita, M.; Nakao, Y.; Matsunaga, S.; van Soest, R. W. M. *Bioorg. Med. Chem. Lett.*, in press.
2. Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 366–369.
3. Jimenez, C.; Crews, P. *J. Nat. Prod*. **1990**, *53*, 978–982.
4. Kong, F.; Faulkner, D. J. *J. Org. Chem.* **1993**, *58*, 970–971.
5. Molinski, T. F.; Searle, P. A. *J. Org. Chem.* **1993**, *58*, 7578–7580.
6. Jares-Erijman, E. A.; Bapat, C. P.; Lithgow-Bertelloni, A.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 5732–5737.
7. Amaminol A (**1**): pale-yellow oil. $[\alpha]_D^{24}$ –170.8° (*c* 0.20, MeOH); IR (film) ν_{max} 3385, 3264, 2957, 2868, 1671, 1615sh, 1522, 1455, 1427, 1390, 1198, 1183, 1136, 1072, 970, 840, 803, 754, 720 cm^{-1}; HRFABMS (matrix: NBA) *m/z* 278.2479 $(M+H)^+$ ($C_{18}H_{32}NO$, Δ −0.5 mmu); ^1H NMR and ^{13}C NMR data, see Table 1.
8. Amaminol B (**2**): pale-yellow oil. $[\alpha]_D^{24}$ –112.4° (*c* 0.20, MeOH); IR (film) ν_{max} 3410, 2957, 2870, 1676, 1525, 1433, 1203, 1138, 970, 839, 800, 723 cm^{-1}; HRFABMS (matrix: NBA) *m/z* 278.2478 $(M+H)^+$ ($C_{18}H_{32}NO$, Δ −0.6 mmu); ^1H NMR (CD_3OD) δ 5.80 (1H, d, *J*=9.8 Hz, H12), 5.49 (1H, dt, 15.2, 6.5, H16), 5.39 (1H, ddd, 9.8, 4.2, 2.3, H13), 5.33 (1H, dd, 15.2, 8.9, H15), 3.67 (1H, ddd, 9.2, 4.2, 3.1, H3), 3.25 (1H, dq, 3.1, 6.7, H2), 2.91 (1H, m, H14), 2.03 (2H, dq, 6.5, 7.4, H17), 1.82 (2H, m, H10α and H11), 1.74 (1H, m, H8β), 1.70 (2H, m, H9), 1.62 (1H, m, H6), 1.58 (1H, m, H4b), 1.47 (1H, m, H5a), 1.41 (1H, m, H5b), 1.35 (1H, m, H4a), 1.27 (1H, m, H7), 1.21 (3H, d, 6.7, H1), 1.18 (1H, m, H8α), 1.15 (1H, m, H10β), 0.98 (3H, t, 7.4 Hz, H18); ^{13}C NMR (CD_3OD) δ 135.0d (C16), 132.0d (C13), 130.2d (C12), 129.9d (C15), 71.7d (C3), 52.6d (C2), 47.7d (C11), 46.0d (C7), 44.3d (C14), 43.8d (C6), 31.2t (C4), 30.4t (C10), 29.0t (C8), 28.2t (C5), 26.7t (C17), 23.1t (C9), 14.4q (C18), 12.1q (C1).
9. Wolf, J.-P.; Pfander, H. *Helv. Chim. Acta* **1986**, *69*, 918–926.
10. NOE experiments: irradiation of CH_3-1(δ 1.25) enhanced H2 (0.3%) and H3 (0.2); irradiation of H2 (δ 3.55) enhanced CH_3-1 (3.2%), NH (1.6), and H3 (0.9); irradiation of H3 (δ 4.07) enhanced CH_3-1 (2.0%) and H2(0.7).
11. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
12. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy — Exciton Coupling in Organic Stereochemistry*; Oxford University Press: Oxford, 1983.