Ancorinoside A: A Novel Tetramic Acid Glycoside from the Marine Sponge, *Ancorina* sp. Which Specifically Inhibits Blastulation of Starfish Embryos

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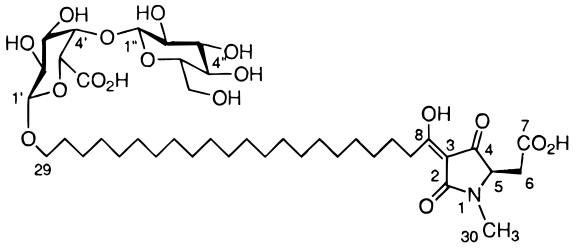
In the course of our search for substances capable of arresting the embryonic development of the starfish at a specific stage, we found that a MeOH extract of the marine sponge *Ancorina* sp. inhibited the blastulation. Bioassay-guided purification of the crude extract resulted in the isolation of a novel tetramic acid glycoside, which was designated ancorinoside A (**1**). This communication reports the structure and biological activities of **1**.



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The marine sponge *Ancorina* sp. (129 g, wet weight) was collected off the coast of Tokushima Prefecture, Japan, and extracted with MeOH. The MeOH extract was partitioned between *n*-BuOH and H_2O, and the *n*-BuOH-soluble fraction was subjected to low-pressure chromatography on ODS using 0−100% MeOH in H_2O as eluent, followed by reversed-phase HPLC (ODS, 60−80% CH_3CN in H_2O) to afford **1** (2.0 mg, 0.16% yield based on the wet weight).[1] A molecular formula of $C_{41}H_{69}NO_{17}$ was established by the HR-FAB mass and NMR spectral data. NMR spectra were obtained in both CD_3OD and C_5D_5N in order to facilitate observation of the spin systems (Table 1). The UV maxima at 283 and 240 nm and chemical shifts (CD_3OD) of four broad carbon signals (δ_C 197.0, 188.9, 175.1, and 103.8 for C4, C8, C2, and C3, respectively) strongly suggested the presence of a tetramic acid skeleton.[2] The presence of an *N*-Me group (C30) was indicated by its chemical shifts (δ_H 2.96 and δ_C 26.9). The ^{1}H NMR spectrum showed signals for a $CHCH_2$ unit (C5−C6) with a broadened methine proton (δ_H 4.05). Interpretation of the HMBC spectrum[3] together with ^{13}C and ^{1}H NMR data led to a partial structure in which the C5 methine locates between N1 and C4 carbonyl carbon constituting the five-membered ring of the tetramic acid moiety: (1) the proton attached to the N1-C30 methyl carbon correlated with the C2 carbonyl carbon (δ_C 175.1) and the C5 methine carbon (δ_C 64.7); (2) both of the two H6 signals at δ_H 2.80 and 2.90 displayed crosspeaks with the C5 methine and the C7 carbonyl carbons (δ_C 174.5) and the former signal showed an additional correlation signal with the C4 carbonyl carbon (δ_C 197.0), as shown in Figure 1. On the other hand, the C6 methylene carbon is located outside the ring and attached to the C7 carbonyl carbon. The NOE correlation between H6 and H30 in the NOESY spectrum (C_5D_5N) confirmed this interpretation.

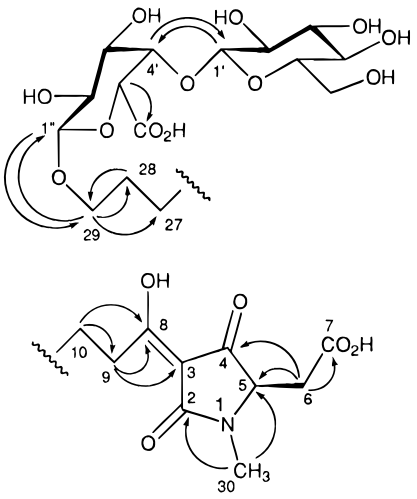

Figure 1. Selected HMBC correlations observed for **1** in CD_3OD.

The NMR data also indicated the presence of a disaccharide moiety composed of a hexopyranose and a hexopyranosyluronic acid, which was supported by the observation of the fragment ion peak at *m*/*z* 339 in the negative FAB mass spectrum. The presence of two anomeric protons was shown in the ^{1}H NMR and COSY spectra (C_5D_5N). Starting from the lower field signal (δ_H 5.30, H1″), we could deduce a glucopyranose structure: H1″ to H5″ were all axial. Another anomeric proton at δ_H 4.81 (H1′) was that of galactopyranosyluronic acid, which was deduced from a combination of COSY, HMBC, and 1D homonuclear Hartmann−Hahn (HOHAHA)[4] spectra. The HMBC correlation from H1″ to C4′ (δ_C 81.8) and the NOE between H1″ and H4′ (δ_H 5.08) indicated that C1″ of glucopyranose attached to C4′ of galactopyranosyluronic acid via a glucosidic linkage. The ^{1}H coupling constant (7.3 Hz) of the anomeric proton (H1″) and the $^1J_{CH}$ value[5] (160.2 Hz) of the anomeric carbon (δ_C 106.9, C1″) indicated that glucopyranose is present in a β-form. The anomeric carbon (C1′) of galactopyranosyluronic acid attached to an oxymethylene (C29) via a β-glycosidic linkage, which was indicated by the HMBC correlation from H1′ to C29 (δ_C 70.1), the ^{1}H coupling constant (8.3 Hz) of H1′, and the $^1J_{CH}$ value (160.2 Hz) of C1′ (δ_C 104.8). The remaining long methylene chain (C9−C28) was located between C8 and C29, which was revealed on the basis of HMBC spectral data, as shown in Figure 1.

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(1) **1**: a viscous colorless oil; $[\alpha]^{25}_D$ −5.5° (*c* 0.09, MeOH); UV (MeOH) 283 (ϵ 7700), 240 nm (ϵ 5600); CD (MeOH) 285 ($\Delta\epsilon$ +0.79), 240 nm ($\Delta\epsilon$ +0.76); IR (film) 3400, 1713, 1680, 1645, 1634 cm^{-1}; HR-FABMS (positive, glycerol matrix) *m*/*z* 870.4445 $[M + Na]^+$ (calcd for $C_{41}H_{69}NO_{17}Na$, 870.4467); FABMS (positive, glycerol matrix) *m*/*z* (relative intensity) 886 $[M + K]^+$ (70), 870 $[M + Na]^+$ (100), 848 $[M + H]^+$ (50); FABMS (negative, glycerol matrix) *m*/*z* 846 $[M - H]^-$, 339; ^{1}H NMR (500 MHz) see Table 1; ^{13}C NMR (125 MHz) see Table 1.

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Table 1. ^{1}H NMR and ^{13}C NMR Data for Ancorinoside A (1)[a]

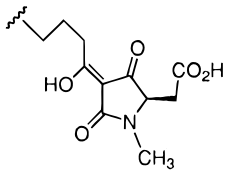
	C_5D_5N		CD_3OD		
no.	δ_C (mult)[b]	δ_H (mult, J (Hz), int)	δ_C (mult)[b]	δ_H (mult, J (Hz), int)	HMBC[d]
2	174.3 (s)		175.1 (s)		
3	101.8 (s)		103.8 (s)		
4	194.1 (s)		197.0 (s)		
5	62.9 (d)	4.30 (m, 1H)	64.7 (d)	4.05 (br s, 1H)	
6	36.5 (t)	3.00 (dd, 6.9, 16.1, 1H)	36.0 (t)	2.80 (m, 1H)	C5, C7
		3.28 (dd, 5.0, 16.1, 1H)		2.90 (dd, 16.5, 4.6, 1H)	C4, C5, C7
7	173.7 (s)		174.5 (s)		
8	191.4 (s)		188.9 (s)		
9	37.5 (t)	3.22 (m, 2H)	35.8 (t)	2.80 (m, 2H)	C3, C8, C10, C11
10	26.3 (t)	1.84 (m, 2H)	27.9 (t)	1.65 (m, 2H)	C8, C9, C11
11	30.0 (t)	1.40 (m, 2H)	31.5 (t)	1.30 (m, 2H)	
12−26	30.0 (t, 15C)	1.30 (m, 30H)	31.5 (t, 15C)	1.30 (m, 30H)	
27	26.4 (t)	1.35 (m, 2H)	27.9 (t)	1.40 (m, 2H)	
28	30.3 (t)	1.67 (m, 2H)	31.5 (t)	1.63 (m, 2H)	C27, C29
29	70.1 (t)	4.20 (dt, 9.2, 6.4, 1H)	72.1 (t)	3.54 (m, 1H)	C27, C28, C1′
		3.73 (dt, 9.2, 6.4, 1H)		3.94 (dt, 9.2, 6.4, 1H)	C27, C28, C1′
30	27.1 (q)	3.09 (s, 1H)	26.9 (q)	2.96 (s, 1H)	C2, C5
1′	104.8 (d)	4.81 (d, 8.3, 1H)	105.5 (d)	4.27 (d, 7.3, 1H)	C29
2′	72.8 (d)	4.47 (t, 8.3, 1H)	73.4 (d)	3.56 (m, 1H)	C3′
3′	75.1 (d)	4.33 (dd, 2.8, 8.3, 1H)	75.8 (d)[c]	3.63 (m, 1H)	C2′
4′	81.8 (d)	5.08 (d, 2.8, 1H)	81.7 (d)	4.33 (d, 2.8, 1H)	C2′, C3′, C5′, C1″
5′	75.3 (d)	4.76 (s, 1H)	75.7 (d)[c]	4.23 (s, 1H)	C1′, C3′, C6′
6′	171.9 (s)		173.0 (s)		
1″	106.9 (d)	5.30 (d, 7.3, 1H)	106.7 (d)	4.47 (d, 7.3, 1H)	C4′
2″	76.0 (d)	3.97 (br t, 8, 1H)	76.4 (d)	3.22 (m, 1H)	C3″
3″	78.4 (d)	4.17 (br t, 9, 1H)	78.8 (d)	3.35 (m, 1H)	C2″, C4″
4″	71.9 (d)	4.02 (br t, 9, 1H)	72.1 (d)	3.30 (m, 1H)	C3″, C5″
5″	78.2 (d)	3.89 (m, 1H)	78.7 (d)	3.20 (m, 1H)	C1″
6″	63.2 (t)	4.07 (dd, 6.4, 11.0, 1H)	63.4 (t)	3.65 (m, 1H)	C4″
		4.31 (dd, 2.7, 11.0, 1H)		3.84 (dd, 11.9, 1.8, 1H)	C4″

[a] Data recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 27 °C. [b] Multiplicities were determined by a DEPT experiment. [c] Assignments interchangeable. [d] HMBC optimized for $^{2,3}J_{CH}$ = 4, 6, 8, and 10 Hz.

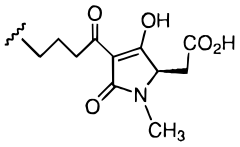
Both the absolute stereochemistries of β-glucopyranose and β-galactopyranosyluronic acid were determined to be D by GLC analysis of the trimethylsilyl (TMS) ethers of 1-(L-α-methylbenzylamino)-1-deoxyalditols[6] which were derived from the hydrolysis product of **1**.

The CD spectrum of **1** exhibited positive Cotton effects at 285 ($\Delta\epsilon$ +0.79) and 240 nm ($\Delta\epsilon$ +0.76). The sign of the Cotton effects of **1** was opposite to that of tenuazonic acid[2a] and equisetin,[2b] suggesting that the chirality of the asymmetric carbon in the tetramic acid ring of **1** was *R*. $NaIO_4/KMnO_4$ oxidation of **1**, followed by acid hydrolysis, afforded *N*-methyl-D-aspartic acid as identified by HPLC after derivatization with Marfey's reagent,[7] thereby confirming that **1** had 5*R* stereochemistry. Thus, the structure of **1** was determined to be (5*R*)-5-(carboxymethyl)-3-{22-*O*-[β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyluronic acid]-1-hydroxydocosylidene}-1-methyl-2,4-pyrrolidinedione. Actually, ancorinoside A exists in an equilibrium of inseparable four tautomers, **1**, **1′**, **1″**, and **1‴**.

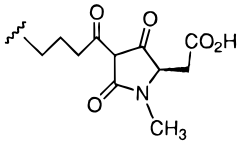
Ancorinoside A (**1**) is the first metabolite having a tetramic acid ring the precursor of which is considered to be a D-amino acid. The structure of the dicarboxylic acid with a 21-methylene chain is also unique to ancorinoside A (**1**).



1′



1″



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When fertilized starfish (*Asterina pectinifera*) eggs were cultured from fertilization in the presence of **1** at or above 0.4 μg/mL, the development proceeded normally to 256−512 cell stage and the embryos ceased to develop further without exhibiting any sign of blastulation. Studies on the mode of the unusual action of **1** are now in progress.

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Supporting Information Available: Copies of the 1D and 2D NMR spectra of compound **1** (7 pages).

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