Aldose Reductase Inhibitors from a Marine Sponge, *Dictyodendrilla* sp.

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Summary: Three potent aldose reductase inhibitors (**1a**, **1b**, **2a**) with a unique pyrrolo[2,3-*c*]carbazole skeleton isolated from a marine sponge, *Dictyodendrilla* sp., were characterized by chemical and physical evidence and X-ray crystallographic analysis.

Aldose reductase catalyzes reduction of aldoses, such as glucose and galactose, to the corresponding polyols, such as sorbitol and galactitol, respectively. According to the polyol pathway hypothesis[1] for the pathogenesis of diabetic complications, intracellular accumulation of the polyols may result in diabetic complications. Aldose reductase inhibitors may therefore provide an effective means for the prevention and treatment of such diseases. Clinical trials of some inhibitors are actually under way.[2]

In order to isolate aldose reductase inhibitors from marine organisms, we systematically screened their extracts for inhibition of bovine aldose reductase. Previously, we reported potent inhibitors of this enzyme in the extracts of a red alga, *Asparagopsis taxiformis*, producing two inhibitors, pentabromopropen-2-yl tribromoacetate and dibromoacetate.[3] Here we report the isolation, characterization, and inhibitory activity of the other active principles[4] (**1a**, **1b**, **2a**) from a marine sponge, *Dictyodendrilla* sp.

A dark green sponge, *Dictyodendrilla* sp. (wet wt. 15.4 kg), collected by scuba diving, 20–30-m deep at Azumacho, Kagoshima in 1990, was extracted with methanol at room temperature. The methanol extract was concentrated under reduced pressure and partitioned between ether and water. The aqueous extract was then extracted with ethyl acetate. The inhibitory activity remained in the ethyl acetate extract. An activity-directed separation of the active ethyl acetate extract gave three potent inhibitors, **1a** (25 mg), **1b** (104.1 mg), and **2a** (31.1 mg).Compound **1a** was fully characterized by elemental analysis, atomic absorption spectrum, and HRFABMS ([M^+ + H] *m/z* 821.1479, Δ +2.6 mmu, $C_{43}H_{29}N_2O_{12}SNa$). In the ^1H NMR spectrum, there were observed two methylene signals [δ 2.37 (2H, m), 2.97 (2H, m)], an ABC-type aromatic signal [δ 5.74 (1H, d, *J* = 8.6 Hz, H_{10}), 6.61 (1H, dd, *J* = 7.9, 8.6 Hz, H_9), 7.15 (1H, d, *J* = 7.9 Hz, H_8)], and unresolved broad signals (22H, m). The ^{13}C NMR spectrum indicated the presence of two sp^3 carbon atoms [δ 36.8 (t), 47.5 (t)] and a carbonyl sp^2 carbon atom [δ 181.6 (s)] accompanied by many unassignable aromatic carbon atoms. **1a** was hydrolyzed chemically and enzymatically to **1c** on respective treatments with trifluoroacetic acid and sulfatase,[5] clearly indicating the presence of a sulfate group. In the ^1H NMR spectrum of **1c**, the ABC-type aromatic protons [H_8 (δ 6.54), H_9 (δ 6.49), H_{10} (δ 5.43)], especially H_8 and H_{10}, were shifted more significantly to upper field than those of **1a**, while the other protons did not shift as much. This indicated that the sulfate group could be located at C-7 in **1a** because H_8 and H_{10} should be situated *ortho* and

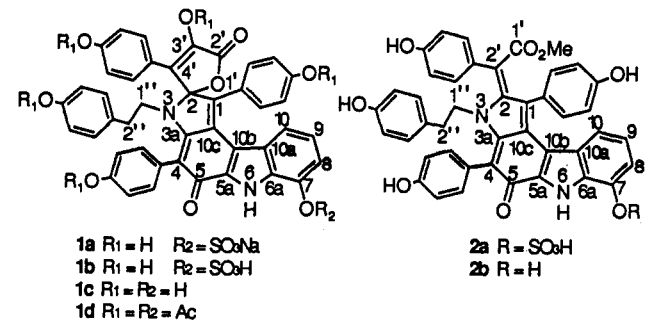

Figure 1.

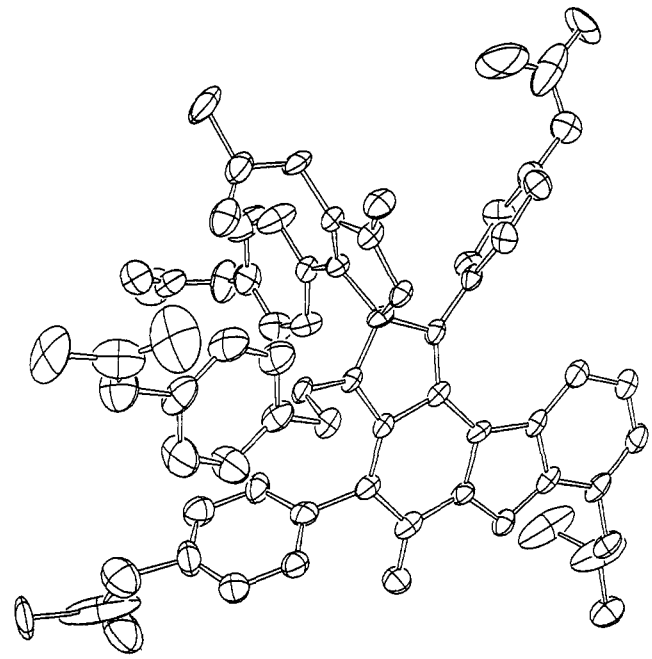

Figure 2. Perspective drawing of **1d**.

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(3) Sugano, M.; Sato, A.; Nagaki, H.; Yoshioka, S.; Shiraki, T.; Horikoshi, H. *Tetrahedron Lett.* **1990**, *31*, 7015.
(4) **1a**: purple solid; mp > 300 °C; UV (EtOH) λ_{max} nm (ε) 475 (1900), 391 (10 700), 324 (22 100), 288 (23 100), 229 (45 800); IR (KBr) ν_{max} cm^{-1} 3396, 1760, 1575, 1512, 1383, 1262, 1237, 1171, 1057. Anal. Calcd for $C_{43}H_{29}N_2SO_{12}Na(H_2O)_9$: C, 52.53; H, 4.82; N, 2.85; S, 3.26. Found: C, 52.45; H, 4.90; N, 2.96; S, 3.32. Atomic absorption analysis of Na: calcd 2.34, found 4.90. **1b**: purple solid; mp > 300 °C; HRFABMS [M^++ H] *m/z* 799.1576 $C_{43}H_{31}N_2O_{12}S$, Δ −2.2 mmu); UV (EtOH) λ_{max} nm (ε) 475 (1200), 391 (9700), 324 (19 800), 289 (20 900), 229 (40 800); IR (KBr) ν_{max} cm^{-1} 3267, 1688, 1608, 1569, 1510, 1268, 1219, 1169. **2a**: purple solid; mp > 300 °C; UV (EtOH) λ_{max} nm (ε) 482 (23 200), 281 (21 000), 231 (41 000); IR (KBr) ν_{max} cm^{-1} 3267, 1688, 1608, 1569, 1510, 1268, 1219, 1169.

(5) The Dupont sulfatase (NEE 154) was used.

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Table I. ^1H and ^{13}C NMR Data of 1b–d

no.	1b (CD_3OD) ^1H (mult, J)	1c (CD_3OD) ^1H (mult, J)	1c (CD_3OD) ^{13}C (mult)	1d (CD_3CN) ^1H (mult, J)
1			143.0 (s)	
2			106.4 (s)	
3a			157.8 (s)	
4			112.9 (s)	
5			180.9 (s)	
5a			142.2 (s)	
6a			130.1 (s)	
7			145.5 (s)	
8	7.17 (d, 8.3)	6.54 (d, 8.5)	109.7 (d)	6.95 (dd, 7.8, 1.1)
9	6.64 (t, 8.3)	6.49 (t, 8.5)	122.6 (d)	6.72 (dd, 7.8, 8.3)
10	5.79 (d, 8.3)	5.43 (d, 8.5)	115.9 (d)	5.56 (dd, 8.3, 1.1)
10a			126.6 (s)	
10b			112.2 (s)	
10c			131.0 (s)	
1′			168.9 (s)	
2′			142.1 (s)	
3′			123.4 (s)	
1″	2.79 (m), 3.05 (m)	2.80 (m), 3.05 (m)	46.6 (t)	3.04 (m), 3.14 (m)
2″	2.37 (2H, m)	2.37 (2H, m)	35.8 (t)	2.56 (2H, m)
1-ph 1			124.6 (s)	
2	7.30 (dd, 8.3, 2.0)	7.27 (dd, 8.3, 2.1)	132.2 (d)	7.25 (dd, 8.3, 2.1)
3	6.91 (dd, 8.3, 2.4)	6.88 (dd, 8.3, 2.4)	116.7 (d)	7.10 (dd, 8.3, 2.5)
4			160.2 (s)	
5	6.70 (dd, 8.3, 2.4)	6.69 (dd, 8.3, 2.4)	116.3 (d)	7.26 (dd, 8.3, 2.5)
6	6.78 (dd, 8.3, 2.0)	6.77 (dd, 8.3, 2.1)	132.4 (d)	7.62 (dd, 8.3, 2.1)
4-ph 1			125.8 (s)	
2	7.10 (dd, 8.3, 2.0)	7.08 (dd, 8.3, 2.1)	134.4 (d)	7.04 (dd, 8.3, 2.5)
3	6.83 (dd, 8.3, 2.0)	6.82 (dd, 8.3, 2.4)	115.4 (d)	6.97 (dd, 8.3, 2.5)
4			158.6 (s)	
5	6.98 (dd, 8.3, 2.0)	6.97 (dd, 8.3, 2.4)	116.4 (d)	7.59 (dd, 8.3, 2.5)
6	7.39 (dd, 8.3, 2.0)	7.34 (dd, 8.3, 2.1)	134.8 (d)	7.59 (dd, 8.3, 2.5)
3′-ph 1			124.6 (s)	
2, 6	7.72 (2H, d, 8.8)	7.72 (2H, d, 8.6)	130.2 (2d)	7.77 (2H, d, 9.3)
4			159.8 (s)	
3, 5	6.84 (2H, d, 8.8)	6.51 (2H, d, 8.6)	116.8 (2d)	7.25 (2H, d, 9.3)
2″-ph 1			130.4 (s)	
2, 6	6.37 (2H, d, 8.8)	6.36 (2H, d, 8.5)	130.5 (2d)	6.60 (2H, d, 8.6)
4			156.8 (s)	
3, 5	6.52 (2H, d, 8.8)	6.51 (2H, d, 8.5)	116.1 (2d)	6.84 (2H, d, 8.6)
Ac				2.19 (3H, s), 2.23 (3H, s), 2.29 (3H, s), 2.32 (3H, s), 2.36 (3H, s), 2.50 (3H, s)

para to the sulfate group, respectively. The structure of **1a** was determined by X-ray crystallographic analysis of an acetate (**1d**),[6] but spectral data were paramount in elucidation of **1a–c**.The structure was determined by the direct methods (Multan 78) and Fourier syntheses. Parameters were refined by succesive block-diagonal least-squares methods with anisotropic temperature factors for non-hydrogen atoms to $R = 0.108$.[7] A perspective drawing of **1d** in Figure 2 shows that the aromatic ring at C-1 and the carbazole ring are nearly perpendicular to each other. This conformation is compatible with the ^1H NMR data of **1a** and **1c** indicating that the high-field resonances of H_{10}'s are due to the ring current of the aromatic ring at C-1. Consequently, **1a** has a unique pyrrolo[2,3-*c*]carbazole spirolactone structure as shown, and **1b** was also determined to be an acid form of **1a** by comparing its spectral data with those of **1a** and its chemical conversion to **1c**. Although **1a** and **1b** have respective asymmetric centers at C-2, they were optically inactive, showing no Cotton effect in the CD spectrum[8] and a symmetric space group ($P\bar{1}$) in the X-ray crystal structure. The racemization may be ascribed to the intervention of nonenzymatically equilibrated intermediates with the sp^2-like carbon atom at C-2 in the sponge.

Compund **2a** was analyzed for $C_{43}H_{33}N_2O_{11}S$ by the HRFABMS ([M^+ + H] m/z 785.1826, Δ +2.3 mmu). **2a** was also hydrolyzed to **2b**[9] on treatment with trifluoroacetic acid at room temperature. Both the ^1H NMR and the UV absorption spectra were consistent with the fact that a sulfate group is located at C-7, analogously to **1a**.

Refluxing of **1c** with trifluoroacetic acid in methanol led to **2b**. The structure of **2a** could therefore be analyzed by comparing the ^1H and ^{13}C NMR spectra of **1c** and **2b** to **2a**. The presence of a methoxycarbonyl group in **2b** is suggested by the IR absorption [ν_{max} cm^{-1} (KBr) 1691] and a long-range coupling between the methoxy protons (δ 3.17) and the carbonyl carbon (δ 171.3) in the HMBC experiment. The HMBC experiment also showed that the C-1″-methylene protons (δ 2.98) are correlated with

(6) **1d**: red brown leaflets; mp 202–205 °C; FABMS [M^+ + H] m/z 981; UV (EtOH) λ_{max} nm (ε) 382 (9400), 338 (15 900), 283 (26 100); IR (KBr) ν_{max} cm^{-1} 1768, 1711, 1505, 1369, 1197, 1165, 1134, 1126, 1014. Crystal data: triclinic, space group $P\bar{1}$, a = 14.227(2) Å, b = 13.551(4) Å, c = 15.776(3) Å, α = 95.42(3)°, β = 115.98(1)°, γ = 87.37(2)°, Z = 2. The author has deposited atomic coordinates for **1d** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

(7) Further improvement of the discrepancy factor failed owing to crystal twinning and to some disorder of the acetyl groups.

(8) The UV absorption band of **1a** near at the Na D-line precluded measurement of its optical rotation.

(9) **2b**: red brown solid; mp > 300 °C; HRFABMS [M^+ + H] m/z 705.2271 (Δ +3.4 mmu); UV (EtOH) λ_{max} nm (ε) 499 (25 400), 279 (24 200), 231 (46 900); IR (KBr) ν_{max} cm^{-1} 3263, 1691, 1607, 1573, 1510, 1468, 1266, 1227, 1170, 1112.

Table II. ^1H and ^{13}C NMR Data of 2a and 2b

	2a (CD_3OD)		2b (CD_3OD)	
no.	^1H (mult, J)	^{13}C (mult)	^1H (mult, J)	^{13}C (mult)
1		141.4 (s)		141.1 (s)
2		151.2 (s)*		151.4 (s)*
3a		157.4 (s)*		157.4 (s)*
4		123.2 (s)		122.6 (s)
5		178.1 (s)		178.4 (s)
5a		135.5 (s)		134.9 (s)
6a		132.8 (s)		130.0 (s)
7		140.0 (s)		145.1 (s)
8	7.16 (d, 7.9)	117.8 (d)	6.50 (d, 7.9)	109.6 (d)
9	6.58 (t, 7.9)	121.6 (d)	6.44 (t, 7.9)	122.3 (d)
10	5.34 (d, 7.9)	121.8 (d)	5.07 (d, 7.9)	116.2 (d)
10a		127.2 (s)		126.7 (s)
10b		113.5 (s)		113.6 (s)
10c		132.4 (s)		132.7 (s)
1′		171.1 (s)		171.3 (s)
2′		115.1 (s)		114.9 (s)
3′				
1″	3.00 (2H, m)	49.9 (t)	2.98 (2H, m)	49.9 (t)
2″	2.27 (2H, m)	34.4 (t)	2.67 (2H, m)	34.4 (t)
1-ph 1		126.2 (s)		126.2 (s)
2, 6	7.17 (2H, d, 8.6)	134.1 (2d)	7.17 (2H, d, 8.0)	134.1 (2d)
3, 5	6.94 (2H, d, 8.6)	116.4 (2d)	6.93 (2H, d, 8.0)	116.2 (2d)
4		159.7 (s)		159.4 (s)
4-ph 1		128.9 (s)		129.0 (s)
2, 6	7.19 (2H, d, 8.5)	133.5 (2d)	7.18 (2H, d, 8.0)	133.3 (2d)
3, 5	6.85 (2H, d, 8.5)	116.8 (2d)	6.84 (2H, d, 8.0)	116.9 (2d)
4		159.9 (s)		158.8 (s)
2′-ph 1		126.4 (s)		126.5 (s)
2, 6	7.35 (2H, d, 7.9)	133.4 (2d)	7.34 (2H, d, 8.5)	133.5 (2d)
3, 5	6.91 (2H, d, 7.9)	116.3 (2d)	6.91 (2H, d, 8.5)	116.2 (2d)
4		158.2 (s)		158.1 (s)
2″-ph 1		130.1 (s)		130.2 (s)
2, 6	6.58 (2H, d, 8.3)	131.0 (2d)	6.58 (2H, d, 8.0)	131.0 (2d)
3, 5	6.49 (2H, d, 8.3)	116.0 (2d)	6.49 (2H, d, 8.0)	115.9 (2d)
4		156.9 (s)		156.8 (s)
OMe	3.17 (3H, s)	52.9 (q)	3.17 (3H, s)	52.9 (q)

*: May be interchangeable.

both C-2 (δ 151.4) and C-3a (δ 157.4), indicating that the amino ketal carbon atom (δ 106.4) at C-2 in **1c** changed its hybridization to the sp^2 one in **2b**. The carbon atom at 114.9 ppm was assigned to C-2′ because of its correlation with the *ortho* protons of the aromatic ring at C-2′. In the NOE experiment, irradiation of the C-1″-methylene proton signal enhanced the signals of all ortho protons of the aromatic rings at C-4 and -2″, which was compatible with *cis* arrangement of these substituents. This arrangement was supported by the diamagnetism of the aromatic ring at C-1 resulting in the high-field shifts of H_{10} and the methoxy protons. Consequently, **2a** could be depicted as shown.

It is well known that oxidative decarboxylations of α-keto acids are effected by peracids,[10] iodosobenzene,[11] and electrolysis.[12] On respective reactions with *m*-chloroperbenzoic and peracetic acids, **1c** did not give **2b**. These results may indicate that since **1c** has an exclusive enol form, it did not undergo an electrophilic attack of the peracids. The reaction, however, was obviously accelerated under oxygen more effectively than under nitrogen, indicating the contribution of other oxygen-involved mechanisms. **2a** and **2b** are presumably biosynthesized by the enzymatic decarboxylation of **1a**, **b**, or their corresponding acid forms with such an enzyme as a pyruvate decarboxylase or a formate lyase if they exist in the sponge.

The inhibitory activities (IC_{50}) of **1a**, **1b**, and **2a** against bovine lens aldose reductase were 4.9×10^{-8} M, 1.25×10^{-7} M, and 1.12×10^{-7} M, respectively. In the sereis of **1**, the sulfate groups did not always influence the activity (**1c**: 1.02×10^{-7} M), while in the series of **2**, the sulfate group potentiated the activity (**2b**: 5.67×10^{-7} M). The acetate (**1d**: $>10^{-5}$ M)) was substantially inactive. The studies on structure–activity relationship and mechanisms of the oxidative decarboxylation are in progress.

Supplementary Material Available: Characterization and general experimental procedures for new compounds and ^1H NMR spectra of **1a–c** and **2a,b** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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