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Aragusterol C: a novel halogenated marine steroid from an Okinawan sponge, *Xestospongia* sp., possessing potent antitumor activity

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Abstract. A novel chlorinated steroid, aragusterol C, was isolated from an Okinawan marine sponge of the genus *Xestospongia*. The compound strongly inhibited the proliferation of KB cells in vitro, and also showed potent in vivo antitumor activity against L1210 cells in mice. The complete structure of aragusterol C was determined by spectroscopic analysis and X-ray crystallographic analysis.
Key words. Marine sponge; halogenated steroid; antitumor substance; *Xestospongia*.

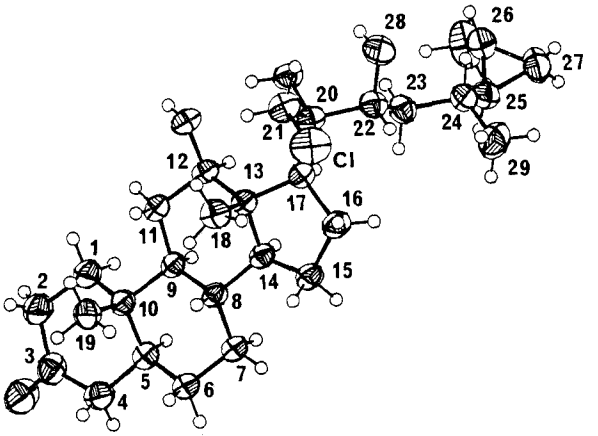
Marine sponges are recognized as a rich source of structurally unique and biologically active substances[1]. In our continuing study[2] on biologically active substances from Okinawan marine invertebrates, aragusterol A (structure **1**), a potent antitumor steroid, was isolated from a sponge of the genus *Xestospongia*, and found to have structure **1** based on the results of spectroscopic analysis, chemical reactions and chemical synthesis[3]. While searching for related steroids from the sponge, a novel halogenated steroid, aragusterol C (structure **2**), was isolated, whose in vivo antitumor activity was stronger than that of aragusterol A. This paper describes the structural elucidation of aragusterol C based on spectroscopic analysis and X-ray crystallographic analysis.

Materials and methods

Wet specimens (44.8 kg) of the sponge, *Xestospongia* sp.[3], collected on the coral reef of Aragusuku Island (Okinawa, Japan) in May 1992, were extracted with MeOH. The MeOH extract (2604 g) was partitioned between EtOAc and H_2O. The EtOAc soluble portion (267 g) was chromatographed on a silica gel column to give three fractions; fraction 1 eluted with hexane-EtOAc = 5:1, fraction 2 eluted with hexane-EtOAc = 1:1, and fraction 3 eluted with EtOAc and then MeOH. Fraction 2 (67.8 g) was chromatographed on an active carbon[4] column to give three fractions; fraction 1 eluted with MeOH, fraction 2 eluted with EtOAc, and fraction 3 eluted with $CHCl_3$. Fraction 2 and 3 were combined and repeatedly subjected to silica gel column chromatography, to give aragusterol C (**2**)[5] as colorless rods (0.32% yield based on the EtOAc soluble portion).

Results

Antitumor activity. Aragusterol C (**2**) strongly inhibited the proliferation of KB cells at IC_{50} 0.041 μg/ml, and expressed potent in vivo antitumor activity against L1210 leukemia in mice (T/C 257%, at 1.6 mg/kg).



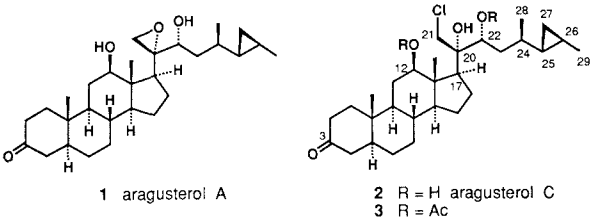


Figure. Computer-generated perspective drawing of aragusterol C (**2**).

Structure. The molecular formula $C_{29}H_{47}ClO_4$ of **2** was determined from elemental analysis and HRMS measurement. The IR spectrum of **2** showed absorptions due to hydroxyl groups (3400, 3266 cm^{-1}) and carbonyl group (1693 cm^{-1}). All 29 carbons appeared in the ^{13}C NMR spectrum and DEPT experiments indicated the presence of 4 methyls, 11 methylenes, 10 methines, 3 sp^3 quaternary carbons and one sp^2 quaternary carbon. In the table are shown ^{13}C and ^1H NMR correlations determined by examination of two-dimensional HMQC

NMR data for aragusterol C (**2**) and its diacetate **3**

No.	**2** ^{13}C[a]	^{1}H (J in Hz)[b]	**3** ^{13}C[a]	^{1}H (J in Hz)[b]
1	38.6 (CH_2)	2.03 (1H, ddd, 2.1, 6.4, 13.1)	38.4 (CH_2)	
2	38.1 (CH_2)	2.32 (1H, brd, 13.5)	37.9 (CH_2)	2.31 (1H, m)
		2.38 (1H, dt, 6.4, 13.5)		2.35 (1H, dt, 6.3, 13.3)
3	211.6 (C)		211.1 (C)	
4	44.6 (CH_2)	2.09 (1H, ddd, 1.7, 3.8, 15.0)	44.5 (CH_2)	2.11 (1H, ddd, 1.9, 4.0, 15.1)
		2.26 (1H, dd, 14.0, 15.0)		2.25 (1H, dd, 13.9, 15.1)
5	46.6 (CH)	1.52 (1H, m)	46.4 (CH)	
6	28.9 (CH_2)		28.8 (CH_2)	
7	31.1 (CH_2)		30.9 (CH_2)	
8	33.9 (CH)		33.9 (CH)	
9	52.5 (CH)		52.1 (CH)	
10	35.7 (C)		35.7 (C)	
11	29.8 (CH_2)		27.5 (CH_2)	
12	77.8 (CH)	3.44 (1H, dd, 4.5, 11.1)	80.0 (CH)	4.68 (1H, dd, 4.8, 11.1)
13	49.2 (C)		47.5 (C)	
14	54.1 (CH)		54.5 (CH)	
15	23.5 (CH_2)[c]		23.4 (CH_2)[c]	
16	23.6 (CH_2)[c]		23.9 (CH_2)[c]	
17	55.0 (CH)		56.6 (CH)	
18	9.0 (CH_3)	0.98 (3H, s)	10.3 (CH_3)	1.01 (3H, s)
19	11.5 (CH_3)	1.03 (3H, s)	11.4 (CH_3)	1.07 (3H, s)
20	77.1 (C)		76.8 (C)	
21	47.4 (CH_2)	3.88 (2H, s)	47.6 (CH_2)	3.76 (1H, d, 11.5)
				3.83 (1H, d, 11.5)
22	71.4 (CH)	3.97 (1H, dd, 3.3, 11.1)	75.4 (CH)	5.26 (1H, brd, 11.0)
23	37.8 (CH_2)		36.9 (CH_2)	
24	35.3 (CH)		35.1 (CH)	0.53 (1H, m)
25	28.0 (CH)	0.27 (1H, m)	27.9 (CH)	0.23 (1H, m)
26	12.3 (CH)	0.53 (1H, m)	12.4 (CH)	0.40 (1H, m)
27	12.6 (CH_2)	0.18 (1H, m)	12.6 (CH_2)	0.15 (1H, m)
		0.27 (1H, m)		0.23 (1H, m)
28	18.9 (CH_3)	0.98 (3H, s)[d]	18.7 (CH_3)	0.99 (3H, d, 6.7)[c]
29	19.3 (CH_3)	1.02 (3H, d, 5.4)	19.1 (CH_3)	1.00 (3H, d, 6.3)[c]
			21.1 ($COCH_3$)	2.04 (3H, s)
			21.7 ($COCH_3$)	2.06 (3H, s)
			170.2 ($COCH_3$)	
			170.8 ($COCH_3$)	

[a] ^{13}C NMR spectra were recorded at 100 MHz in $CDCl_3$. Carbon multiplicities were determined by DEPT experiments.
[b] ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$. Proton and carbon assignments were made based on the results of HMQC and HMBC experiments.
[c] The signals may be interchanged in each column.
[d] The methyl signal (H-28) appeared as a somewhat broad singlet, since the chemical shift of H-28 was close to that of H-24. In the ^{1}H NMR spectrum of diacetate **3**, the methyl signal (H-28) appeared as a doublet.

and HMBC spectra. The ^{1}H and ^{13}C NMR data indicated two secondary hydroxyl groups [δ_H 3.44 (1H, dd, $J = 4.5$, 11.1 Hz), 3.97 (1H, dd, $J = 3.3$, 11.1 Hz), δ_C 77.8 (CH), 71.4 (CH)], a tertiary hydroxyl group [δ_C77.1 (C)], a chloromethyl group [δ_H3.88 (2H, s), δ_C47.4 (CH_2)], a ketone [δ_C211.6 (C)], and a 1,2-disubstituted cyclopropyl group [δ_H 0.18 (1H, m), 0.27 (2H, m), 0.53 (1H, m)] to be present. The presence of two secondary hydroxyl groups was confirmed by acetylation. Treatment of **2** with acetic anhydride in pyridine at room temperature for 71 h gave diacetate **3**[6] [δ_H 2.04 (3H, s, $OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 4.68 (1H, dd, $J = 4.8$, 11.1 HZ, $CHOCOCH_3$), 5.26 (1H, br d, $J = 11.0$ Hz, $CHOCOCH_3$), δ_C 170.2 (C, $OCOCH_3$), 170.8 (C, $OCOCH_3$)]. The NMR data of **2** were closely related to those of aragusterol A (**1**)[2] except for characteristics due to the 20 and 21 positions, suggesting aragusterol C to have the structure shown as **2**. Analysis of the two-dimensional HMBC spectrum of **2** supported this structure. For complete determination of the structure, X-ray crystallographic analysis was conducted on **2**. The structure was solved by the direct method (SHELXS 86) refined by full-matrix least-squares to $R = 0.031$[7]. The computer-generated perspective drawing shown in the figure presents the complete structure of **2** with the absolute stereochemistry. The structure was confirmed by treating **2** with methanolic KOH to give aragusterol A (**1**).

Halogenated steroids are very rare in nature[8,9]. Kiheisterones[9], recently isolated from the Maui sponge (*Strongylacidon* sp.) and chlorinated at C-4 in the steroidal nucleus, were the first halogenated steroids to be obtained from a marine source. Aragusterol C (**2**) is thus the first marine steroid found to have the chlori-

nated side chain. The present result established the complete structure of aragusterol A (**1**).

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3 The sponge was identified by Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam. Specimens are deposited in his collection (registered number: ZMA Por. 7842).
4 Trade Mark; Shirasagi. For separation of 13.2 g of fraction 2, 60 g of the active carbon was used.
5 **2**: $[\alpha]_D + 20.1°$ (*c* 0.35, $CHCl_3$). mp 204–205 °C. CIMS *m/z* 497 ($M^+ + 1$, $C_{29}H_{47}{}^{37}ClO_4 + H$), 495 ($M^+ + 1$, $C_{29}H_{47}{}^{35}ClO_4 + H$).
6 **3**: colorless powder. $[\alpha]_D - 0.7°$ (*c* 1.08, $CHCl_3$). CIMS *m/z* 581 ($M^+ + 1$, $C_{33}H_{51}{}^{37}ClO_6 + H$), 579 ($M^+ + 1$, $C_{33}H_{51}{}^{35}ClO_6 + H$). IR (KBr) 3490, 1736, 1718, 1250 cm^{-1}.
7 Crystal data for **2**: orthorhombic, space group $P2_12_12_1$, $Z = 4$, lattice constants $a = 14.877$ (3) Å, $b = 15.680$ (3) Å, $c = 11.662$ (3) Å, $Dc = 1.21$ g/cm^3, $V = 2720$ (1) Å^3, crystal size $= 0.60 \times 0.40 \times 0.40$ mm^3.
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