Marine Natural Products. XXXVII.[1] Aragusteroketals A and C, Two Novel Cytotoxic Steroids from a Marine Sponge of *Xestospongia* sp.

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Aragusteroketals A (1) and C (2), two new marine steroids having dimethylketal structure, were isolated from an Okinawan marine sponge of *Xestospongia* sp. The chemical structures of 1 and 2 were determined on the basis of spectroscopic analysis and chemical evidence. Compounds 1 and 2 showed potent cytotoxic activity with the same IC_{50} value of 4 ng/ml against KB cells.

Key words marine sponge; *Xestospongia*; steroid; cytotoxic; dimethylketal

In the past 20 years, a number of steroids with unusual structures have been isolated from marine invertebrates. In 1993, Yamada and his group[3] first reported aragusterol A (**3**) from an Okinawan marine sponge of *Xestospongia* sp.; **3** strongly inhibited the cell proliferation of tumor cells *in vitro* and also showed potent *in vivo* anti-tumor activities against P388 and L1210 in mice. Since then, several side-chain analogues, aragusterols B,[4] C (**4**),[5] and D[4] and xestokerols A, B, and C,[6] have been isolated. In our continuing search[1] for new biologically active marine natural products, we have investigated the chemical constituents of an Okinawan marine sponge of *Xestospongia* sp. and have isolated two new aragusterol analogues with dimethylketal structures named aragusteroketals A (**1**) and C (**2**), together with aragusterols A (**3**) and C (**4**). Aragusteroketals A (**1**) and C (**2**) exhibited potent cytotoxic activity against KB cells. In this paper, we describe the structure elucidation of aragusteroketals A (**1**) and C (**2**).

The acetone extract of a marine sponge of *Xestospongia* sp. (collected on the reef (−10 m) of Iriomote Island, Okinawa Prefecture), which showed cytotoxic activity against KB cells, was subjected to bioassay-guided separation (based on cytotoxicity towards L1210 and KB cells). The acetone extract was partitioned into water–EtOAc mixture to give the cytotoxic EtOAc-soluble portion, which was then separated by repeated SiO_2 column chromatography (*n*-hexane–EtOAc and $CHCl_3$–MeOH–H_2O) to give two active fractions (fraction A and fraction B). Fraction A was separated by SiO_2 column chromatography and reversed-phase HPLC to provide aragusteroketal A (**1**) (0.08% yield from the EtOAc-soluble portion) and aragusterol A (**3**) (0.28%). Fraction B was separated by SiO_2 column chromatography and reversed-phase HPLC to provide aragusteroketal C (**2**) (0.28%) and the major steroid, aragusterol C (**4**) (2%).

Aragusteroketal A (**1**) was obtained as an amorphous solid. The FAB-MS of **1** showed a quasi-molecular $(M+Li)^+$ ion peak at *m/z* 511 and the molecular formula was determined as $C_{31}H_{52}O_5$ by HR FAB-MS and NMR analysis. The ^1H- and ^{13}C-NMR spectra of **1** defined the presence of an ketal (δ_C 100.3), two secondary hydroxyls [δ 3.36 (dd, J=4, 11 Hz), 3.49 (d-like, J=10 Hz); δ_C 77.8 (d), 72.2 (d)], an epoxide [δ 2.91 (d, J=4 Hz), 3.08 (d, J=4 Hz); δ_C 50.5 (t), 65.7 (s)], a 1,2-disubstituted cyclopropane ring [δ 0.17, 0.25 (2H), 0.51], two methoxyls [δ 3.14, 3.19; δ_C 47.8 (q) × 2], and four methyls [δ 0.70, 0.79, 0.95, 1.02 (3H each)]. The COSY and HMQC spectra of **1** revealed four partial structures (fragment A, C-1—C-2; fragment B, C-4—C-9 and C-11—C-12; fragment C, C-14—C-17; fragment D, C-22—C-29). The connectivity of these partial structures was figured out on the basis of the heteronuclear multiple bond connectivity (HMBC) correlations depicted in Fig. 1. For example, the ketal carbon at C-3 was correlated with $OC\underline{H}_3$, H_{ab}-2, and H_b-4. The H_3-19 was correlated with C-1, C-5, and C-9. These correlations defined the A and B ring structure in **1**. The correlations between the H_3-18 proton and C-12, C-13, C-14, and C-17 revealed the connectivity between rings C and D. Furthermore, the connectivity of fragment

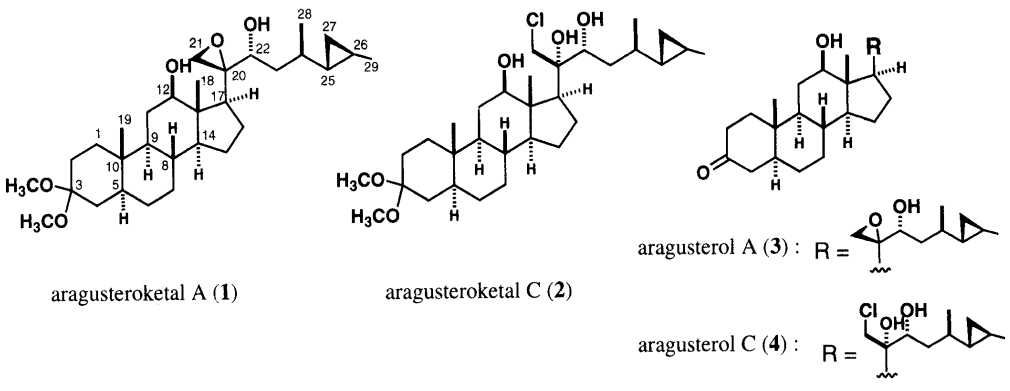

Chart 1

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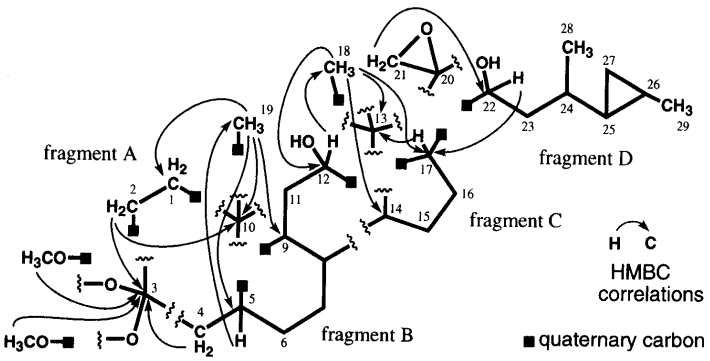

Fig. 1. HMBC Correlations for Aragusteroketal A (**1**)

Table 1. ^{1}H- and ^{13}C-NMR Data for Aragusteroketal A (**1**) and Aragusteroketal C (**2**)

Atom No.	**1**		**2**	
	δ_C [a]	δ [b]	δ_C [a]	δ [b]
1	34.9 (t)	a 1.07 (m), b 1.56 (m)	35.0 (t)	a 1.09 (ddd, 3, 13, 14), b 1.57 (ddd, 3, 3, 13)
2	28.3 (t)	a 1.42 (ddd, 4, 14, 18), b 1.90 (m)	28.3 (t)	a 1.44 (ddd, 4, 14, 14), b 1.88 (m)
3	100.3 (s)		100.3 (s)	
4	35.4 (t)	a 1.28 (m), b 1.65 (m)	35.3 (t)	a 1.31 (m), b 1.63 (m)
5	42.4 (d)	1.30 (m)	42.3 (d)	1.28 (m)
6	28.3 (t)	a 1.20 (m), b 1.28 (m)	28.3 (t)	a 1.20 (m), b 1.29 (m)
7	31.3 (t)	a 0.88 (m), b 1.65 (m)	31.3 (t)	a 0.88 (m), b 1.68 (m)
8	33.8 (d)	1.30 (m)	33.8 (d)	1.32 (m)
9	52.4 (d)	0.82 (m)	52.6 (d)	0.83 (m)
10	35.7 (s)		35.6 (s)	
11	29.3 (t)	a 1.28 (m), b 1.79 (m)	29.7 (t)	a 1.27 (m), b 1.73 (m)
12	77.8 (d)	3.36 (dd, 4, 11)	77.9 (d)	3.43 (dd, 5, 11)
13	48.9 (s)		49.0 (s)	
14	54.5 (d)	1.02 (m)	54.2 (d)	0.94 (m)
15	23.7 (t)	a 1.28 (m), b 1.73 (m)	23.5 (t)	a 1.25 (m), b 1.70 (m)
16	26.8 (t)	a 1.58 (m), b 1.95 (m)	23.5 (t)	a 1.68 (m), b 1.93 (m)
17	48.2 (d)	2.12 (dd, 10, 10)	55.0 (d)	1.92 (m)
18	8.3 (q)	0.70 (s)	8.8 (q)	0.95 (s)
19	11.5 (q)	0.79 (s)	11.5 (q)	0.81 (s)
20	65.7 (s)		76.9 (s)	
21	50.5 (t)	a 2.91 (d, 4), b 3.08 (d, 4)	47.3 (t)	a 3.87 (s), b 3.88 (s)
22	72.2 (d)	3.49 (d-like, 10)	71.3 (d)	3.94 (dd, 11, 5)
23	40.2 (t)	a 1.50 (m), b 1.62 (m)	37.6 (t)	a 1.34 (m), b 1.68 (m)
24	34.9 (d)	0.94 (m)	35.3 (d)	0.98 (m)
25	27.6 (d)	0.25 (m)	27.9 (d)	0.26 (m)
26	12.5 (d)	0.51 (m)	12.3 (d)	0.53 (m)
27	12.4 (t)	a 0.17 (m), b 0.25 (m)	12.5 (t)	a 0.17 (m), b 0.26 (m)
28	18.9 (q)	0.95 [c]	18.8 (q)	0.95 (s)
29	19.1 (q)	1.02 (d, 6)	19.2 (q)	1.02 (d, 6)
OCH_3	47.8 (q)	3.19 (s)	47.5 (q)	3.19 (s)
OCH_3	47.8 (q)	3.14 (s)	47.5 (q)	3.14 (s)

a) 125 MHz in $CDCl_3$; *b*) 500 MHz in $CDCl_3$, *J* value in Hz. *c*) Observed at δ 1.08 (d, 7) in C_5D_5N.

D and the epoxide was defined by the HMBC correlations between H-21 and C-22; H-22 and C-17. Thus, the plane structure of aragusteroketal A has been constructed as **1**, which is presumed to be a 3-dimethylketal analogue of aragusterol A (**3**). This presumption was supported by the following evidence. When aragusteroketal A (**1**) was kept in $CDCl_3$ at room temperature, **1** was gradually converted to aragusterol A (**3**), the physicochemical properties of which were identical with those of the compound isolated from the sponge. From these findings, the absolute stereostructure of aragusteroketal A was confirmed to be **1**.

Aragusteroketal C (**2**), a colorless amorphous solid, gave a quasi-molecular $(M+Na)^+$ ion peak at *m/z* 563 in the FAB-MS, the composition being defined as $C_{31}H_{53}ClNaO_5$ by HR FAB-MS analysis. The ^{1}H- and ^{13}C-NMR spectra of **2** also showed signals ascribable to a ketal (δ_C 100.3), two secondary hydroxyls [δ 3.43 (dd, $J=5$, 11 Hz), 3.94 (dd, $J=5$, 11 Hz); δ_C 77.9 (d), 71.3 (d)], a 1,2-disubstituted cyclopropane ring [δ 0.17, 0.26 (2H), 0.53], two methoxyls [δ 3.14, 3.19; δ_C 47.5 (q) × 2], and four methyls [δ 0.81, 0.95, 0.98, 1.02 (3H, each)]. The NMR spectra of **2** lacked the oxymethylene signal assignable to the epoxide in **1** and showed characteristic signals at δ 3.88 and 3.87 (both 1H, s) and δ_C 47.3 (t), which were similar to those of the chlorohydrin moiety in aragusterol C (**4**). Furthermore, aragusteroketal C (**2**) was gradually converted in $CDCl_3$ to aragusterol C (**4**). Consequently, aragusteroketal C (**2**) has been elucidated to be the 3-dimethylketal analogue of aragusterol C (**4**).

Aragusteroketals A (**1**) and C (**2**) have also been obtained from the acetone extract of the sponge by use of a separation procedure not involving methanol. Aragusteroketals A (**1**) and C (**2**) are rare examples of natural products having a dimethylketal structure. Aragusteroketals A (**1**) and C (**2**) and aragusterols A (**3**) and C (**4**) exhibited potent cytotoxic activities with IC_{50} values of 0.004, 0.004, 0.03, and 0.02 μg/ml against KB cell, respectively.

Experimental

The IR spectra were obtained with a JASCO FT-IR 5300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL GX-500 spectrometer and with TMS as the internal standard. The FAB-MS were recorded on a JEOL JMS SX-102 mass spectrometer.

Extraction and Isolation The frozen sponge of *Xestospongia* sp. (5 kg), which was collected in July, 1993 off Iriomote Island, Okinawa, was initially steeped in acetone. The residue obtained by evaporation of the solvent under reduced pressure was partitioned into EtOAc–H_2O mixture (1 : 1), and the EtOAc layer was taken and evaporated to give the EtOAc-soluble portion (15 g). The EtOAc-soluble portion (6 g) was separated by SiO_2 column (*n*-hexane : EtOAc = 3 : 1) chromatography to give the cytotoxic fraction (750 mg). This fraction was further separated on an SiO_2 column ($CHCl_3$: MeOH : H_2O = 100 : 3 : 1, lower phase) to give fractions A (109 mg) and B (310 mg). Fraction A (109 mg) was then separated by SiO_2 column (*n*-hexane : acetone = 4 : 1) chromatography and reversed-phase HPLC [Cosmosil 5C_{18} AR 10 × 250 mm, flow rate 3 ml/min; eluent MeOH–H_2O–CH_2Cl_2 (500 : 100 : 6)] to afford aragusteroketal A (**1**, 5 mg) and aragusterol A (**3**, 17 mg). Fraction B (310 mg) was also separated by SiO_2 column (*n*-hexane : acetone = 4 : 1) chromatography and reversed-phase HPLC [Cosmosil 5C_{18} AR 10 × 250 mm, flow rate 4 ml/min; eluent MeOH–H_2O–CH_2Cl_2 (900 : 100 : 10)] to give aragusteroketal C (**2**, 17 mg) and aragusterol C (**4**, 119 mg).

Aragusteroketal A (**1**): Amorphous solid, $[\alpha]_D$ +25.3° (c = 0.12, $CHCl_3$, 25 °C). IR ν_{max} (KBr): 3300, 2945 cm^{-1}. FAB-MS (LiCl) *m/z*: 511 $(M+Li)^+$. HR FAB-MS *m/z*: Calcd for $C_{31}H_{52}LiO_5$: 511.3975. Found: 511.3957. ^{1}H-NMR (500 MHz, $CDCl_3$, δ), ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): as shown in Table 1. HMBC correlations of **1**: C-1/H_a-2, H_3-19; C-3/H_{ab}-2, OC$\underline{H}_3$ × 2, H_b-4; C-4/H_b-2; C-5/H_b-6, H_3-19; C-8/H_b-11; C-9/H-8, H_{ab}-11, H_3-19; C-12/H_b-11, H_3-18; C-13/H-17, H_3-18; C-14/H_a-15, H_3-18; C-17/H_3-18, H-22; C-18/H-12, H-14; C-20/H_{ab}-21; C-22/H_{ab}-21; C-23/H_3-28; C-24/H_{ab}-23, H-25, H-26, H_{ab}-27, H_3-28; C-25/H_{ab}-27, H_3-28, H_3-29; C-26/H_a-27, H_3-29; C-28/H_{ab}-23; C-29/H-25, H_{ab}-27.

Aragusteroketal C (**2**): Amorphous solid, $[\alpha]_D$ +8° (c = 1.5, $CHCl_3$, 25 °C). IR ν_{max} (KBr): 3289, 2947 cm^{-1}. FAB-MS *m/z*: 565, 563 $(M+Na)^+$. HR FAB-MS *m/z*: Calcd for $C_{31}H_{52}{}^{37}ClNaO_5$: 565.3450 and $C_{31}H_{52}{}^{35}ClNaO_5$: 563.3479. Found: 565.3446, 563.3473. ^{1}H-NMR (500 MHz, $CDCl_3$, δ), ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): as shown in Table 1. HMBC correlations of **2**: C-1/H_a-2, H_3-19; C-3/H_b-1, H_{ab}-2, OC$\underline{H}_3$ × 2; C-5/H_{ab}-4, H_3-19; C-8/H_b-7; C-9/H-8, H_{ab}-11, H_3-19; C-10/H_{ab}-1, H_b-11, H_3-19; C-12/H_{ab}-11; C-13/H_b-11, H_b-15, H_3-18; C-14/H-8, H_3-18; C-17/H_3-18, H_{ab}-21; C-18/H-12; C-20/H-17, H_{ab}-21; C-22/H_{ab}-21; C-23/H-22, H_3-28; C-24/H-22, H_{ab}-27, H_3-28; C-25/H_{ab}-27, H_3-29; C-26/H-25, H_{ab}-27, H_3-29; C-28/H_a-23; C-29/H_{ab}-27.

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References and Notes

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