Tetrahedron Letters, Vol. 34, No. 39, pp. 6277-6280, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

# Aragusterol A: A Potent Antitumor Marine Steroid from the Okinawan Sponge of the Genus, *Xestospongia*

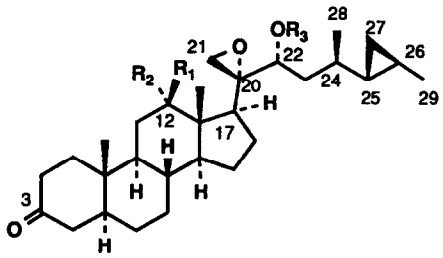
**Kazuo Iguchi,* Michinari Fujita, Hiroto Nagaoka, Hidemichi Mitome, and Yasuji Yamada***

Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan

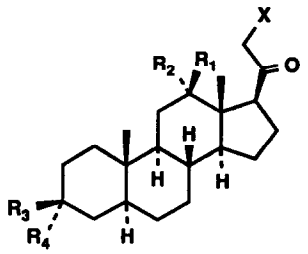
***Abstract:*** Aragusterol A, a new marine steroid possessing potent antitumor activity, was isolated from the Okinawan sponge of the genus *Xestospongia* and its structure was determined by spectroscopic analysis and chemical evidence. The compound strongly inhibited the cell proliferation of KB, HeLaS3, P388 and LoVo cells *in vitro*, and also showed potent *in vivo* antitumor activity toward P388 in mice and L1210 in mice.

While conducting research on the biologically active substances of Okinawan marine invertebrates, the authors isolated a new steroid, aragusterol A,[1] possessing potent antitumor activity from the sponge of the genus, *Xestospongia*. This compound strongly inhibited the cell proliferation of KB, HeLaS3, P388 and LoVo cells *in vitro* at $IC_{50}$ 0.042, 0.16, 0.022 and 0.0079 μg/ml, respectively. It also showed potent *in vivo* antitumor activity toward P388 in mice (T/C 172%, at 6.25 mg/kg) and L1210 in mice (T/C 220%, at 1.6 mg/kg).[2] This paper describes the full structure of aragusterol A based on spectroscopic analysis and chemical evidence.

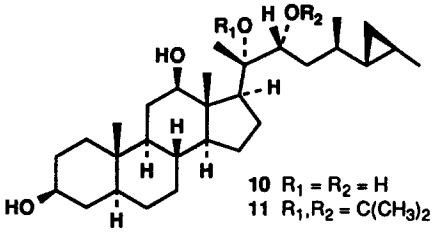
The MeOH extract of wet specimens of the sponge,[3] collected on the coral reef of Aragusuku Island (Okinawa, Japan), was partitioned between EtOAc and $H_2O$. Repeated chromatographic separation of the EtOAc soluble portion gave aragusterol A[4] (**1**, $C_{29}H_{46}O_4$, 0.81% yield based on the EtOAc soluble portion). IR, $^1H$ and $^{13}C$ NMR spectra[4] demonstrated the presence of a ketone, two secondary hydroxyls, an epoxide, a cyclopropane and four methyls. Acetylation of **1** with $Ac_2O$ in pyridine gave only monoacetate **2** ($[\alpha]_D$ +28.2°),[5] showing one of the two secondary hydroxyl groups to be located at the hindered position. The structure of the steroidal nucleus of **1** was determined by the following chemical conversion. PCC oxidation of **1** gave diketone **3** ($[\alpha]_D$ +63.6°), chlorodiketone **6** ($[\alpha]_D$ +32.0°) and chlorotriketone **7** ($[\alpha]_D$ +70.0°) in 31%, 16% and 11% yields, respectively. Reduction of **7** with zinc dust in AcOH quantitatively gave triketone **8** ($[\alpha]_D$ +135.8°), which was shown to be identical with **8**[6] obtained by treating known diketone **9**[7] with methanolic $K_2CO_3$ followed by PCC oxidation. The formation of **6** and **7** can be explained as due to opening of the epoxide ring of the initially formed 22-keto epoxide by attack of chloride anion at C21 followed by cleavage of the bond between C20 and C22 of the resulting β-chloro-α-hydroxy ketone. This reaction also suggested the presence of the epoxide at C20 and C21, and a secondary hydroxyl group at C-22 on the side chain. The structure of the side chain was further elucidated by the following chemical conversion. $LiAlH_4$ reduction of **1** gave tetraol **10** ($[\alpha]_D$ +7.3°) which was converted to acetonide **11** ($[\alpha]_D$ +11.4°) by treatment with dimethoxypropane in the presence of PPTS in DMF and acetone. $H_5IO_6$ oxidation of **10** followed by $NaBH_4$



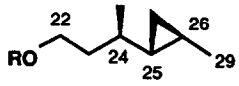
1 $R_1$ = OH, $R_2$ = $R_3$ = H aragusterol A
2 $R_1$ = OH, $R_2$ = H, $R_3$ = Ac
3 $R_1$,$R_2$ = O, $R_3$ = H
4 $R_1$ = OH, $R_2$ = H, $R_3$ = *(R)*-MTPA
5 $R_1$ = OH, $R_2$ = H, $R_3$ = *(S)*-MTPA



6 $R_1$ = OH, $R_2$ = H, $R_3$, $R_4$ =O, X = Cl
7 $R_1$,$R_2$ = $R_3$,$R_4$ = O, X = Cl
8 $R_1$,$R_2$ = $R_3$,$R_4$ = O, X = H
9 $R_1$, $R_2$ = O, $R_3$ =OAc, $R_4$ = X = H



10 $R_1$ = $R_2$ = H
11 $R_1$,$R_2$ = $C(CH_3)_2$



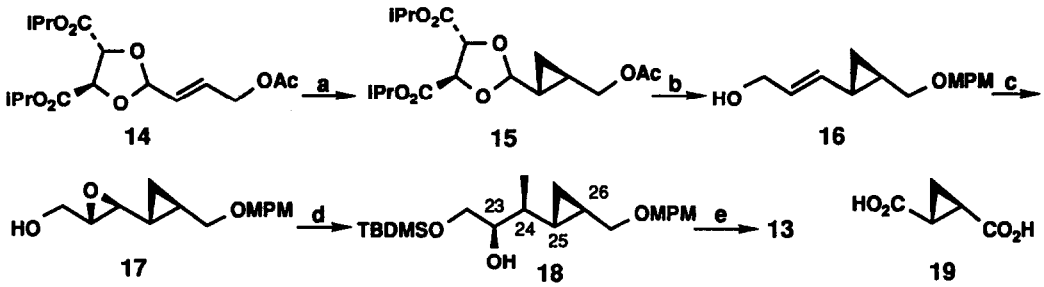
12 R = H
13 R = $COC_6H_4(pNO_2)$

reduction gave alcohol **12** as a side chain fragment which was converted to its *p*-nitrobenzoate **13** ($[\alpha]_D$ -25.1°),[8] clarifying the structure from C22 to C29 involving a methyl at C24 and a cyclopropyl at C25, C26 and C27. These chemical evidences coupled with analysis of correlations observed in the 2D NMR spectra (HOHAHA, $^{13}C$-$^1H$ COSY, COLOC and INADEQUATE) led unambiguously to the planar structure of **1**.

The stereochemistry of chiral centers on the nucleus of **1** except for C12 bearing a secondary hydroxyl group was established based on the above-mentioned chemical correlation of triketone **8** with **9** derived from hecogenin[9] (complete structure already established). The β configuration of the hydroxyl at C12 was determined from $^1H$ NMR coupling constants ($J$ = 4.6, 11.0 Hz) of H12 [δ 3.39 (dd)] and NOE correlation between H12 and H14. The configuration at C22 bearing a secondary hydroxyl group was shown to be *R* by the modified Mosher's method[10]; *(R)*-(+)-MTPA ester **4** and *(S)*-(-)-MTPA ester **5** were prepared from **1** and Δδ (Δ of **5** - Δ of **4**) was calculated by comparing $^1H$ NMR spectra.[11] The *R* configuration at C20 was indicated by NOE correlation between H21 [δ 1.38 (3H, s)] and H22 [δ 3.77 (1H, dd)] of acetonide **11**. The relative stereochemistry of the cyclopropane moiety was derived from the NOE correlation between H25 and H29. However determination of the relative stereochemistry of the remaining chiral center at C24 was difficult from the analysis of NOE correlations. Relative and absolute stereochemistries at C24, C25 and C26 were thus determined by the synthesis of fragment **13** and its enantiomer **23**.[12]

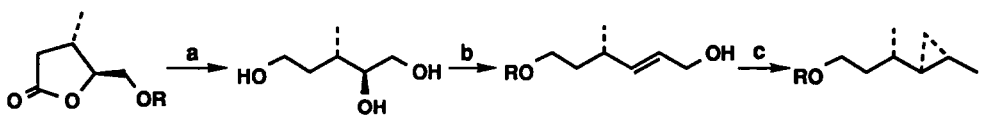
Compound **13** was synthesized as shown in Scheme I. Stereoselective cyclopropanation of **14**[13] ($[\alpha]_D$ -23.6°) using asymmetric Simmons-Smith reaction ($CH_2I_2$, $Et_2Zn$)[14] gave **15** ($[\alpha]_D$ -50.9°) in 86% yield. Absolute configurations at C25[15] and C26 in **15** were confirmed by converting **15** into dicarboxylic acid **19**[16,17] ($[\alpha]_D$ -233°; lit.[16] $[\alpha]_D$ -247°). Compound **15** was converted to allylic alcohol **16** ($[\alpha]_D$ -57.5°) followed by Sharpless epoxidation to give epoxide **17** in 32% overall yield from **15**. Treatment of **17** with

***Scheme I***




***Reagents***: (a) $CH_2I_2$, $Et_2Zn$, hexane, -20°C. (b) i) TsOH, MeOH-$H_2O$; ii) $(EtO)_2P(O)CH_2CO_2Me$, $K_2CO_3$; iii) TBDMSCl, imidazole; iv) DIBAH, $CH_2Cl_2$, -78°C; v) TBDPSCl, imidazole; vi) AcOH-THF-$H_2O$ (2:1:1); vii) MPMBr, NaH; viii) $Bu_4NF$, THF. (c) $tBuO_2H$, L-(+)-DIPT, $Ti(OiPr)_4$, $CH_2Cl_2$, -20°C. (d) i) $Me_3Al$, $CH_2Cl_2$, 0°C; ii) TBDMSCl, $Et_3N$. (e) i) BzCl, pyridine; ii) AcOH-THF-$H_2O$ (2:1:1); iii) PDC, DMF; iv) $CH_2N_2$; v) $SmI_2$, HMPA, MeOH, THF; vi) DDQ; vii) $CBr_4$, $Ph_3P$, $CH_2Cl_2$; viii) $LiBHEt_3$, THF; ix) *p*-$NO_2C_6H_4COCl$, pyridine.

***Scheme II***




***Reagents***: (a) i) $LiAlH_4$, THF, 0°C; ii) AcOH-$H_2O$ (4:1). (b) i) $Pb(OAc)_4$,$K_2CO_3$, $C_6H_6$; ii) $Ph_3P{=}CHCO_2Me$, $C_6H_6$; iii) TBDPSCl, imidazole; iv) DIBAH, $CH_2Cl_2$, -78°C. (c) i) $CH_2I_2$, $Et_2Zn$, hexane, -20°C to room temperature; ii) $CBr_4$, $Ph_3P$, $CH_2Cl_2$; iii) $LiAlH_4$, $Et_2O$, THF; iv) $Bu_4NF$, THF; v) *p*-$NO_2C_6H_4COCl$, pyridine.

$Me_3Al$[18] and then protection of the primary hydroxyl group gave **18** ($[\alpha]_D$ -12.4°) and its diastereomer at C24 in 56% and 27% overall yields, respectively.[19] Compound **18** was converted to *p*-nitrobenzoate **13** ($[\alpha]_D$ -27.0°) which was shown identical with **13** from the natural product. By this synthesis, the absolute configurations (25*R* and 26*R*) of the chiral centers on the cyclopropyl moiety were established, but the absolute configuration at C24 remained ambiguous.[19] Synthesis starting from the compound possessing definite stereochemistry at this chiral center was thus required, and compound **23** was synthesized using lactone **20** as the starting material initially on hand. As shown in Scheme II, lactone **20**[20] prepared from D-mannitol was reduced with $LiAlH_4$ followed by treatment with 80% AcOH to give triol **21** ($[\alpha]_D$ -11.3°), which was converted to allylic alcohol **22** ($[\alpha]_D$ +8.2°) in 61% overall yield from **20**. Cyclopropanation of **22** with $CH_2I_2$ and $Et_2Zn$ gave two diastereomers in about a 1:1 ratio. Each compound was converted to the corresponding *p*-nitrobenzoate, **23** and its diastereomer having different configurations at C25 and C26 in 23% and 10% overall yields from **22**, respectively. The physical properties of **23** were observed to be identical with those of **13** except for the sign of the optical rotation ($[\alpha]_D$ +24.1°). From above synthetic evidences, the 24*R* configuration of **1** was established.

Steroids with a 26-methyl-26,27-cycloergostane skeleton are very rare.[22]

**Acknowledgment.** We thank Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam, for identification of the sponge. We also thank Dr. S. Nakaike, Research Center, Taisho Pharmaceutical Co., Ltd., for measurement of biological activity.

## References and Notes


1. The compound was named after the island, Aragusuku. The sponge was collected on the coral reef near the island.
2. The biological activity of aragusterol A has been investigated by Dr. S. Nakaike and his coworkers in Taisho Pharmaceutical Co., Ltd. The details on the biological activity will be described in a separate paper.
3. The sponge was identified as the genus *Xestospongia* by Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam. Specimens are on deposit in his collection (registered number: ZMA Por.7842).
4. **1**; colorless needles, mp 157-158°C; $[\alpha]_D$ +37.6° (*c* 1.06, $CHCl_3$); IR (KBr) 3490, 3394, 1708 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$, *J* in Hz) δ 0.16 (1H, m, H27), 0.26 (2H, m, H25, H27), 0.50 (1H, heptet, *J* = 5.9, H26), 0.73 (3H, s, H18), 0.95 (3H, s, H28, the signal was found to be a doublet at δ 1.00 ppm (*J* = 6.5 ) in $C_6D_6$), 0.96 (1H, m, H24), 1.01 (3H, s, H19), 1.02 (3H, d, *J* = 6.1, H29), 1.07 (1H, dd, *J* = 7.8, 11.0, H17), 1.71 (1H, qd, *J* = 3.1, 13.1, H7), 1.79 (1H, td, *J* = 4.6, 13.0, H11), 2.03 (1H, ddd, *J* = 2.2, 6.5, 14.0, H1), 2.09 (1H, ddd, *J* = 2.1, 3.8, 15.0, H4), 2.16 (1H, dd, *J* = 9.0, 10.8, H14), 2.25 (1H, dd, *J* = 13.6, 15.0, H4), 2.31 (1H, br d, *J* = 13.5, H2), 2.37 (1H, dt, *J* = 6.5, 13.5, H2), 2.92 (1H, d, *J* = 3.9, H21), 3.08 (1H, d, *J* = 3.9, H21), 3.39 (1H, dd, *J* = 4.6, 11.0, H12), 3.46 (1H, dd, *J* = 2.4, 10.6, H22); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 8.2 (C18), 11.4 (C19), 12.4 (C27), 12.5 (C26), 18.9 (C28), 19.1 (C29), 23.7 (C15), 26.8 (C16), 27.6 (C25), 28.8 (C6), 29.4 (C11), 31.0 (C7), 33.7 (C8), 34.9 (C24), 35.6 (C10), 38.1 (C2), 38.4 (C1), 40.1 (C23), 44.5 (C4), 46.5 (C5), 48.1 (C14), 48.8 (C13), 50.6 (C21), 52.2 (C9), 54.3 (C17), 65.8 (C20), 72.2 (C22), 77.4 (C12), 211.8 (C3).
5. All optical rotation data were obtained in $CHCl_3$ solution, except for **19** (in $H_2O$).
6. **8**; $[\alpha]_D$ +158.7°. Somewhat small $[\alpha]_D$ value of **8** from **1** may be attributed to observation error in the measurement caused by its small amount.
7. Mueller, G. P. ; Stobaugh, R. E.; Winniford, R. S. *J. Am. Chem. Soc.* **1953**, *75*, 4888-4892.
8. **13**; $^1H$ NMR (400 MHz, $CDCl_3$, *J* in Hz) δ 0.18 (1H, m), 0.23 (2H, m), 0.50 (1H, m), 0.93 (1H, m), 1.02 (3H, d, *J* = 6.6), 1.03 (3H, d, *J* = 6.0), 1.85 (2H, br q, *J* = 7.0), 4.47 (1H, td, *J* = 7.0, 10.9), 4.48 (1H, td, *J* = 7.0, 10.9), 8.20 (2H, d, *J* = 8.9), 8.28 (2H, d, *J* = 8.9).
9. Marker, R. E. ; Wagner, R. B.; Ulshafer, P. R.; Wittbecker, E. L.; Goldsmith, D. P. J.; Ruof, C. H. *J. Am. Chem. Soc.* **1947**, *69*, 2167-2230: Mazur, Y.; Danieli, N.; Sondheimer, F. *J. Am. Chem. Soc.* **1960**, *82*, 5889-5908.
10. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092-4096.
11. Δδ values (Δ of **5** - Δ of **4**): -49 Hz (H21), -40 Hz (H21), -8 Hz (H-18), +31 Hz (H24), +24 Hz (H25), +11 Hz (H28), +4 Hz (H29). These values clearly indicated the *R* configuration at C22.
12. Structural determination using X-ray crystallographic analysis was not possible, since crystals of **1** as well as several of its derivatives were inadequate for this purpose.
13. Compound **14** was prepared from 2-butene-1,4-diol by a three-step sequence in 56% overall yield: i) $Ac_2O$, pyridine, to give monoacetate; ii) PCC, $CH_2Cl_2$; iii) diisopropyl L-(+)-2,3-*O*-bis(trimethylsilyl)-tartrate, TMSOTf, $CH_2Cl_2$.
14. Arai, I.; Mori, A.; Yamamoto, H. *J. Am. Chem. Soc.* **1985**, *107*, 8254-8256.
15. Numbering is in accordance with that for aragusterol A (**1**).
16. Inouye, Y.; Sugita, T.; Walborsky, H. M. *Tetrahedron* **1964**, *20*, 1695-1699.
17. Compound **15** was converted to **19** by treatment with TsOH followed by Jones oxidation.
18. Suzuki, T.; Saimoto, H.; Tomioka, H.; Oshima, K.; Nozaki, H. *Tetrahedron Lett.* **1982**, *23*, 3597-3600.
19. The major product **18** was initially predicted to have the methyl group with an α configuration at C24, based on the $S_N2$ type reaction path for stereoselective methylation using $Me_3Al$.[18] However the formation of a significant amount of the diastereomer at C24 suggested the $S_N1$ type reaction path for the present methylation, throwing a doubt on the stereochemistry at C24. The β configuration of the methyl group in **18** was later established by the synthesis of **23**.
20. Lactone **20** was stereoselectively synthesized by methylation of (*S*)-5-(*tert*-butyldimethylsilyloxy)-2-penten-4-olide[21] (derived from D-mannitol) with lithium dimethylcuprate in $Et_2O$ at -78°C in 54% yield. Similar stereoselective reactions were reported. For example: Hanessian, S.; Murray, P. J.; Sahoo, S. P. *Tetrahedron Lett.* **1985**, *26* , 5627-5630: Nagaoka, H.; Iwashima, M.; Abe, H.; Iguchi, K.; Yamada, Y. *Chem. Pharm. Bull.* **1992**, *40*, 1742-1749.
21. Hafele, B; Jager, V. *Liebigs Ann. Chem.* **1987**, 85-87.
22. Mattia, C. A.; Mazzarella, L.; Pulti, R.; Sica, D.; Zollo, F. *Tetrahedron Lett.* **1978**, 3953-3954: Cho, J.-H.; Djerassi, C. *J. Chem. Soc. Perkin Trans. I* **1987**, 1307-1318.


(Received in Japan 21 June 1993)