Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 6, pp. 691-692, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00089-9

SYNTHESIS OF 5-EPIARAGUSTEROL A

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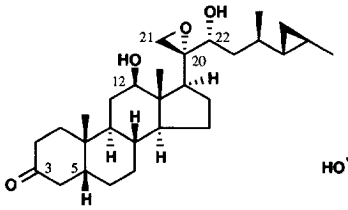
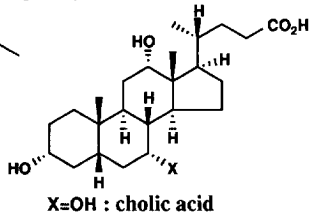
Abstract: Synthesis of 5-epiaragusterol A (**1**) from deoxycholic acid was achieved. 5-Epiaragusterol A (**1**) showed antiproliferative activity against KB cells, which was equivalent to that of aragusterol A.

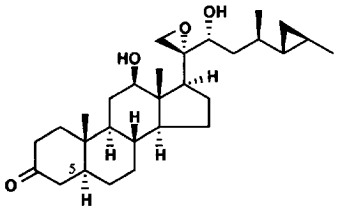
We have previously reported the isolation and structural determination of the antitumor marine steroids aragusterols A-D, from the Okinawan marine sponge genus, *Xestospongia*.[1] Aragusterol A was noted to very strongly inhibit the proliferation of KB cells at IC_{50} 0.042 μg/mL and express potent *in vivo* antitumor activity toward L1210 leukemia in mice (T/C 220% at 1.6 mg/kg).[1a] We have also achieved the synthesis of aragusterols A-D; the method features the enantioselective formation of a side chain segment and stereoselective coupling of a steroid nuclear segment, prepared starting from (+)-hecogenin, with this side chain segment.[2] The large scale synthesis of these marine steroids is difficult owing to the scarcity of (+)-hecogenin in nature. Consequently, A/B *cis* juncture 5-epiaragusterol A was designed and synthesized from bile acid (deoxycholic acid), which is abundantly present in nature.[3] 5-Epiaragusterol A may serve as a substitute for aragusterol A. This paper presents the synthesis of 5-epiaragusterol A and assessment of its biological activity.



5-epiaragusterol A (1)



X=OH : cholic acid
X=H : deoxycholic acid



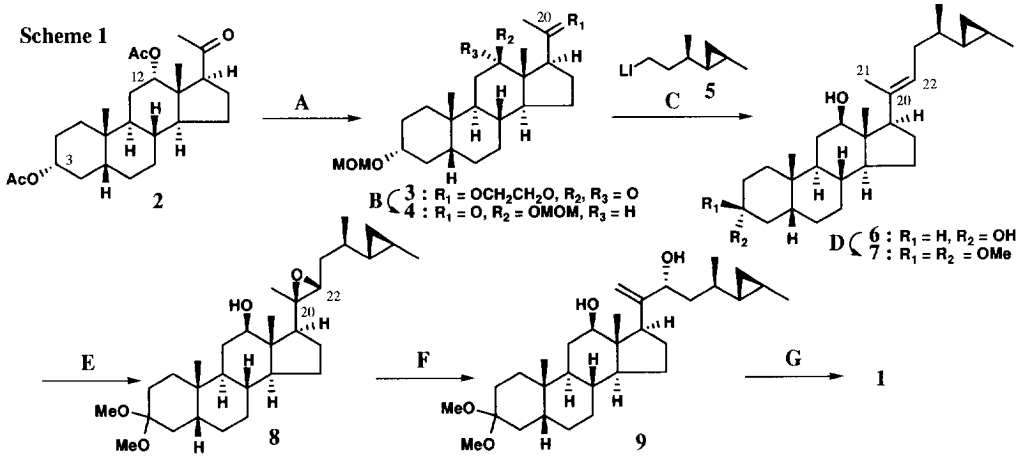
aragusterol A

A/B *cis* juncture steroid **2**[4] was prepared by degradation of the side chain from deoxycholic acid according to the method of Miescher.[5] Steroid **2** was converted to ketone **3** in five steps as follows: i) protection of the ketone as a ketal; ii) regioselective methanolysis of the acetate at the C-3 position; iii) protection of the hydroxyl group as a MOM ether; iv) reductive cleavage of the acetate at the C-12 position and v) oxidation of the hydroxyl group. Reduction of the ketone at the C-12 position in **3** with L-Selectride® afforded β-alcohol as the sole product, and hydrolysis of the ketal and protection of the hydroxyl group as a MOM ether afforded 20-oxo steroid **4**. Reaction of 20-oxo steroid **4** with alkyllithium **5**[2] in THF at -78°C, followed by treatment with conc. HCl gave 20(22)*E* olefin **6** and the 20(22)*Z* isomer in a 7:1 ratio accompanied by the 20(21) isomer formation in trace amount. Alcohol **6** was converted to ketal **7** in the following five steps: i)

acetylation of two hydroxyl groups; ii) regioselective methanolysis of the acetate at the C-3 position; iii) PCC oxidation of the hydroxyl group; iv) protection of the ketone as a ketal and v) reductive cleavage of acetate. Epoxidation of **7** with ^{t}BuOOH in the presence of $VO(acac)_2$[6] gave (20*R*, 22*R*) epoxide **8** along with (20*S*, 22*S*) isomer in a 3:1 ratio. Epoxide **8** was converted to allylic alcohol **9** by treatment with $^i Pr_2NMgBr$,[7] prepared from diisopropylamine and methylmagnesium bromide. The stereoselective epoxidation of allylic alcohol **9** with *m*CPBA at 0°C in the presence of Na_2HPO_4, followed by acid hydrolysis of the ketal with AcOH-H_2O afforded 5-epiaragusterol A (**1**).[8]

5-Epiaragusterol A (**1**) showed strong antiproliferative activity at IC_{50} 0.041 μg/mL toward KB cells, which was comparable to that of aragusterol A. It is thus shown that 5-epiaragusterol A (**1**) may be used in place of aragusterol A as an antitumor steroid.

Scheme 1



Reagents and conditions: A. i) $HOCH_2CH_2OH$, TsOH, benzene, reflux, 92%, ii) K_2CO_3, MeOH, rt, 84%, iii) MOMCl, $^i Pr_2NEt$, $ClCH_2CH_2Cl$, rt, 96%, iv) $LiAlH_4$, Et_2O, 0°C, 99%, v) PCC, 4Å MS, $ClCH_2CH_2Cl$, rt, quant.; **B**. i) L-Selectride®, THF, 0°C, 77%, ii) AcOH:H_2O (4:1), rt, iii) MOMCl, $^i Pr_2NEt$, $ClCH_2CH_2Cl$, rt, 84% (2 steps); **C**. i) **5**, THF, -78°C, ii) conc. HCl, MeOH, reflux, 69% (2 steps); **D**. i) Ac_2O, Py, DMAP, rt, ii) K_2CO_3, MeOH, rt, quant. (2 steps), iii) PCC, 4Å MS, CH_2Cl_2, rt, 99%, iv) $(MeO)_3CH$, TsOH, MeOH, rt, 99%, v) $LiAlH_4$, THF, 0°C, quant.; **E**. TBHP, $VO(acac)_2$, benzene, rt, 67%; **F**. $^i Pr_2NMgBr$, THF, rt, 98%; **G**. i) *m*CPBA, Na_2HPO_4, CH_2Cl_2, 0°C, ii) AcOH:H_2O (4:1), rt, 66% (2 steps).

Acknowledgments: The authors express their appreciation to Taisho Pharmaceutical Co., Ltd. for biological activity measurement. This work was supported in part by a Grant-in-Aid for Scientific Research (Grant No. 07274104) from the Ministry of Education, Science and Culture of Japan.

References and Notes

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3. As a result of having 12βOH take the form 12αOH the activity of aragusterol A was noted to be reduced (unpublished data).
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8. **1**: colorless powder, mp 66-67 °C; $[\alpha]_D^{26}$ +23.1° (*c* 0.1, $CHCl_3$); IR (KBr) 3402, 2949, 1708 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.16 (1H, m), 0.25 (2H, m), 0.50 (1H, heptet, J = 6.0 Hz), 0.73 (3H, s), 0.94 (3H, br s), 1.01 (3H, d, J = 5.6 Hz), 1.01 (3H, s), 2.30 (1H, dt, J = 5.3, 14.6 Hz), 2.61 (1H, t, J = 14.2 Hz), 2.92 (1H, d, J = 3.9 Hz), 3.07 (1H, d, J = 3.9 Hz), 3.46 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.2, 12.4, 12.5, 18.9, 19.1, 22.5, 23.7, 25.2, 26.5, 26.9, 27.5, 29.1, 33.8, 34.8, 34.9, 36.9, 37.0, 39.2, 40.1, 42.2, 43.9, 48.1, 49.0, 50.6, 54.6, 65.8, 72.2, 77.6, 213.0; FABMS *m/z*: 497(M^++K); HRFAB M^++K *m/z* obsd 497.3029, $C_{29}H_{46}O_4K$ required 497.3033.

(*Received in Japan* 6 *January* 1997; *accepted* 6 *February* 1997)