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First synthesis of 3,16,20-polyoxygenated cholestanes, new cytotoxic steroids from the gorgonian *Leptogorgia sarmentosa*

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ABSTRACT

Using tigogenin as starting material, (20*S*)-20-hydroxycholestane-3,6-dione (**1**), (16*S*, 20*S*)-16,20-dihydroxycholestan-3-one (**2**), (20*S*)-20-hydroxycholest-1-ene-3,16-dione (**3**) and (20*S*)-20-hydroxycholest-4-ene-3,16-dione (**4**), natural polyoxygenated steroids from the gorgonian, *Leptogorgia sarmentosa*, were synthesized in four steps. Antitumor activity against three tumor cell lines (breast cancer, MCF7, lung cancer NCI and oral cancer KB) was evaluated. Two compounds (**3** and **4**) showed strong activity against NCI (IC_{50} 6.16 and 10.51 μM) and moderate activity against MCF7 and KB, the IC_{50} being in the range 30.65–47.22 μM. Compound **2** showed moderate activity against NCI (IC_{50} 42.68 μM) but was inactive against MCF7 and KB whereas compound **1** showed no activity against all tested cells.

1. Introduction

For many years marine organisms have become increasingly recognized as important sources of steroid metabolites many of which are novel compounds and some of which are extremely toxic against tumor cells [1,2]. A series of polyoxygenated steroids that uncommonly present oxidation both at C-16 and C-20 of the cholestane nucleus have been isolated from the gorgonian, *Leptogorgia sarmentosa* [3–5] and similar cholestanes have also been obtained from the anthozoen, *Antipathies subpinnala* [6,7]. Recently three new steroids of this type (**1–3**) together with the known steroid (**4**) (Fig. 1) were isolated from the gorgonian, *L. sarmentosa* [8]. Compounds **1** and **2** and a fraction that contained **3** as the major component have been reported to exhibit cytotoxicity against P-388 of mouse lymphoid neoplasma human lung carcinoma (A 549), human colon carcinoma (HTG) and human melanoma (MEL 28) with ED_{50} value of 1 μg/ml in all cases.

The fact that insufficient amounts of these compounds were available for further pharmacological studies, coupled with the need to evaluate the mechanism of action, prompted us to undertake the synthesis of all of them.

2. Experimental

2.1. General

Reactions were monitored by TLC on aluminum sheets SIL G/UV254 from Merck. Chromatographic plates were sprayed with vanillin solution and heated until color developed. Melting points were determined on an electro thermal SMP-10 apparatus and are uncorrected. Infrared spectra were recorded on a PerkinElmer 2000 Fourier transform infrared spectrophotometer. The NMR spectra were recorded in $CDCl_3$ on a Bruker Advance DPX-400 spectrometer operating at 400 MHz (proton)

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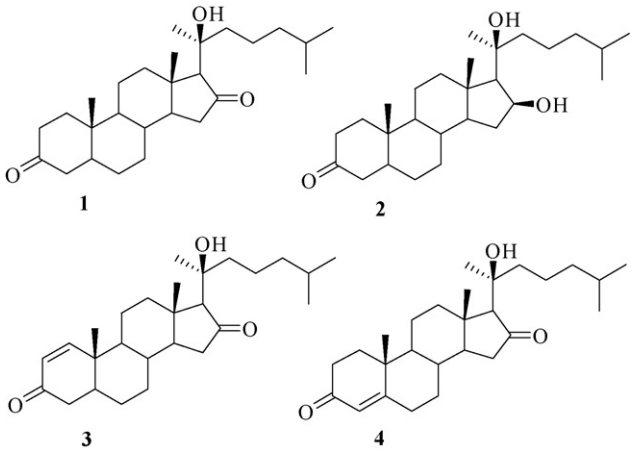

Fig. 1 – Steroids isolated from *Leptogorgia sarmentosa*.

and 100 MHz (carbon-13); the chloroform peak was used as standard. Chemical shifts are expressed in ppm and coupling constants (*J*) in Hz. Mass spectra were obtained on an Agilent Technology 1100 series LL/MSD Trap; the first number denotes *m/z* value and the ion assignment and abundance are given in parentheses. Tigogenin was isolated from the waste of *Agave sisalana* leaves [9]. All chemicals and solvents were purchased from the Fluka Co. as analytical grade and solvents were purified by general methods before being used. *m*-Iodoxybenzoic acid was prepared as described by Vogel [10] and Barton [11].

2.2. *Synthetic procedures*

2.2.1. *3β-Acetoxy-16β-γ-acetoxymethylvaleroyloxy-5α-pregnan-20-one* (6)

A mixture of tigogenin (1 g, 2.4 mmol), acetic anhydride (17 ml), ammonium chloride (256 mg, 4.8 mmol) and pyridine (1.4 ml) was heated to 135 °C and kept at that temperature for 16 h. After cooling down the reaction mixture, acetic acid (2 ml), 1,2-dichloroethane (95 ml) and water (0.5 ml) were added and the mixture cooled to 0 °C. A solution of chromium trioxide (424 mg) in water (0.6 ml) and acetic acid (0.2 ml) was added dropwise and the mixture then stirred at room temperature until the reaction was completed. Then a solution of sodium chloride (480 mg) in water (7.2 ml) and methanol (0.1 ml) was introduced and the mixture was stirred for 1 h. The reaction mixture was extracted with methylene chloride and the organic phase was washed with water and dried over anhydrous sodium sulphate. The residue from removal of the solvent in vacuo was purified by flash column chromatography (7:3; ethyl acetate:hexane) to provide **6** (580 mg, 45%) as a white solid, m.p. 97–98 °C [lit. 99–100 °C] [12] IR (KBr) υ_{max} 1749, 1739, 1733, 1717 cm^{-1}. ^1H NMR ($CDCl_3$): 0.62 (m, 1H, H-14), 0.77 (s, 3H, H-18), 0.84 (m, 2H, H-1, H-9), 0.85 (d, *J* = 6.7 Hz, 3H, H-26), 0.94 (m, 1H, H-12), 0.95 (s, 3H, H-19), 1.10 (m, 1H, H-5), 1.22 (m, 2H, H-6), 1.24 (m, 1H, H-8), 1.38 (m, 1H, H-23), 1.40 (m, 1H, H-11), 1.50 (m, 3H, H-2, H-4), 1.56 (m, 1H, H-24), 1.60 (m, 1H, H-11), 1.62 (m, 2H, H-2, H-23), 1.65 (m, 1H, H-1), 1.68 (m, 1H, H-25), 1.70 (m, 1H, H-12), 1.80 (m, 1H, H-24), 1.95 (s, 3H, H-21), 1.98 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.0 (m, 2H, H-7), 2.32 (d, *J* = 7.6 Hz, 1H, H-17), 2.34 (m, 2H, H-15), 3.80 (d, *J* = 6.1 Hz, 2H, H-27), 4.61 (m, 1H, H-3), 5.43 (dt, *J* = 7.7, 4.34 Hz, 1H, H-16). ^{13}C NMR ($CDCl_3$): 12.2 (C-18), 13.6 (C-19), 16.4 (C-26), 2.5 (C-11), 20.8 ($CH_3C{=}O$), 21.4 ($CH_3C{=}O$), 27.4 (C-24), 28.2 (C-6), 28.3 (C-23), 30.6 (C-21), 31.7 (C-7), 31.8 (C-15), 31.9 (C-25), 33.9 (C-2), 34.3 (C-8), 35.0 (C-4), 35.5 (C-1), 36.6 (C-12), 38.1 (C-10), 42.5 (C-13), 44.6 (C-5), 53.9 (C-9), 54.2 (C-14), 66.6 (C-17), 68.7 (C-27), 73.5 (C-3), 74.4 (C-16), 17.7 (C=O), 171.1 (C=O), 172.6 (C=O), 205.5 (C=O).

2.2.2. *3,16,20-Trihydroxycholestane* (7)

To a mixture of Mg turnings (338 mg), I_2 (catalytic amount) and dry THF (30 ml) was added 1-bromo-4-methylpentane (1.4 ml, 9.39 mmol) at room temperature under N_2. The mixture was stirred at room temperature for 3 h and then a solution of **6** (1 g, 1.87 mmol) in dry THF (20 ml) was added dropwise at 0 °C. The reaction was stirred at 0 °C for 20 min and then quenched with ice-water followed by neutralization with dilute HCl. The mixture was then extracted with methylene chloride, the combined organic layers were washed with water, dried over anhydrous sodium sulphate, and concentrated in vacuo. The crude product was filtered through a short column (eluting with 3:7; ethyl acetate:hexane) to provide product (**7**) which was used in the next step without further purification.

2.2.3. *(20S)-20-Hydroxycholestane-3,16-dione* (1) *and (16S, 20S)-16,20-dihydroxy-cholestan-3-one* (2)

To a stirred solution of **7** in methylene chloride (50 ml) was added NaOAc (596 mg, 7.27 mmol) and PCC (2 g, 7.97 mmol) at room temperature. After stirring for 3 h, the mixture was filtered through a celite pad. The filtrate was evaporated and purified by flash column chromatography (15:85; ethyl acetate:hexane) to yield **1** (195 mg, 25% from two steps) as a white solid, m.p. 145–146 °C and **2** (1.5 mg, 2.2%) m.p. 172–173 °C.

Compound **1**. IR (KBr) υ_{max} 3440, 1727, 1717 cm^{-1}. ^1H NMR ($CDCl_3$): 0.80 (d, *J* = 6.6 Hz, 6H, H-26, H-27), 0.87 (s, 3H, H-18), 0.98 (s, 3H, H-19), 1.08 (m, 4H, H-7, H-9, H-24), 1.19 (m, 3H, H-21), 1.29 (m, 2H, H-23), 1.32 (m, 1H, H-1), 1.36 (m, 1H, H-14), 1.42 (m, 2H, H-11), 1.43 (m, 1H, H-12), 1.44 (m, 2H, H-22), 1.45 (m, 1H, H-25), 1.47 (m, 2H, H-6), 1.52 (m, 1H, H-8), 1.57 (m, 1H, H-5), 1.59 (m, 1H, H-7), 1.81 (dd, *J* = 18.5, 13.5 Hz, 1H, H-15), 2.04 (m, 1H, H-1), 2.02 (m, 1H, H-12), 2.06 (m, 1H, H-4), 2.13 (s, 1H, H-17), 2.20 (m, 1H, H-15), 2.27 (m, 1H, H-4), 2.32 (m, 1H, H-2), 2.37 (m, 1H, H-2). ^{13}C NMR ($CDCl_3$): 11.4 (C-19), 14.7 (C-18), 21.0 (C-11), 22.4 (C-23), 22.6 (C-26), 22.7 (C-27), 25.4 (C-21), 27.9 (C-25), 28.6 (C-6), 31.6 (C-7), 33.8 (C-8), 35.7 (C-10), 38.0 (C-2), 38.1 (C-1), 39.3 (C-12), 39.4 (C-15), 39.6 (C-24), 42.7 (C-13), 44.3 (C-22), 44.5 (C-4), 46.4 (C-5), 50.7 (C-14), 53.5 (C-9), 71.4 (C-17), 73.9 (C-20), 211.5 (C-3), 221.2 (C-16). CIMS: 417 [$(M+H)^+$, 7], 399 [$(M+H)^+ - H_2O$, 100)], 381, 331 ($M^+ - C_6H_{13}$, 5).

Compound **2**. IR (KBr) υ_{max} 3439, 1700 cm^{-1}. ^1H NMR ($CDCl_3$): 0.66 (m, 1H, H-9), 0.77 (m, 1H, H-14), 0.80 (d, *J* = 6.6 Hz, 6H, H-26, H-27), 0.86 (m, 1H, H-7), 0.96 (s, 3H, H-19), 1.09 (s, 3H, H-18), 1.18 (m, 1H, H-15), 1.13 (m, 2H, H-24), 1.16 (m, 2H, H-12, H-17), 1.21 (s, 3H, H-21), 1.27 (m, 4H, H-6, H-23), 1.28 (m, 1H, H-1), 1.37 (m, 1H, H-11), 1.41 (m, 1H, H-8), 1.42 (m, 1H, H-5), 1.46 (m, 1H, H-11), 1.47 (m, 1H, H-22), 1.48 (m, 1H, H-25), 1.64 (m, 1H, H-7), 1.70 (m, 1H, H-22), 1.96 (m, 1H, H-1), 2.00 (m, 2H, H-4), 2.07 (m, 1H, H-12), 2.17 (m, 1H, H-15), 2.20 (m, 1H, H-2), 2.31 (m, 1H, H-2), 4.51 (m, 1H, H-16). ^{13}C NMR ($CDCl_3$): 11.5 (C-19), 15.0 (C-18), 20.9 (C-23),