Pergamon

Tetrahedron Letters 41 (2000) 6035–6040

TETRAHEDRON LETTERS

New antiproliferative epoxysecosterols from *Pseudopterogorgia americana*

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Received 4 May 2000; revised 5 June 2000; accepted 6 June 2000

Abstract

Two new 9(11)-secogorgosterols containing oxirane rings at the C-5/C-6 position (**1** and **2**) were isolated from *Pseudopterogorgia americana* along with a known secogorgosterol (**3**). The structures were elucidated using detailed spectroscopic studies and the epoxysecosteroids were found to exhibit activity against prostate (LnCap) and lung cancer (Calu-3) cell lines.

Keywords: NMR; mass spectrometery; steroids; anti-tumor compounds and anti-inflammatory compounds.

Pseudopterogorgia americana, an abundant marine gorgonian on reefs in Florida and the Caribbean, produces 9(11)-secosterols and polyoxygenated sterols, which exhibit inhibitory activity against protein kinase C, potent anti-proliferative and promising anti-inflammatory activity.[1–3] Our recent detailed chemical studies on *P. americana* have resulted in the isolation of two new 9(11)-secogorgosterols containing an epoxide ring at the C-5/C-6 position, 1β,3β-dihydroxy-5α,6α-epoxy-9-oxo-9,11-secogorgostan-11-ol (**1**), 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-secogorgostan-11-ol (**2**) and the known 3β-hydroxy-9-oxo-9,11-secogorgostan-11-ol (**3**). Their structures were established on the basis of spectral studies.

Methanol extraction of the freeze-dried gorgonian (88.9 g) yielded a crude extract (26.8 g) which was fractionated by solvent partitioning between water and $CHCl_3$:MeOH (2:1). The organic fraction was subjected to silica gel column chromatography and reversed-phase HPLC to afford two new secosterols with an unusual oxygenation pattern **1** (4.2 mg) and **2** (3.6 mg) along with a known metabolite, secogorgosterol **3** (5.1 mg).[6]

1β,3β-Dihydroxy-5α,6α-epoxy-9-oxo-9,11-secogorgostan-11-ol (**1**), $C_{30}H_{50}O_5$, was isolated as a colorless gum. The UV spectrum showed a terminal absorption, indicating the lack of a

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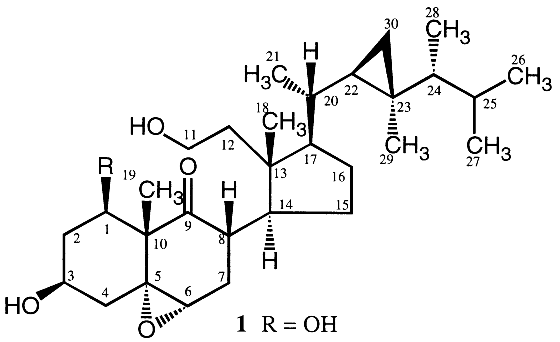
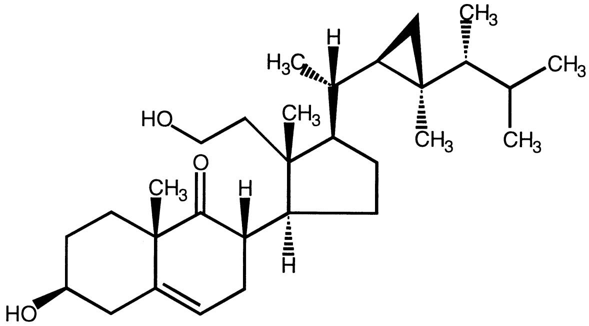
0040-4039/00/$ - see front matter
PII: S0040-4039(00)01015-7



1 R = OH
2 R = H



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conjugated π system. The IR spectrum displayed intense absorption bands at 3317 (O–H), 1710 (C=O), and 1103 (C–O–C) cm^{-1}. The FABMS of **1** showed a molecular ion peak at *m*/*z* 490.3653 which is consistent with the elemental formula $C_{30}H_{50}O_5$ (calcd 490.3658) and indicated the presence of six degrees of unsaturation, ascribed to one carbonyl group, one epoxide ring, and the four secogorgosterol rings. A signal at *m*/*z* 472 ($C_{30}H_{48}O_4$) was ascribed to M^+–H_2O.

The presence of a secogorgosterol derivative was evident from the ^1H, ^{13}C, and COSY data (Table 1). The ^1H NMR spectrum (500 MHz, $CDCl_3$) of **1** featured signals as doublets of doublets at δ –0.14 and 0.45 (J=9.0, 4.5 Hz), integrating for one hydrogen each, which are characteristic signals of the C-30 methylene protons of a cyclopropyl ring, while a two-hydrogen resonance at δ 0.27 was ascribed to the C-22 and C-24 methine protons. These characteristic signals suggested the presence of a cyclopropane system substituted at C-22 and C-23 of the side chain of a secogorgosteroidal skeleton.[4] Three singlets, integrating for three hydrogens each, resonated at δ 0.67, 0.85 and 1.24 were ascribed to hydrogens of the C-18, C-29 and C-19 methyl groups,[4,5] while four three-hydrogen doublets at δ 0.82 (J=6.5 Hz), 0.89 (J=7.0 Hz), 0.91 (J=6.5 Hz) and 1.01 (J=6.5 Hz) were assigned to the protons of C-26, C-28, C-27 and C-21 methyl groups, respectively.[4,5] Another one-proton signal appeared as a broad doublet of triplets at δ 2.81 was diagnostic of the C-8 methine proton,[4,5] while another downfield signal at δ 3.22 (d, J=4.5 Hz) was assigned to the C-6 methine proton. A four-hydrogen multiplet at δ 3.65–3.76 was assigned to the C-1 methine, C-3 methine, and C-11 methylene protons. Their downfield chemical shift values suggested the presence of a geminal oxygen functionality at each above mentioned carbon atom. The chemical shift values of the C-11 methylene protons (δ 3.72–3.76) are characteristic of 9(11)-secosterols with a hydroxyl functionality at C-11.[4–6]

Table 1
^{1}H and ^{13}C NMR chemical shift assignments of **1** and **2**

Carbon No.	**1** ^{1}H-NMR (δ)	Multiplicities (APT)	^{13}C-NMR (δ)	**2** ^{1}H-NMR (δ)	Multilpicities (APT)	^{13}C-NMR (δ)
1	3.65 (m)	CH	69.9	1.79 (m)	CH_2	29.9
				1.39 (m)		
2	2.12 (m)	CH_2	35.0	2.10 (m)	CH_2	34.8
	1.50 (m)			1.45 (m)		
3	3.67 (m)	CH	69.4	3.66 (m)	CH	69.5
4	1.93 (m)	CH_2	40.3	1.90 (m)	CH_2	39.9
	1.45 (m)			1.47 (m)		
5	---	-C-	61.0	---	-C-	60.9
6	3.22 (d,J = 4.5 Hz)	CH	60.4	3.21 (d, J = 4.5 Hz)	CH	60.0
7	2.40 (m)	CH_2	31.8	2.38 (m)	CH_2	32.0
	1.80 (m)			1.76 (m)		
8	2.81 (dt)	CH	41.5	2.80 (dt)	CH	41.6
9	---	-C-	214.1	---	-C-	214.0
10	---	-C-	46.4	---	-C-	46.7
11	3.76 (m)	CH_2	59.1	3.76 (m)	CH_2	59.0
	3.72 (m)			3.71 (m)		
12	1.79 (m)	CH_2	39.4	1.75 (m)	CH_2	39.6
	1.40 (m)			1.41 (m)		
13	---	-C-	45.6	---	-C-	45.5
14	2.60 (m)	CH	40.4	2.66 (m)	CH	40.2
15	1.40 (m)	CH_2	25.8	1.41 (m)	CH_2	26.0
	1.25 (m)			1.23 (m)		
16	1.99 (m)	CH_2	27.7	1.90 (m)	CH_2	27.9
	1.65 (m)			1.64 (m)		
17	1.23 (m)	CH	50.2	1.20 (m)	CH	50.0
18	0.67 (s)	CH_3	17.2	0.66 (s)	CH_3	17.0
19	1.24 (s)	CH_3	17.5	1.24 (s)	CH_3	17.4
20	0.99 (m)	CH	35.0	0.95 (m)	CH	34.9
21	1.01 (d, J = 6.5Hz)	CH_3	20.6	1.00 (d, J = 6.6 Hz)	CH_3	20.8
22	0.27 (d, J = 9.0Hz)	CH	32.0	0.26 (d, J = 9.0 Hz)	CH	31.9
23	---	-C-	25.8	---	-C-	25.9
24	0.27 (d, J = 9.0Hz)	CH	50.7	0.26 (d, J = 9.0 Hz)	CH	50.5
25	1.50 (m)	CH	31.5	1.48 (m)	CH	31.4
26	0.82 (d, J = 6.5Hz)	CH_3	22.2	0.80 (d, J = 6.5 Hz)	CH_3	22.3
27	0.91 (d, J = 6.5Hz)	CH_3	21.4	0.90 (d, J = 6.5 Hz)	CH_3	21.5
28	0.89 (d, J = 7.0Hz)	CH_3	15.3	0.88 (d, J = 7.0 Hz)	CH_3	15.2
29	0.85 (s)	CH_3	14.3	0.84 (s)	CH_3	14.2
30	0.45 (dd, J = 9.0, 4.5 Hz), -0.14 (dd, J = 9.0, 4.5 Hz)	CH_2	21.3	0.44 (dd, J = 9.0, 4.4 Hz), -0.15 (dd, J = 9.0, 4.4 Hz)	CH_2	21.2

The COSY-45° spectrum was used for the complete ^{1}H NMR chemical shift assignments and identified four isolated spin systems in **1**. The first spin system consisted of the C-1 to C-4 carbon fragment. H-1 (δ 3.65) exhibited COSY-45° interactions with the C-2 methylene protons (δ 1.50 and 2.12). The latter further exhibited vicinal couplings with the C-3 methine proton (δ 3.67) which showed ^{1}H–^{1}H spin correlations with the C-4 methylene protons (δ 1.45 and 1.93). The second spin system is comprised of C-11 and C-12. The COSY-45° spectrum featured cross-peaks of the C-11 methylene protons (δ 3.72 and 3.76) with the C-12 methylene protons (δ 1.40 and 1.79). Geminal couplings of the C-11 and C-12 methylene protons were also observed. The third, and largest fragment traced from C-6 to C-30 and started with the C-6 methine proton (δ 3.22)

which showed vicinal coupling with the C-7 methylene protons (δ 1.80 and 2.40) which in turn exhibited cross-peaks with the C-8 methine proton (δ 2.81). The latter further showed cross-peaks with the C-14 methine proton (δ 2.60) which exhibited vicinal couplings with the C-15 methylene protons (δ 1.25 and 1.40). The C-15 methylene protons showed COSY-45° interactions with the C-16 methylene protons (δ 1.65 and 1.99), which in turn exhibited vicinal couplings with the C-17 methine proton (δ 1.23). The C-17 methine proton further showed cross-peaks with the C-20 methine proton (δ 0.99). Cross-peaks of the C-20 methine proton with the C-21 methyl protons (δ 1.01) and C-22 methine proton (δ 0.27) were also observed in the COSY-45° spectrum. The latter exhibited vicinal couplings with the C-30 methylene protons (δ -0.14 and 0.45). Geminal couplings between the C-7, C-15, C-16 and C-30 methylene protons were also observed in the COSY-45° spectrum. A fourth spin system composed of the C-24 to C-28 fragment and begins with the C-24 methine proton (δ 0.27) which showed cross-peaks with the C-25 methine proton (δ 1.50) and C-28 methyl protons (δ 0.89). The former further exhibited vicinal couplings with the C-26 (δ 0.82) and C-27 (δ 0.91) methyl protons. ^{1}H NMR chemical shift assignments of **1** are summarized in Table 1.

The ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **1** showed distinct resonances of all 30 carbons. An attached proton test (APT) experiment indicated the presence of ten CH, eight CH_2, seven CH_3 and five quaternary carbon atoms in **1**. Interpretation of ^{13}C NMR data further suggested that the compound is a secosterol as chemical shift values of the majority of the carbon atoms were found to be nearly identical to those of previously reported secogorgosterols of the series,[4–6] greatly facilitating the ^{13}C NMR chemical shift assignments of **1**. A resonance at δ 69.9 was assigned to C-1 while a signal at δ 69.4 was due to C-3. Other downfield signals at δ 61.0 and 60.4 were diagnostic signals for the epoxy-bearing C-5 and C-6. A resonance at δ 59.1 is characteristic for the C-11 hydroxy-bearing carbon in secosterols.[2,4–6] Complete ^{13}C NMR chemical shift assignments of **1** are shown in Table 1.

The stereochemistry at various chiral centers was established with the aid of NOESY, coupling constants, optical rotations and by comparing the ^{13}C NMR chemical shift values with known secosterol (**3**) of the series. Compound **1** showed an $[\alpha]_D^{20}$ value of +21. The positive sign of optical rotation suggested that the C-3 methine proton has an α-orientation, while the C-8 methine proton, C-18 and C-19 methyl groups are β-oriented as in other secosterols of this series. The ^{13}C NMR chemical shift values of C-3, C-8, C-10, C-13, C-14, C-17, C-20, C-22, C-23 and C-24 were identical to those reported for secosterol **3** which further suggested that these centers have the same orientation as in **3**.[4–6] NOE cross-peaks between H-8 (δ 2.81), H_3-18 (δ 0.67) and H_3-19 (δ 1.24) were also observed in the NOESY spectrum which indicated that they are *cis* to each other and thus all oriented β. With the stereochemistry at these chiral centers established, the NOESY spectrum was helpful for the assignment of stereochemistry at other chiral centers. The C-1 methine proton (δ 3.65) showed a cross-peak with the C-3 methine proton (δ 3.67) which suggested the α-stereochemistry for the C-1 methine proton and thus the β-stereochemistry for the C-1 hydroxyl. The C-6 methine proton (δ 3.22) exhibited an NOE with the C-8 methine proton (δ 2.81) and the C-19 methyl protons (δ 1.24), indicating that the C-5/C-6 epoxy functionality has the α-orientation. Tori and co-workers[7] have shown that for 9β,11β-epoxy steroids, $J_{11\alpha,12\alpha}$ and $J_{11\alpha,12\beta}$ are ~1.5 Hz while for the 9α,11α isomer, $J_{11\beta,12\alpha}$ ~0, $J_{11\beta,12\beta}$ ~4.5 Hz. Hence the single 4.5 Hz coupling observed for the H-6 resonance in **1** further confirmed the α stereochemistry for the oxirane ring. Probable conformation of **1** and its important NOE interactions are shown in Fig. 1. Based on these spectral data, structure **1** was established for this new natural product.

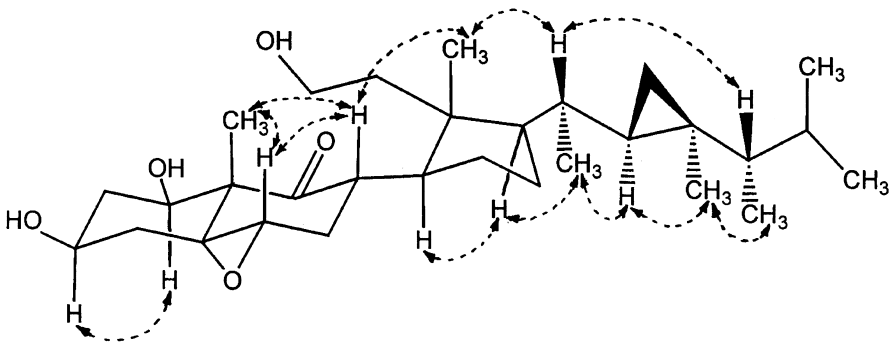

Figure 1. Probable conformation of compound **1** and its important NOE interactions as observed in the NOESY spectrum

The second compound, 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-secogorgostane-11-ol (**2**), was also isolated as a colorless gum. The UV and IR spectra of compound **2** were identical to those of **1**. The EIMS of **1** showed a molecular ion peak at *m/z* 474, which is consistent with an elemental formula $C_{30}H_{50}O_4$ and indicated the presence of six degrees of unsaturation.

The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **2** was very similar to that of **1** except the multiplet at δ 3.66–3.76 integrated for three hydrogens instead of four hydrogens as discussed previously for compound **1**. This indicated that the signal for the C-1 methine proton, geminal to the hydroxyl group, is absent in this region. The C-1 methylene protons were observed as a multiplet at δ 1.79 and 1.39 as observed in the COSY-45° spectrum by tracing ^{1}H–^{1}H coupling frame work of the C-3 methine proton (δ 3.66) in order to assign complete ^{1}H NMR chemical shift assignments of **2** (Table 1). The ^{13}C NMR spectrum of **2** (Table 1) indicates that chemical shift values of all the carbon atoms are nearly identical to those as previously discussed for compound **1** except for C-1 which resonated at δ 29.9. The high resolution electron impact mass spectrum of **2** showed an M^+ at *m/z* 474.3715, which is in agreement with the molecular formula $C_{30}H_{50}O_4$ (calcd 474.3709). The mass spectrum also exhibited a similar fragmentation pattern to that of **1** with many ions observed at 16 amu lower than that of **1**. This combination of ^{1}H, ^{13}C NMR and MS data confirmed that compound **2** is the C-1 deoxy derivative of **1**. The optical rotation, NOESY and ^{13}C NMR spectra also favors similar stereochemistry at all chiral centers as established for compound **1**. Based on these spectroscopic studies, structure **2** was established for this new secosterol.

Compound **1** exhibited significant activity against prostate cancer (LnCap) and lung cancer cell lines (Calu-3) in an MTT assay with observed IC_{50} values of 15.49 μg/ml and 11.0 μg/ml, respectively. Compound **2** also showed similar bioactivity against the above mentioned cancer cell lines with observed IC_{50} values of 18.43 μg/ml and 12.0 μg/ml, respectively. Interestingly, compound **3** exhibited IC_{50} values of 41.0 μg/ml and 38.12 μg/ml against the LnCap and Calu-3 cell lines, respectively, suggesting that the epoxide ring is at least partly responsible for the observed activity. The similarity of the IC_{50} values of **1** and **2** indicates that the C-1 hydroxyl is not responsible for the observed activity.

Acknowledgements

We are very grateful to the Camille and Henry Dreyfus Foundation for providing financial support for this study. We also thank Harbor Branch Oceanographic Institution, Florida for

permitting us to use their polarimeter for measuring optical rotations, and the Midwest Center for Mass Spectrometry at the University of Nebraska–Lincoln for mass measurements.

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