

## 12-ACETOXYPSEUDOPTEROLIDE: A NEW DITERPENE FROM *PSEUDOPTEROGORGIA ELISABETHAE*

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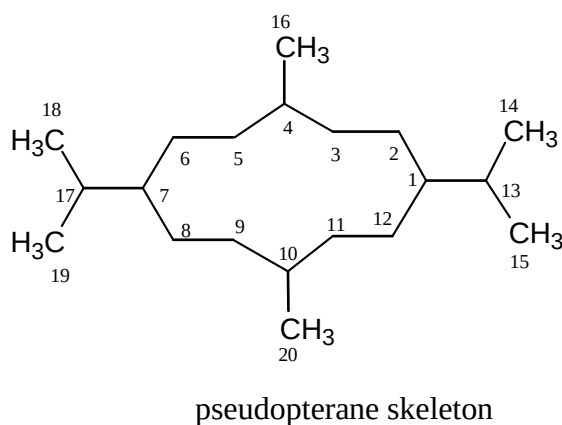
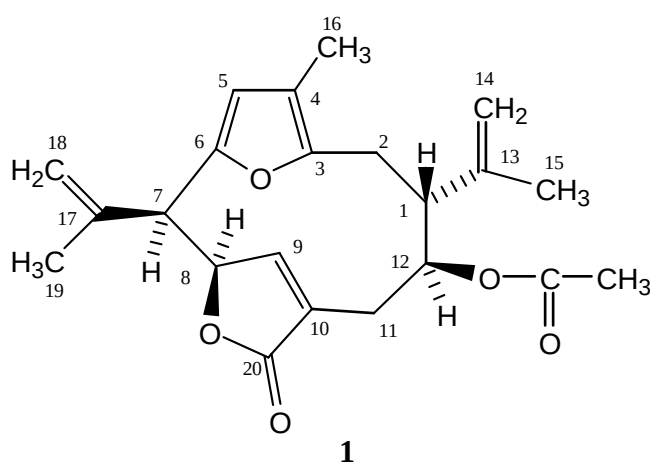
**Abstract** - Chemical studies on the non polar fraction of the methanolic extract of *Pseudopterogorgia elisabethae* Bayer collected from the Florida Keys has yielded 12-acetoxypseudopterolide (**1**), a new diterpene of the pseudopterane series. The structure was established through spectroscopic analysis. Compound (**1**) was shown to exhibit modest anti-cancer activity against a human prostate cancer cell line (LnCap).

Soft corals belonging to the genus *Pseudopterogorgia* provide a very rich source of biomedically significant natural products.<sup>1-2</sup> For instance, pseudopterolins isolated from *P. elisabethae* in the Bahamas exhibit potent anti-inflammatory activity, and seco-pseudopterolins from *Pseudopterogorgia* sp. collected in the Florida Keys exhibit similar anti-inflammatory as well as anti-bacterial activity.<sup>3-5</sup> Secosterols from *P. americana* have been shown to exhibit inhibitory activity against protein kinase C as well as anti-proliferative activity.<sup>6</sup> Recently, diterpenes as well as diterpene alkaloids purified from *P. elisabethae*, of Colombian origin have shown anti-cancer and anti-tuberculosis activities.<sup>7-9</sup>

In the course of a detailed examination of the non-polar metabolites of *P. elisabethae* collected in the central Florida Keys, we have isolated 12-acetoxypseudopterolide (**1**), a new pseudopterane diterpene. The structure of compound (**1**) was elucidated through the use of spectroscopic studies. 12-Acetoxypseudopterolide (**1**) showed mild anti-cancer activity against a prostate cancer cell line (LnCap).

12-Acetoxypseudopterolide (**1**), C<sub>22</sub>H<sub>26</sub>O<sub>5</sub>, was isolated as yellow colored gum. Its UV spectrum displayed an absorption maximum at 246 nm, characteristic of an  $\alpha,\beta$ -unsaturated carbonyl moiety,<sup>10</sup>

and the IR spectrum showed intense absorption bands at 2928 (C-H), 1753 and 1745 ( $\alpha,\beta$ -unsaturated  $\gamma$ -lactone), 1726 (ester carbonyl) and 1659 (C=C)  $\text{cm}^{-1}$ . The electron-impact MS spectrum featured a molecular ion peak at  $m/z$  370, consistent with molecular formula  $\text{C}_{22}\text{H}_{26}\text{O}_5$ , and indicate the presence of ten degrees of unsaturation. A signal at  $m/z$  355 ( $\text{C}_{21}\text{H}_{23}\text{O}_5$ ) was ascribed to  $\text{M}^+ - \text{CH}_3$ , and a signal at  $m/z$  310 ( $\text{C}_{20}\text{H}_{23}\text{O}_3$ ) was ascribed to  $\text{M}^+ - \text{AcOH}$ . The base peak at  $m/z$  154 is due to the fragment  $\text{C}_9\text{H}_{14}\text{O}_2$ , and could arise by the cleavage of the C-2/C-3 and C10-/C-11 bonds.



The  $^1\text{H}$ -NMR spectrum ( $\text{CDCl}_3$ , 500 MHz) of **1** showed three singlets, each integrating for 3 hydrogens, at  $\delta$  1.70, 1.89, and 1.99 which we assigned to the C-15, C-16 and C-19 methyl groups. Another singlet (3H) resonating at  $\delta$  2.00 was ascribed to the methyl protons of an acetoxy functionality at C-12. Four singlets, integrating for one-proton each, at  $\delta$  4.80, 4.89, 4.99 and 5.14 were assigned to the olefinic C-14 and C-18 methylene protons, respectively. A resonance at 5.52 ( $J_1 = 12$  Hz,  $J_2 = 10.6$  Hz and  $J_3 = 4.0$  Hz) was consistent with the C-12 methine proton, geminal to the acetoxy group, and the C-8 methine proton appeared as doublet at  $\delta$  5.38 ( $J = 5.0$  Hz). A doublet

(1H) at  $\delta$  3.68 ( $J = 5.0$  Hz) due to the C-7 proton and a multiplet (1H) at  $\delta$  3.00 due to the C-1 methine proton, were also observed in the  $^1\text{H}$ -NMR spectrum of compound (**1**). A singlet at  $\delta$  6.63 was ascribed to the C-5 aromatic proton and the signal at  $\delta$  6.67 was assigned to the C-9 olefinic proton.

Table-1:  $^1\text{H}$  and  $^{13}\text{C}$ -NMR Chemical Shift Assignments of **1**.

| Carbon No.         | $^{13}\text{C}$ -NMR $\delta$ | Multiplicity (APT) | $^1\text{H}$ -NMR $\delta$ ( $J$ value in Hz)            |
|--------------------|-------------------------------|--------------------|----------------------------------------------------------|
| 1                  | 42.8                          | CH                 | 3.00 (m)                                                 |
| 2                  | 32.4                          | CH <sub>2</sub>    | 1.77 (m)<br>2.45 (m)                                     |
| 3                  | 146.6                         | -C-                | ---                                                      |
| 4                  | 114.4                         | -C-                | ---                                                      |
| 5                  | 112.1                         | CH                 | 6.63 (s)                                                 |
| 6                  | 152.0                         | -C-                | ---                                                      |
| 7                  | 49.6                          | CH                 | 3.68 (d, $J = 5.0$ Hz)                                   |
| 8                  | 81.6                          | CH                 | 5.35 (d, $J = 5.0$ Hz)                                   |
| 9                  | 147.1                         | CH                 | 6.67 (s)                                                 |
| 10                 | 130.1                         | -C-                | ---                                                      |
| 11                 | 34.9                          | CH <sub>2</sub>    | 1.61 (br d, $J = 13.0$ Hz)<br>2.06 (br d, $J = 13.0$ Hz) |
| 12                 | 72.6                          | CH                 | 5.52 (ddd, $J = 12, 10.6, 4.0$ Hz)                       |
| 13                 | 142.0                         | CH                 | 3.00 (m)                                                 |
| 14                 | 112.1 <sup>a</sup>            | CH <sub>2</sub>    | 4.80 (br s)<br>4.89 (br s)                               |
| 15                 | 18.5 <sup>b</sup>             | CH <sub>3</sub>    | 1.70 (s)                                                 |
| 16                 | 10.1 <sup>b</sup>             | CH <sub>3</sub>    | 1.89 (s)                                                 |
| 17                 | 145.8                         | -C-                | ---                                                      |
| 18                 | 122.0 <sup>a</sup>            | CH <sub>2</sub>    | 4.99 (br s)<br>5.14 (br s)                               |
| 19                 | 22.0 <sup>b</sup>             | CH <sub>3</sub>    | 1.99 (s)                                                 |
| 20                 | 176.0                         | -C-                | ---                                                      |
| CH <sub>3</sub> CO | 22.1 <sup>b</sup>             | CH <sub>3</sub>    | 2.00 (S)                                                 |
| CH <sub>3</sub> CO | 170.1                         | -C-                | ---                                                      |

a,b Assignments are interchangeable

A COSY-45° spectrum was used for the  $^1\text{H}$ -NMR chemical shift assignments of compound (**1**). The C-1 methine proton ( $\delta$  3.00) showed vicinal coupling with C-2 methylene ( $\delta$  1.77 and 2.45) and C-12 methine ( $\delta$  5.52). The latter in turn exhibited  $^1\text{H}$ - $^1\text{H}$  spin correlations with the C-11 methylene protons ( $\delta$  1.61 and 2.06). These observations suggested that the acetoxy functionality was located at C-12. The geminal couplings between C-2 ( $\delta$  1.77 and 2.45) as well as C-11 ( $\delta$  1.61 and 2.06)

methylene protons were also observed in the COSY-45° spectrum. Some of the signals in the COSY-45° spectrum did not exhibit  $^1\text{H}$ - $^1\text{H}$  spin correlations, and they were assigned based on the comparison of the chemical shift of these signals with the reported compounds of the series.<sup>11</sup> Thus, a combination of the COSY-45° spectrum and literature data for diterpenes of the pseudopterane series allowed for the completion of the  $^1\text{H}$ -NMR chemical shift assignments of **1** (Table-1).

The  $^{13}\text{C}$ -NMR spectrum of (**1**) showed distinct resonances for all twenty two carbon atoms. An attached proton test established that compound (**1**) consists of six CH, four CH<sub>2</sub>, four CH<sub>3</sub> and eight quaternary carbons. The interpretation of the  $^{13}\text{C}$ -NMR spectrum also suggested that the compound is of pseudopterane series.<sup>11,12</sup> The chemical shifts of the majority of the carbon atoms were found to be nearly identical to those of pseudopterane diterpenes which greatly facilitated the  $^{13}\text{C}$ -NMR chemical shift assignments of compound (**1**).<sup>11,12</sup> Resonances at  $\delta$  42.8 and 72.6 were assigned to C-1 and C-12 respectively. The downfield value of the latter suggested the presence of a geminal acetoxy functionality at C-12. The resonance at  $\delta$  81.6 was ascribed to C-8 while other signals at  $\delta$  147.1, 142.0, 112.1, 145.8, and 122.0 were assigned to the C-9, C-13, C-14, C17, and C-18 respectively. Complete  $^{13}\text{C}$ -NMR Chemical shift assignments of **1** is presented in Table-1.

The stereochemistry at various chiral centers was established by chemical shift comparison of **1** with previously reported compounds of the series, measurement of optical rotation and analysis of the NOESY spectrum. The  $[\alpha]_D^{20}$  value of the compound (**1**) was found to be +18°. The positive sign of the optical rotation and  $^1\text{H}$ -NMR chemical shifts of H-1 ( $\delta$  3.00) and H-7 ( $\delta$  3.68), which were nearly identical to those of reported diterpenes of the series, suggested that H-1 is  $\alpha$ -oriented while H-7 is  $\beta$ -oriented as in other pseudopterane diterpenes of the series.<sup>12</sup> With the stereochemistry for these two centers established, the NOESY spectrum of compound (**1**) helped to establish the stereochemistry at other stereocenters of the molecule. H-7 ( $\delta$  3.68) showed an NOE cross-peak with H-8 ( $\delta$  5.38), suggesting the  $\alpha$ -stereochemistry for the C-8 methine proton. The C-1 methine proton ( $\delta$  3.00) showed an NOE interaction with the C-2 $\beta$  methine proton ( $\delta$  2.45). This suggested the chemical shift value of H-2 $\beta$  ( $\delta$  2.45) and the  $^1\text{H}$ - $^1\text{H}$  spin correlations of H-2 $\alpha$ /H-2 $\beta$ , representing geminal coupling between them, observed in the COSY-45° spectrum, helped to identify the chemical shift value of H-2 $\alpha$  ( $\delta$  1.77). The NOE between H-12 ( $\delta$  5.52) and H-2 $\alpha$  ( $\delta$  1.77) and the absence of a cross-peak between the C-12 and C-1 methine protons in the NOESY spectrum suggested  $\alpha$ -stereochemistry for the C-12 methine proton and  $\beta$ -stereochemistry for the C-12 acetoxy functionality. Based on these spectroscopic data, structure (**1**) was proposed for this new natural product. This is the first example of a pseudopterane type diterpene isolated from *P. elisabethae*;

previously this type of diterpene has been isolated from *P. acerosa* and *P. kallos*.<sup>11-13</sup> Compound (**1**) exhibited mild anti-cancer activity against a prostate cancer cell line with an IC<sub>50</sub> value of 47.9 µg/mL observed using an MTT assay.<sup>14</sup>

## EXPERIMENTAL

**General Experimental Procedures.** Optical rotations were measured on a Jasco polarimeter. The UV spectra were recorded on a Shimadzu UV 240 instrument, and IR spectra recorded on a Galaxy FT-IR spectrophotometer. The <sup>1</sup>H-NMR spectra (one- and two-dimensional) were recorded in CDCl<sub>3</sub> on an Inova Varian 500 NMR spectrometer at 500 MHz, while <sup>13</sup>C-NMR spectra were recorded on the same instrument at 125 MHz. MS spectral measurements were conducted at the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln. TLC was performed using GF<sub>254</sub> precoated plates and HPLC was performed using a Perkin Elmer Series 410 LC pump and Hitachi UV detector monitoring 265 nm with a gradient elution of acetonitrile-water (90-10) to 100% acetonitrile on an analytical reverse phase C18 column (Vydac).

**Collection, Extraction and Isolation:** *P. elisabethae* was collected from the central Florida Keys during February 1999 by scuba from a depth of 24 m. The organism was identified as *Pseudopeterogorgia elisabethae* by Frederick M. Bayer, Department of Invertebrate Zoology, Smithsonian National Museum of Natural History. A voucher specimen (USNM100302) has been deposited in this institute. *P. elisabethae* (99.0 g) was freeze dried and extracted with methanol (400 mL), and then with chloroform (1.5 L) for 12 h at room temperature. The solvent was evaporated under reduced pressure to produce a gum (12.1 g). This was loaded onto a silica gel column and eluted with hexane, hexane with increasing amount of ethyl acetate (0-100%) and then with ethyl acetate-methanol (0-100%). The fraction (9.7 mg) obtained on elution with hexane-ethyl acetate (40:60) was subjected to HPLC using a reverse phase column and a gradient elution of acetonitrile-water (90-100) to 100% acetonitrile as mobile phase to afford compound (**1**) as a yellow colored gum (4.5 mg, 4.5x10<sup>-3</sup>% yield). This compound was homogeneous on TLC in various solvent systems (*R<sub>f</sub>* = 0.67 with hexane-ethylacetate, 65:35).

**12-Acetoxypseudopterolide (1).** [ $\alpha$ ]<sub>D</sub><sup>20</sup> +18° (c 0.9, CHCl<sub>3</sub>). UV (MeOH)  $\lambda_{\max}$  246 (log  $\epsilon$  2.68) nm. IR  $\nu_{\max}$  (CHCl<sub>3</sub>): 2928 (C-H), 1753, 1745 ( $\alpha,\beta$ -unsaturated  $\gamma$  lactone), 1726 (ester carbonyl) and 1659 (C=C) cm<sup>-1</sup>. EIMS *m/z* (rel. int., %) 370 (C<sub>22</sub>H<sub>26</sub>O<sub>5</sub>, 5), 355 (C<sub>21</sub>H<sub>23</sub>O<sub>5</sub>, 3), 310 (C<sub>20</sub>H<sub>23</sub>O<sub>3</sub>, 9), and 154 (C<sub>9</sub>H<sub>14</sub>O<sub>2</sub>, 100). <sup>1</sup>H- and <sup>13</sup>C NMR data see Table-1.

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