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

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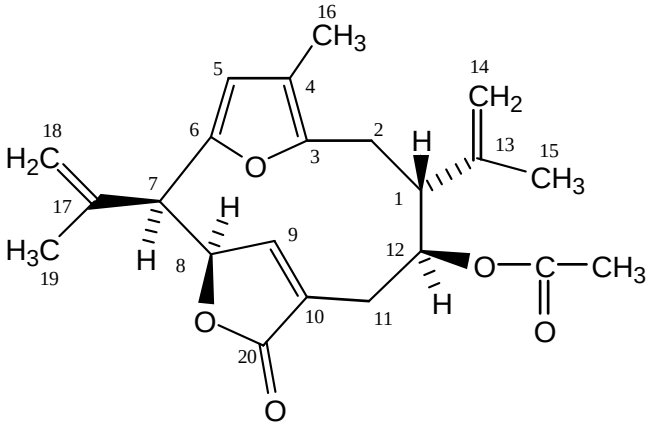
Abstract - Chemical studies on the non polar fraction of the methanolic extract of *Pseudopterogorgia elisabethae* Bayer collected from the Florida Keys has yielded 12-acetoxypseudopterolide (**1**), a new diterpene of the pseudopterane series. The structure was established through spectroscopic analysis. 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.[1-2] For instance, pseudopterosins isolated from *P. elisabethae* in the Bahamas exhibit potent anti-inflammatory activity, and seco-pseudopterosins from *Pseudopterogorgia* sp. collected in the Florida Keys exhibit similar anti-inflammatory as well as anti-bacterial activity.[3-5] Secosterols from *P. americana* have been shown to exhibit inhibitory activity against protein kinase C as well as anti-proliferative activity.[6] Recently, diterpenes as well as diterpene alkaloids purified from *P. elisabethae*, of Colombian origin have shown anti-cancer and anti-tuberculosis acitivities.[7-9]

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**), $C_{22}H_{26}O_5$, was isolated as yellow colored gum. Its UV spectrum displayed an absorption maximum at 246 nm, characteristic of an α,β-unsaturated carbonyl moiety,[10]

and the IR spectrum showed intense absorption bands at 2928 (C-H), 1753 and 1745 (α,β-unsaturated γ–lactone), 1726 (ester carbonyl) and 1659 (C=C) cm^{-1}. The electron-impact MS spectrum featured a molecular ion peak at *m/z* 370, consistent with molecular formula $C_{22}H_{26}O_5$, and indicate the presence of ten degrees of unsaturation. A signal at *m/z* 355 ($C_{21}H_{23}O_5$) was ascribed to M^+-CH_3, and a signal at *m/z* 310 ($C_{20}H_{23}O_3$) was ascribed to M^+-AcOH. The base peak at *m/z* 154 is due to the fragment $C_9H_{14}O_2$, and could arise by the cleavage of the C-2/C-3 and C10-/C-11 bonds.



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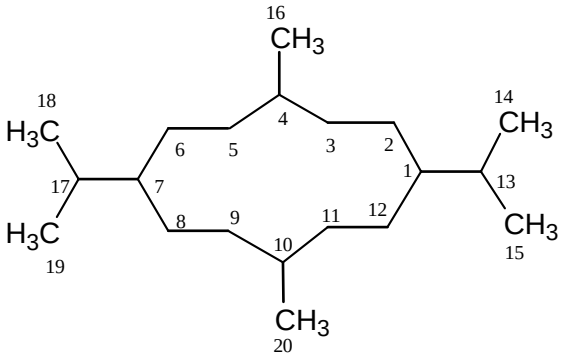

pseudopterane skeleton

The ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of **1** showed three singlets, each integrating for 3 hydrogens, at δ 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 δ 2.00 was ascribed to the methyl protons of an acetoxy functionality at C-12. Four singlets, integrating for one-proton each, at δ 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 δ 5.38 (J = 5.0 Hz). A doublet

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

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

Carbon No.	^{13}C-NMR δ	Multiplicity (APT)	^{1}H-NMR (*J* value in Hz) δ
1	42.8	CH	3.00 (m)
2	32.4	CH_2	1.77 (m)
			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_2	1.61 (br d, *J*= 13.0 Hz)
			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[a]	CH_2	4.80 (br s)
			4.89 (br s)
15	18.5[b]	CH_3	1.70 (s)
16	10.1[b]	CH_3	1.89 (s)
17	145.8	-C-	---
18	122.0[a]	CH_2	4.99 (br s)
			5.14 (br s)
19	22.0[b]	CH_3	1.99 (s)
20	176.0	-C-	---
$\underline{CH_3}CO$	22.1[b]	CH_3	2.00 (S)
$CH_3\underline{C}O$	170.1	-C-	---

a,b Assignmnets are interchangeable

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

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

The ^{13}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_2, four CH_3 and eight quaternary carbons. The interpretation of the ^{13}C-NMR spectrum also suggested that the compound is of pseudopterane series.[11,12] 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}C-NMR chemical shift assignments of compound (**1**).[11,12] Resonances at δ 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 δ 81.6 was ascribed to C-8 while other signals at δ 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}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^{o}. The positive sign of the optical rotation and ^{1}H-NMR chemical shifts of H-1 (δ 3.00) and H-7 (δ 3.68), which were nearly identical to those of reported diterpenes of the series, suggested that H-1 is α-oriented while H-7 is β-oriented as in other pseudopterane diterpenes of the series.[12] With the stereochemistry for these two centers established, the NOESY spectrum of compound (**1**) helped to establish the streochemistry at other stereocenters of the molecule. H-7 (δ 3.68) showed an NOE cross-peak with H-8 (δ 5.38), suggesting the α-stereochemistry for the C-8 methine proton. The C-1 methine proton (δ 3.00) showed an NOE interaction with the C-2β methine proton (δ 2.45). This suggested the chemical shift value of H-2β (δ 2.45) and the ^{1}H-^{1}H spin correlations of H-2α/H-2β, representing geminal coupling between them, observed in the COSY-45^{o} spectrum, helped to identify the chemical shift value of H-2α (δ 1.77). The NOE between H-12 (δ 5.52) and H-2α (δ 1.77) and the absence of a cross-peak between the C-12 and C-1 methine protons in the NOESY spectrum suggested α-stereochemistry for the C-12 methine proton and β-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*.[11-13] Compound (**1**) exhibited mild anti-cancer activity against a prostate cancer cell line with an IC_{50} value of 47.9 μg/mL observed using an MTT assay.[14]

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 ^{1}H-NMR spectra (one- and two-dimensional) were recorded in $CDCl_3$ on an Inova Varian 500 NMR spectrometer at 500 MHz, while ^{13}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_{254} 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, 4.5×10^{-3}% yield). This compound was homogeneous on TLC in various solvent systems (R_f = 0.67 with hexane-ethylacetate, 65:35).

12-Acetoxypseudopterolide (1). $[\alpha]_D^{20}$ +18° (c 0.9, $CHCl_3$). UV (MeOH) λ_{max} 246 (log ε 2.68) nm. IR ν_{max} ($CHCl_3$): 2928 (C-H), 1753, 1745 (α,β-unsaturated γ lactone), 1726 (ester carbonyl) and 1659 (C=C) cm^{-1}. EIMS *m/z* (rel. int., %) 370 ($C_{22}H_{26}O_5$, 5), 355 ($C_{21}H_{23}O_5$, 3), 310 ($C_{20}H_{23}O_3$, 9), and 154 ($C_9H_{14}O_2$, 100). ^{1}H- and ^{13}C NMR data see Table-1.

ACKNOWLEDEGMENTS

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