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Cembranoids from *Eunicea* sp. enhance insulin-producing cells proliferation

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ABSTRACT

Nine new cembranoids **1**–**9** containing an α–methylene–γ–butyrolactone together with the known diterpenes **10**–**15** have been isolated from a crude extract of *Eunicea* sp. and their structures were established by spectroscopic methods. The proliferative effect of six of these compounds on insulin-producing cells (beta–cells) was evaluated.

1. Introduction

Species of *Eunicea* are commonly found on Caribbean Sea coral reefs. The genus is comprised of approximately fifteen documented species, and the most studied have been: *Eunicea* sp., *E. tourneforti*, *E. succinea*, *E. mammosa*, *E. knighti*, *E. pinta*, *E. asperula* and *E. calyculata*. This genus biosynthesizes diterpenoids by a network of oxidation processes that generate the chemical structures of the metabolites depicted in Fig. 1.

Our interest in studying octocorals from both sides of the Isthmus of Panama[1–3] led us to analyze specimens of *Eunicea* sp. collected from the Caribbean coast of Panama. From a crude extract of *Eunicea* sp., the cembranoids **1**–**15** have been isolated after flash chromatography followed by HPLC, Fig. 1. The new compounds **1**–**9** together with the known metabolites euniolide (**10**)[4] and peunicin (**11**)[5] typify a class of cembranoids with an oxidized isopropenyl group involved in the formation of an α–methylene–γ–butyrolactone ring. In 14–deoxycrassin (**12**),[6], the isopropenyl group forms part of a six memebered lactone ring, whereas compounds **13**–**15**[7–9] lack the lactone ring.

2. Results and discussion

Compound **1** was obtained as an oil whose EIMS spectrum showed a peak at *m/z* $[M]^+$ 332, which corresponds to the chemical formula $C_{20}H_{28}O_4$ (HREIMS) (*m/z* 332.1994 $[M]^+$, calcd 332.1988). This corroborated the ^{13}C NMR spectrum, which displayed correlations in the HSQC spectrum indicative of five quaternary, six methine, six methylene, and three methyl carbons (Table 1). Absorptions for a hydroxyl group at 3441 cm^{-1} and a carbonyl at 1742 cm^{-1} were observed in the IR spectrum.

Connectivity information obtained from COSY, HSQC, and HMBC experiments unambiguously determined the planar structure of compound **1** as a cembranolide with an epoxide at C-3–C-4, two methyl groups of trisubstituted olefins at C-8 and C-12 respectively, an allylic alcohol at C-13 and a C-14–C-16 α–methylene–γ–lactone moiety. ^1H–^1H COSY experiments

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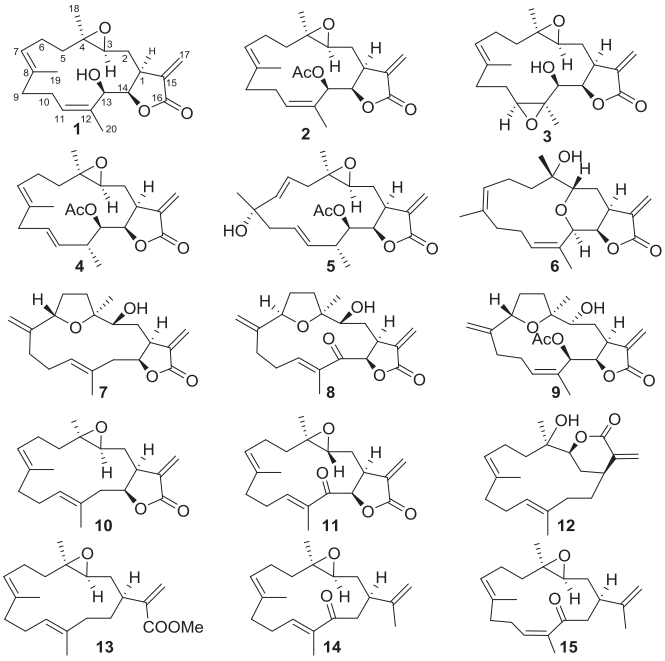

Fig. 1. Cembranolides isolated from *Eunicea* sp.

established three spin systems: H-3–H-13 (fragment **I**), H_2-5–H-7 (fragment **II**) and H_2-10–H-11 (fragment **III**). HMBC correlations allowed us to join these fragments. Correlations of H_3-18/C-3, C-4 and C-5 connected fragment **I** and **II** through C-4. HMBC correlations of H_3-19 with C-7, C-8 and C-9 place H_3-19 at C-8 and the correlations of H-10a (δ_H 1.80 ppm) with C-9 and of H-9b (δ_H 2.30 ppm) with C-10 bind fragments **II** and **III**, whereas the correlations H_3-20/C-11, C-12 and C-13 connect fragments **I** and **III**. The isopropenyl group at C-1, characteristic of marine cembranoids, forms part of an α–methylene–γ–lactone ring between C-1 and C-14, in virtue of the HMBC correlations H_2-17/C-1, C-15, C-16 and H-14/C-15, C-16. The ^{13}C chemical shifts of C-3 (δ 60.4), C-4 (δ 60.2) and C-13 (δ 68.8) indicated that there is an epoxide between C-3 and C-4 and an allylic alcohol at C-13. Thus, the structure of **1** was established, with seven degrees of unsaturation as shown in Fig. 2.

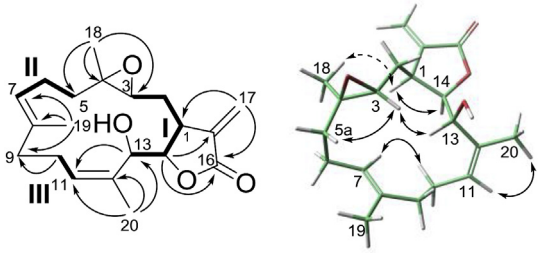

Fig. 2. COSY (–), HMBC (→) correlations and selected NOE effects (↔) of **1**.

The relative configuration of **1** is assigned based on NOESY experiments and coupling constants. The *cis*-fusion of the lactone ring was established by a strong NOE interaction between H-1 and H-14, which is consistent with the coupling constant $^3J_{1\text{-}14} = 8.4$ Hz.[10] NOE correlations of H-3 with H-5a, as well as of H_3-18 with H-1, suggested a *trans*-substituted epoxide ring. The high field signal of Me-19 ($\delta_C = 15.7$ ppm) suggested an *E* geometry for the olefin at C-7/C-8,[11] which was confirmed by the NOE observed between H-7 with H-10a ($\delta_H = 1.80$ ppm). The NOE interaction of H_3-20 with H-11 established the *Z* geometry for the olefin at C-11/C-12. Finally, the observed NOE between H-13 and H-3 fix the configuration of the hydroxyl group as depicted in Fig. 2. The relative configuration of **1** is thus 1*S**, 3*S**, 4*R**, 7*E*, 11*Z*, 13*R**, 14*R**.

Compound **2** was obtained as a colorless oil, and its molecular formula was determined as $C_{22}H_{30}O_5$ by HREIMS [*m/z* 374.2104 $[M]^+$, calculated 374.2093]. Absorption for carbonyl groups at 1753 cm^{-1} was observed in the IR spectrum.

The ^1H and ^{13}C NMR data (Table 1) resembled those of compound **1**, with the primary difference being an acetoxy group

Table 1
^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data for compounds **1**–**3** in $CDCl_3$.

Pos.	1		2		3	
	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C
1	3.17 m	40.4	3.17 m	40.6	3.16 m	40.6
2	1.80 m 2.47 ddd (5.1, 11.7, 14.7)	29.0	1.75 m 2.02 m	28.3	1.75 ddd (2.8, 9.8, 14.8) 2.42 m	28.8
3	3.06 dd (4.8, 9.3)	60.4	3.05 dd (4.8, 9.6)	59.8	2.77 dd (4.7, 10.0)	60.2
4	–	60.2	–	60.1	–	60.0
5	*a* 1.20 ddd (3.6, 13.2, 13.2) *b* 2.15 ddd (2.7, 5.1, 13.2)	39.6	*a* 1.24 m *b* 2.15 m	39.3	*a* 1.07 ddd (3.5, 13.2, 13.2) *b* 2.16 ddd (2.8, 4.7, 13.2)	39.9
6	1.98 m 2.31 m	23.2	1.91 m 2.40 m	23.0	1.95 m 2.31 m	23.2
7	5.21 dd (7.5, 7.5)	123.2	5.21 dd (7.5, 7.5)	123.1	5.23 dd (8.2, 8.2)	123.1
8	–	138.5	–	138.6	–	137.7[a]
9	*a* 1.79 m *b* 2.30 m	40.3	1.77 m 2.36 m	40.2	1.99 dd (12.3, 12.3) 2.30 m	35.7
10	*a* 1.80 m *b* 2.31 m	29.1	1.90 m 2.37 m	29.4	0.90 m 2.42 m	29.2
11	5.55 m	127.7	5.59 dd (4.2, 11.9)	128.4	2.96 dd (3.5, 11.9)	63.6
12	–	135.8	–	132.6	–	63.2
13	4.94 br d (4.8)	68.8	5.86 s	70.4	3.82 s	72.0
14	4.70 d (8.4)	83.5	4.84 d (8.4)	82.3	4.83 d (8.5)	79.3
15	–	138.1	–	138.2	–	137.5[a]
16	–	169.9	–	169.2[a]	–	169.4
17	5.42 d (3.3) 6.19 d (3.6)	118.7	5.36 d (3.4) 6.19 d (3.6)	117.5	5.41 d (3.2) 6.21 d (3.5)	118.6
18	1.35 s	16.0	1.34 s	16.0	1.34 s	15.6
19	1.60 s	15.7	1.61 s	15.6	1.61 s	15.3
20	1.85 s	19.6	1.72 s	19.9	1.48 s	18.6
21			–	169.1[a]		
22			1.96 s	20.5		
OH	2.73 br d (4.8)					

[a] Interchangeable.