Regular Article

Four New Pregnan-10,2-carbolactones from an Epipolasis sp. Marine Sponge

Unwoo Kang, Dongdong Wang, Heidi R. Bokesch, and Kirk R. Gustafson

* Molecular Targets Program, Center for Cancer Research, National Cancer Institute; Frederick, Maryland 21702–1201, U.S.A.; and Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research; Frederick, Maryland 21702–1201, U.S.A.

Introduction

Marine sponges have been a rich source of biologically active secondary metabolites, including a large number of highly functionalized terpenes and steroids.1–5 Sponges from the genus Epipolasis have not been investigated extensively, but they have provided a number of unique terpenes and steroids with unusual side chains. This includes reduced azulene type diterpenes,6–8 tricyclic or tetracyclic verrucosane type diterpenes, 9–11 and steroids with a t-butyl group, an additional isopropyl group, or multi degrees of unsaturation in the side chain.12–14 In the current study, an extract of an Epipolasis sp. marine sponge provided four rare pregnane-10,2-carbolactones, which is a type of steroid that has only been reported twice before.15,16 The isolation and structure determination of these new compounds are described herein.

Results and Discussion

The Epipolasis sp. aqueous extract was sequentially chromatographed on an Oasis SPE cartridge and C18 reversed-phase HPLC to yield four new (1–4) compounds (Fig. 1).

Compound 1 was obtained as a white amorphous solid. The molecular formula C21H28O5 was determined by (+)-high resolution-electrospray ionization (HR-ESI)-MS measurement of a sodium adduct at m/z 383.1830 [M + Na]+ in positive ion mode. The IR spectrum showed strong absorptions for hydroxy (3384 cm−1) and carbonyl (1770 cm−1) groups. The 1H- and 13C-NMR spectra (Tables 1, 2), together with analysis of the heteronuclear single quantum coherence (HSQC) spectrum, revealed a methyl singlet (δH 1.05, H-18), a methyl doublet (δH 1.71, H-21), four oxygenated methines (δH 4.66, H-2; δH 3.83, H-3; δH 4.36, H-4; δH 4.69, H-16), two trisubstituted olefins (δC 137.8, C-5; δC 131.4, C-6; δC 154.9, C-17, δC 117.7, C-20), and an ester carbonyl (δC 178.7, C-19). Ring A of a classic steroid nucleus (C-1 through C-5 and C-10) was defined by correlation spectroscopy (COSY) correlations between H-1/H-2, H-3/H-4, and heteronuclear multiple bond correlation (HMBC) cross peaks of H-1/C-5, H-2/C-10, H-3/C-2, and H-4/C-10. HMBC data defined the olefin in ring B by correlating H-4 to C-6. The remaining portion of the steroidal ring system was assembled using a network of COSY correlations between vicinal protons (Fig. 2). Substitution of a methyl group on the second olefin was supported by a COSY correlation between H-21 and H-20, and this olefin fragment was located at C-17/C-20 by HMBC correlations of H-21/C-16, H-20/C-17, and H-20/C-13. HMBC correlations between H-18/C-12, C-13, C-14, and C-17 indicated that the C-18 singlet methyl was attached at C-13. Finally, the observation of HMBC correlations from H-1, H-2, and H-9 to C-19 established an ester linkage between C-2 and C-10. The structure of compound 1 was very similar to that of 3β,4β-dihydroxypregna-5,17-diene-10,2-carbolactone, which was previously isolated from Myrmekioderma sp.,15 and Petrosia (Stongylophora) sp.16 marine sponges. The 1H- and 13C-NMR data recorded for 1 were in good agreement with those reported for this dihydroxy analogue, except for the deshielded chemical shifts of H-16 and C-16, due to the presence of an additional hydroxy group in 1.

The relative configuration of compound 1 was determined by proton-proton coupling constant analysis and nuclear Overhauser effect (NOE) experiments (Fig. 2). Specifically, the multiplicity of H-8 (qd, J = 11.0, 4.5 Hz) indicated that H-8, H-9, and H-14 are all axial, and the relatively small couplings of J 3,4 = 1.5 Hz and J 15,16 = 5.9 Hz suggested that H-2, H-3 and H-4 are on the same face of the molecule. Nuclear Overhauser effect spectroscopy (NOESY) correlations between H-8/H-11, H-8/H-15, H-8/H-18, H-12/H-18, and H-20/H-18 established the β orientation of these protons and the methyl
group. 1D-NOEs of H-14 to H-9 and H-16 in addition to NOE correlations between H-9/H_α-1 and H_α-1/H-3 established the α orientation of the other methine protons. Very close correspondence between the NMR data for compound 1 and the previously reported 3,4-dihydroxy analogue, the structure of which was confirmed by X-ray crystallography, suggested these two compounds share the same relative configuration.

Attempts to assign the absolute configuration of 1 by Mosher’s ester analysis were unsuccessful due to instability of 1.

Compound 2 was obtained as a white amorphous solid and the (+)-HR-ESI-MS spectrum displayed a sodium adduct ion [M + Na]^+ at m/z 425.1918, corresponding to a molecular formula of C_{23}H_{30}O_{6}. The IR, 1H-NMR, and 13C-NMR spectra of 2 showed signals that were similar to those of 1, while the molecular formula represented an addition of C_{2}H_{2}O to the molecular formula of 1. Comparison of 1H- and 13C-NMR data of these two compounds revealed the presence of an additional acetoxy group at δH/δC 2.04/20.7 and δC 172.6 in 2. The position of the acetoxy group was confirmed at C-16 by the deshielded chemical shift of H-16 (δH 5.65) and an HMBC correlation from H-16 to the carbonyl carbon of the acetoxy group (Fig. 3). Compound 2 showed similar NOE correlations as 1 and thus has the same relative configuration.

The structures of two other pregnane analogues were easily identified by comparing 1H- and 13C-NMR data of compounds

Table 1. 1H-NMR Data (600MHz, CD3OD) for Compounds 1–4

| Position | 1   | 2   | 3   | 4   |
|----------|-----|-----|-----|-----|
| 1        | a 1.61 (d, 12.1) | 1.62 (d, 12.1) | 1.64 (d, 12.1) | 1.65 (d, 12.3) |
|          | β 2.68 (dd, 12.1, 6.7) | 2.68 (dd, 12.1, 6.7) | 2.61 (dd, 12.1, 6.7) | 2.61 (dd, 12.1, 6.7) |
| 2        | 4.66 (bd, 6.7) | 4.66 (bd, 6.7) | 4.65 (bd, 6.7) | 4.65 (m) |
| 3        | 3.83 (dd, 5.9, 1.5) | 3.83 (dd, 5.9, 1.5) | 3.83 (d, 5.9) | 3.83 (dd, 5.9, 1.5) |
| 4        | 4.36 (d, 5.9) | 4.36 (d, 5.9) | 4.37 (d, 5.9) | 4.37 (d, 5.9) |
| 5        | 5.99 (dd, 6.3, 1.9) | 5.99 (dd, 6.3, 1.9) | 6.00 (dd, 6.3, 1.9) | 5.99 (dd, 6.3, 1.9) |
| 6        | a 1.65 (o) | 1.68 (o) | 1.64 (o) | 1.69 (m) |
|          | β 2.19 (ddd, 17.9, 6.3, 4.5) | 2.17 (ddd, 17.8, 6.3, 4.5) | 2.20 (ddd, 18.0, 6.3, 4.6) | 2.18 (ddd, 17.9, 6.1, 4.5) |
| 7        | 2.02 (qd, 11.0, 4.5) | 2.02 (m) | 1.99 (qd, 11.2, 4.6) | 1.99 (qd, 11.1, 4.5) |
| 8        | 2.17 (td, 11.8, 4.7) | 1.30 (o) | 1.77 (q, 12.2) | 1.78 (td, 12.7, 11.1) |
| 9        | a 1.65 (o) | 1.68 (o) | 1.91 (dt, 12.5, 4.9) | 1.94 (dt, 12.5, 4.9) |
|          | β 1.81 (m) | 1.83 (qd, 13.2, 4.3) | 1.77 (q, 12.2) | 1.78 (td, 12.7, 11.1) |
| 10       | 1.20 (td, 13.0, 4.3) | 1.24 (td, 12.9, 4.3) | 4.73 (dd, 11.2, 4.9) | 4.78 (dd, 11.1, 4.9) |
|          | β 1.85 (m) | 1.89 (ddd, 12.4, 4.3, 2.5) |  |  |
| 11       | 0.87 (ddd, 13.6, 11.2, 6.0) | 0.97 (ddd, 13.5, 11.2, 6.3) | 0.98 (ddd, 13.4, 11.2, 6.1) | 1.09 (ddd, 13.4, 11.3, 6.4) |
|          | 2.22 (ddd, 12.2, 7.4, 6.0) | 2.42 (ddd, 12.7, 8.0, 6.3) | 2.27 (ddd, 12.9, 6.9, 6.3) | 2.46 (ddd, 12.8, 8.1, 6.4) |
|          | β 1.39 (ddd, 13.6, 12.2, 7.0) | 1.33 (o) | 1.50 (td, 12.9, 7.1) | 1.44 (o) |
| 12       | 4.69 (bt, 7.4) | 5.65 (bt, 7.2) | 4.68 (bt, 7.3) | 5.64 (bt, 7.2) |
| 13       | 1.05 (s) | 1.04 (s) | 1.20 (s) | 1.18 (s) |
| 14       | 5.29 (qd, 6.9, 2.0) | 5.37 (qd, 6.9, 1.9) | 5.49 (qd, 6.9, 1.9) | 5.55 (qd, 6.9, 1.9) |
| 15       | 1.71 (d, 6.9) | 1.58 (d, 6.9) | 1.69 (d, 6.9) | 1.55 (d, 6.9) |
| 16-OAc   | 2.08 (s) | 2.09 (s) |  |  |
| 17       | 2.08 (s) | 2.09 (s) |  |  |

Table 2. 13C-NMR Data (150MHz, CD3OD) for Compounds 1–4

| Position | 1 | 2 | 3 | 4 |
|----------|---|---|---|---|
| 1        | 39.0 | 38.7 | 38.9 | 38.9 |
| 2        | 81.3 | 81.2 | 81.4 | 81.4 |
| 3        | 70.6 | 70.3 | 70.5 | 70.5 |
| 4        | 72.3 | 71.9 | 72.2 | 72.2 |
| 5        | 137.8 | 137.7 | 137.7 | 137.7 |
| 6        | 131.4 | 131.4 | 131.3 | 131.2 |
| 7        | 32.2 | 31.7 | 31.5 | 31.4 |
| 8        | 33.5 | 33.4 | 32.5 | 32.5 |
| 9        | 43.7 | 43.4 | 42.2 | 42.1 |
| 10       | 48.3 | 48.0 | 48.0 | 48.0 |
| 11       | 21.8 | 21.4 | 27.6 | 27.6 |
| 12       | 37.0 | 36.7 | 80.2 | 79.8 |
| 13       | 44.4 | 44.3 | 47.4 | 47.5 |
| 14       | 52.1 | 52.0 | 50.4 | 50.6 |
| 15       | 36.0 | 34.0 | 35.7 | 33.5 |
| 16       | 71.7 | 73.9 | 71.1 | 73.6 |
| 17       | 154.9 | 151.0 | 153.3 | 149.5 |
| 18       | 19.9 | 19.1 | 15.0 | 14.6 |
| 19       | 178.7 | 178.7 | 178.4 | 178.4 |
| 20       | 117.7 | 119.1 | 119.6 | 121.2 |
| 21       | 13.7 | 13.4 | 14.0 | 13.9 |
| 12-OCHOCH₃ | 172.5 | 172.48 |  |  |
| 12-OOCOCH₃ | 21.3 | 21.3 |  |  |
| 16-OOCOCH₃ | 172.6 | 172.54 |  |  |
| 16-OOCOCH₃ | 20.7 | 20.9 |  |  |

Chemical shifts (δ) are shown in ppm, (o) overlapped signal.

Fig. 2. Selected COSY, HMBC, and NOESY Correlations of 1
3 and 4 with the basic structural framework elucidated for 1 and 2. Compound 3 was readily determined to be a 12-acetoxy derivative of 1 by analysis of its MS and NMR data. The molecular formula of C\textsubscript{23}H\textsubscript{30}O\textsubscript{7}, which was established by (+)-HR-ESI-MS measurements, revealed the presence of an additional acetoxy group in 3. Its substitution at C-12 was assigned from the deshielded nature of H-12 (\(\delta_H\) 4.73) and C-12 (\(\delta_C\) 80.2), and an HMBC of H-12/C-O\textsubscript{CH3} (Fig. 3).

Similarly, 4 was identified as the 12-acetoxy derivative of 2. The \(\alpha\) orientation of the proton at C-12 in 3 and 4 was determined by 1D-NOE correlations observed from H-14 to H-12.

Compounds 1–4 were tested for cytotoxic activity against two colon carcinoma cell lines (COLO 205 and HT29) but were inactive at a high test concentration of 40 \(\mu\)M.

Conclusion

In the present study, we isolated four new pregnane 10,2-carbolactones which were identified as, \(3\beta, 4\beta, 16\beta\)-trihydroxypregna-5,17-diene-10,2-carbolactone (1), \(16\beta\)-acetoxy-\(3\beta, 4\beta\)-dihydroxypregna-5,17-diene-10,2-carbolactone (2), \(12\beta\)-acetoxy-\(3\beta, 4\beta, 16\beta\)-trihydroxypregna-5,17-diene-10,2-carbolactone (3), and \(12\beta, 16\beta\)-diacetoxy-\(3\beta, 4\beta\)-dihydroxypregna-5,17-diene-10,2-carbolactone (4) from an extract of an Epipolasis sp. marine sponge. The structures of these rare pregnane analogues were fully elucidated by interpretation of one- and two-dimensional NMR spectroscopic data. While a number of pregnane steroids have been described from marine sponges and other invertebrates,17–22 pregnanes possessing a 10,2-carbolactone ring have only been reported twice, from Hawaiian collections of Myrmekioderma sp.15) and Petrostia (Strongylophora) sp.16) The compounds reported herein were isolated from Epipolasis sp. collected in the Republic of Palau, which is geographically and taxonomically distinct from these previous sponge sources. Compounds 1–4 reveal new sites of oxidation at C-12 and C-16 in this family of pregnanes, and their occurrence in collections from Palau suggests that pregnane 10,2-carbolactone steroids are more widely distributed in sponges than previously recognized.

Experimental

General Experimental Procedures Optical rotations were measured on a Rudolph research analytical AUTOPOL IV polarimeter. ECD spectra were recorded on a JASCO J-1500 spectrophotometer. UV spectra were measured with a PerkinElmer, Inc. Lambda 465 UV/Vis photodiode array spectrophotometer. IR spectra were recorded with a Bruker ALPHA II FT-IR spectrometer. NMR spectra were obtained with a Bruker Avance III NMR spectrometer equipped with a 3 mm cryogenic probe and operated at 600 MHz for \(^1H\) and 150 MHz for \(^1\)C. (+)-HR-ESI-MS data were acquired on an Agilent Technology 6530 Accurate-mass Q-TOF LC/MS. HPLC was performed using a Varian ProStar 215 solvent delivery module equipped with a Varian ProStar 340 UV-Vis detector, operating under Star 6.41 chromatography workstation software. A solid phase extraction (SPE) was carried out with a Waters Oasis HLB (6 cc) cartridge.

Animal Material Specimens of the Epipolasis sp. sponge were collected on the west side of Tobi Island, Republic of Palau in December 1996 and kept frozen until extraction. The collection was carried out by the Coral Reef Research Foundation under contract with the Natural Products Branch, U.S. National Cancer Institute. A voucher specimen (voucher ID # 0CDN9998) was deposited at the Smithsonian Institution, Washington, D.C. The animal material (245.4 g, dry weight) was ground and processed using the standard NCI method for marine samples to provide 98.8 g of aqueous extract (NSC # C016988).23)

Isolation of Compounds A portion of the extract (5.5 g) was dissolved in MeOH and filtered to get a MeOH soluble fraction. The MeOH soluble fraction was dried and fractionated on an Oasis cartridge eluting in a stepwise manner...
with 100% H₂O, MeOH–H₂O (1:3), MeOH–H₂O (3:1), and MeOH–CH₂Cl₂ (9:1). The 75% MeOH eluted fraction and the MeOH–CH₂Cl₂ (9:1) eluted fraction were combined and separated by reversed-phased C₁₈ HPLC (Phenomenex Luna C₁₈(2) 5 µ, 100 Å, 250 × 21.2 mm) using a linear gradient of MeCN–H₂O (50:50 to 100:0) over 50 min to afford compounds 1 (4.3 mg), 2 (0.8 mg), 4 (2.9 mg), and fraction A. Fraction A was further purified by HPLC (Phenomenex Luna C₁₈(2) 5 µ, 100 Å, 250 × 21.2 mm) with MeCN–H₂O (30:70 to 80:20) for 50 min to obtain compound 3 (0.7 µg).

3β,4β,16β-Trihydroxypregna-5,17-diene-10,2-carbolactone (1) White amorphous powder. [α]D²⁵ -70 (c 0.1, CHCl₃); ECD (MeOH) nm (Δε): 205 (−1.6), 224 (−0.8); UV λmax (MeOH) nm (log ε): 197 (4.1); IR (neat) cm⁻¹: 3384, 2922, 1770, 1735; 1H-NMR data (Table 1); 13C-NMR data (Table 2); HR-ESI-MS m/z 441.1883 [M + Na]⁺ (Calcd for C₂₃H₃₀NaO₇, 441.1890).

16β-Acetoxy-3β,4β-dihydroxypregna-5,17-diene-10,2-carbolactone (2) White amorphous powder. [α]D²⁵ -107 (c 0.1, CHCl₃); ECD (MeOH) nm (Δε): 201 (−1.9), 225 (−0.7); UV λmax (MeOH) nm (log ε): 196 (4.3); IR (neat) 3349, 2924, 1770, 1729; 1H-NMR data (Table 1); 13C-NMR data (Table 2); HR-ESI-MS m/z 425.1918 [M + Na]⁺ (Calcd for C₂₁H₂₈NaO₅, 425.1935).

12β-Acetoxy-3β,4β,16β-trihydroxypregna-5,17-diene-10,2-carbolactone (3) White amorphous powder. [α]D²⁵ -104 (c 0.1, CHCl₃); ECD (MeOH) nm (Δε): 201 (−2.5), 225 (−0.9); UV λmax (MeOH) nm (log ε): 194 (4.3); IR (neat) 3349, 2922, 1772, 1733; 1H-NMR data (Table 1); 13C-NMR data (Table 2); HR-ESI-MS m/z 483.1991 [M + Na]⁺ (Calcd for C₂₅H₃₂NaO₈, 483.1995).

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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