



ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

New Bioactive Pregnadiene-derived Glycosides from the Gulf of California Gorgonian Muricea cf. austera

Jesús I. Murillo-Álvarez & Rosalba Encarnación-Dimayuga

To cite this article: Jesús I. Murillo-Álvarez & Rosalba Encarnación-Dimayuga (2003) New Bioactive Pregnadiene-derived Glycosides from the Gulf of California Gorgonian Muricea cf. austera, Pharmaceutical Biology, 41:7, 531-535, DOI: 10.1080/13880200308951348

To link to this article: https://doi.org/10.1080/13880200308951348



Published online: 29 Sep 2008.



Submit your article to this journal 🕑



View related articles

New Bioactive Pregnadiene-derived Glycosides from the Gulf of California Gorgonian *Muricea* cf. *austera*

Jesús I. Murillo-Álvarez and Rosalba Encarnación-Dimayuga

Departamento de Agronomía, Universidad Autónoma de Baja California Sur, Km 5.5 Carretera al Sur. 23080 La Paz, BCS, México

Abstract

Two new steroidal glycosides, pregna-5,20-diene-3-O- β -glucopyranoside (1), and pregna-5,20-diene-3-O- β -(6'-O-acetyl) glucopyranoside (2), and the known trihydroxy sterol, pregna-5-ene-3 β ,20 α ,21-triol (3) have been isolated from the Gulf of California gorgonian *Muricea* cf. *austera*. The structures of the new compounds were established on the basis of chemical and spectral studies. Compound 2, and its peracetylated derivative, 4, displayed moderate in vitro cytotoxicity (IC₅₀ = 17.3 and 14.3 µg/mL) toward HCT-116 human colon carcinoma. Compound 3 showed growth inhibition of *Staphylococcus aureus* and *Bacillus subtilis* at 250 µg/disk, in the agar disk-diffusion assay.

Keywords: Antibacterial activity, cytotoxicity, *Muricea austera*, steroidal glycosides, pregnadiene, Gulf of California.

Introduction

Marine octocorals of the order Gorgonacea are recognized as producing a extensive range of biologically active secondary metabolites (Faulkner, 1999). Recently, our research group assayed a number of octocorals from the Gulf of California belonging to the genus *Muricea*, and found interesting antibacterial activity (Encarnación-Dimayuga et al., 2000). On that basis, the gorgonian *Muricea* cf. *austera* Verrill (Plexauridae) was selected for study with the goal of the isolation of antibacterial compounds. Species of the genus *Muricea* have been shown to be rich sources of novel steroids (Block, 1974; Benito-Pruna et al., 1983; Popov et al., 1983; Bandurraga & Fenical, 1985), and sesquiterpenoids (Izac et al., 1982; Jeffs & Lytle, 1974). Recently, the isolation of degraded pregnanes from *Muricea* sp. was reported (Ortega et al., 2002). In this paper, we describe the isolation, identification, and biological activity of two new sterol glycosides, **1** and **2**, and the isolation of the previously reported trihydroxy sterol **3** (Encarnación & Rios, 1990).

Materials and methods.

General experimental procedures

Optical rotations were measured with an AUTOPOL III polarimeter (Rudolph Research Analytical, Flanders, NJ). Melting points were measured with a MELTEMP II (Laboratory Devices, USA) and are uncorrected. IR spectra were recorded in KBr pellets with a Perkin Elmer Paragon 500 spectrometer. MS data were obtained on an Agilent 1100LC-MS using electrospray ionization. Proton and carbon NMR spectra were recorded in a mixture of 50% CDCl₃ in CD₃OD with Varian Unity INOVA and Varian GEMINI spectrometers operating at 300 MHz for ¹H and 100 MHz for ¹³C. Tetramethylsilane was used as the internal reference. Column chromatography was done on silica gel 60 (230-400 mesh, Whatman) and ODS 60 (40 µm, Baker Analyzed). Preparative HPLC experiments were made using Partial 10 (20 \times 480mm, Whatman) as the stationary phase using a differential refractometer R401 (Water Associates, Inc.) as detector. TLC analyses were obtained using precoated silica gel 60 F_{254} and C_{18} reversed-phase plates (200 μ m thick, Whatman), and spots were visualized by ultraviolet illumination and by spraying with 10% sulfuric acid with 0.25% vanillin. Organic solvents (Productos Químicos de Monterrey, S. A. de C. V.) were glass distilled before using, excepting those that were HPLC grade.

Accepted: 21 August 2003

Address correspondence to: Dra. Rosalba Encarnación-Dimayuga, UABCS. Departamento de Agronomía. A. P. 19-B. 23080 La Paz, BCS, México. Tel.: (612) 128 05 69/147; Fax: (612) 128 08 80; E-mail: rosalba@uabcs.mx

Animal material

Several colonies of *M. austera* (1.52 kg) were collected in Bahía de La Paz (BCS, Mexico) in April 1999. The organisms were collected by scuba diving at a depth of 10 m, and kept in plastic bags at $-20 \,^{\circ}$ C until extraction. Martin Garcia performed taxonomic identification according to Verrill (1868). A voucher specimen (RED-9911-34) is deposited at the Laboratorio de Farmacognosia, Universidad Autónoma de Baja California Sur, for reference.

Extraction and isolation

The freshly chopped colonies (1.5 kg) were extracted 3-times with 2.5 L of 70% CH₂Cl₂ in MeOH at room temperature to give, after rotoevaporation, a crude extract (72g) that was suspended in the extraction mixture $(200 \text{ mL} \times 3 \text{ times})$ and filtered to remove inorganic salts and particles. The supernatant gave 45.3 g of a brown gum. The crude extract and the supernatant showed inhibitory activity against Bacillus subtilis and Staphylococcus aureus at 2.0 mg/disc by the agar disk-diffusion assay and moderate cytotoxicity against HCT-116 cells by the microdilution method. For this reason, the supernatant was bioguided-fractionated over silica gel in a step-gradient fashion (50% hexane in toluene, toluene 100%, 50% toluene in EtOAc, EtOAc 100%, and 50% MeOH in H_2O) to give 6 fractions (fraction 1 to 6), which were subjected to antibacterial and cytotoxic assay. Just fraction 4 (1.34 g) was active against B. subtilis and S. aureus, whereas fraction 5 and 6 displayed IC₅₀ values of 20 and $35 \mu g/mL$ against HCT-116 cells. Fraction 6 (14.235 g) was partitioned between EtOAc and H_2O . The organic layer (1.29 g) was chromatographed on silica gel (CH₂Cl₂ to MeOH) to yield nine fractions (fractions 6-1 to 6-9). The cytotoxic fraction 6-5 eluted with CH₂Cl₂: MeOH (93:7) was subjected to column chromatography on C₁₈ reverse-phase (70% MeOH in H₂O) to give 17.1 mg of the new pregna-5,20-diene-3-O- β -glucopyranoside (1), which was inactive against HCT-116 cells. Traces of a less polar compound (2) were identified as the cytotoxic principle in fraction 6-5. The same compound 2 was present in a larger amount in fraction 5 (400 mg), which was chromatographed over silica gel [toluene: EtOAc :MeOH (80:15:5) to (40:50:10)] to yield four fractions (fractions 5-1 to 5-4). Fraction 5-3 (220 mg), eluted with toluene: EtOAc: MeOH (40:50:10), was repeatedly chromatographed over ODS (with MeOH: H₂O mixtures) to give 20 mg of the new cytotoxic pregna-5,20-diene-3-O-β-(6'-Oacetyl)-glucopyranoside (2). Finally, the antibacterial fraction 4 eluted with 50% hexane in toluene was crystallized from cold MeOH to give 233 mg of the active pregna-5-ene- 3β ,20 α ,21-triol (3).

Pregna-5,20-diene-3-*O*-β-glucopyranoside (1)

White amorphous powder; mp 244–246 °C; IR (KBr) λ_{max} 3429, 2938, 1638, 1457, 1371, 1022, 904 cm⁻¹; ¹H NMR

(300 MHz) and ¹³C NMR (100 MHz) see Table 1; ¹H-¹H COSY (50% CDCl₃ in CD₃OD, 300 MHz) *H* no. cross-peaks *H*-1*a*: H-1b, -2b; *H*-2*a*: H-3; *H*-2*b*: H-1, -3; *H*-3: H-2ab, -4ab; *H*-4*a*: H-3, -6; *H*-6: H-4a, H-7ab: *H*-17: H-20; *H*-20: H-21ab; *H*-1': H-2'; *H*-5': H-6'ab; *H*-6'a: H-6'b; HMBC (50% CDCl₃ in CD₃OD, 300 MHz) *C* no. ²J and ³J correlations *C*-1: H-2a, -19; *C*-3: H-2ab, -4ab, -1'; *C*-5: H-4b; -19; *C*-6: H-4b, -7b; *C*-7: H-6, -8, -9; *C*-8: H-14, -15a; *C*-9: H-11b, -19; *C*-10: H-2a, -4b, -6, -19; *C*-11: H-9; *C*-13: H-15, -18; *C*-14: H-15b; *C*-16: H-17; *C*-17: H-16ab, -18, -20, -21ab; *C*-18: H-14; *C*-19: H-9; *C*-20: H-17, -21ab; *C*-21: H-17, -20; *C*-1': H-3, -2'; *C*-3': H-2'; *C*-5': H-4', -6'ab; *C*-6': H-5', -4'; ESIMS *m*/z 485 [M + Na]⁺, 463 [M + H]⁺, 283 [M – glucose]⁺, 230 (36).

Pregna-5,20-diene 3-*O*-β-(6'-*O*-acetyl)glucopyranoside (2)

White amorphous powder; mp 175–178 °C; IR (KBr) λ_{max} 3408, 2942, 1720, 1638, 1457, 1371, 1265, 1090, 1036, 904 cm⁻¹; ¹H NMR (300 MHz) and ¹³C NMR (100 MHz) see Table 1; ¹H-¹H COSY (50% CDCl₃ in CD₃OD, 300 MHz) *H* no. cross-peaks *H*-1*a*: H-2b; *H*-2*a*: H-3; *H*-3: H-2a, -4ab; *H*-6: H-7ab; *H*-7*b*: H-8; *H*-8: H-9, -14; *H*-9: H-11b: *H*-17: H-16b, -20; *H*-20: H-21ab, -17; *H*-1': H-2'; *H*-4': H-5'; *H*-5': H-6'ab; HMBC (50% CDCl₃ in CD₃OD, 300 MHz) *C* no. ²*J* and ³*J* correlations *C*-1: H-19; *C*-3: H-2b, -4ab, -1'; *C*-5: H-2b, -4a, -19; *C*-6: H-4b; *C*-7: H-6, -8, -9; *C*-8: H-14, -15a; *C*-9: H-7a, -19; *C*-10: H-4b, -6, -12b; *C*-11: H-9; *C*-13: H-14, -15b, -18; *C*-14: H-15b, -18; *C*-16: H-17; *C*-17: H-20,



Figure 1. Structure of compounds 1, 2, 3, and 4.

	1		2	
Carbon	$\delta_{\rm C}$ (DEPT)	$\delta_{\rm H} (J \text{ in Hz})$	$\delta_{\rm C}$ (DEPT)	$\delta_{\rm H} (J \text{ in Hz})$
1	38.3 (CH ₂)	1.10 m, 1.85 m	38.2 (CH ₂)	1.13 m, 1.86 m
2	30.4 (CH ₂)	1.64 m, 1.96 m	30.4 (CH ₂)	1.62 m 1.94 m
3	79.6 (CH)	3.60 m	80.2 (CH)	3.55 m
4	39.5 (CH ₂)	2.27 m, 2.43 m	39.5 (CH ₂)	2.29 m, 2.42 m
5	141.3 (C)		141.2 (C)	
6	122.3 (CH)	5.37 brd (4.5)	122.3 (CH)	5.38 brd (5.4)
7	32.9 (CH ₂)	1.60 m, 1.98 m	32.8 (CH ₂)	1.63 m, 1.98 m
8	33.0 (CH)	1.44 m	32.9 (CH)	1.51 m
9	51.6 (CH)	0.98 m	51.5 (CH)	0.99 m
10	37.7 (C)		37.6 (C)	
11	21.6 (CH ₂)	1.45 m, 1.56 m	21.5 (CH ₂)	1.44 m, 1.55 m
12	38.3 (CH ₂)	1.68, 1.94 m	38.2 (CH ₂)	1.72 m, 1.92 m
13	44.2 (C)		44.1 (C)	
14	56.9 (CH)	1.04 m	56.8 (CH)	1.09 m
15	25.9 (CH ₂)	1.17 m, 1.65 m	25.6 (CH ₂)	1.13 m, 1.74 m
16	28.1 (CH ₂)	1.55 m, 1.78 m	28.0 (CH ₂)	1.55 m, 1.76 m
17	56.4 (CH)	1.95 m	56.3 (CH)	1.98 m
18	13.2 (CH ₃)	0.62 s	13.1 (CH ₃)	0.63 s
19	19.8 (CH ₃)	1.03 s	19.8 (CH ₃)	1.03 s
20	140.2 (CH)	5.75 m	140.1 (CH)	5.75 m
21	114.7 (CH ₂)	4.95 d (15.3)	114.7 (CH ₂)	4.95 d (14.7)
		4.96 d (12.3)		4.96 d (12.3)
1′	102.0 (CH)	4.40 d (7.8)	102.2 (CH)	4.39 d (7.7)
2'	74.6 (CH)	3.19 dd (8, 7.8)	74.6 (CH)	3.21 dd (8, 7.7)
3'	77.3 (CH)	3.30 m	77.4 (CH)	3.32 m
4′	71.2 (CH)	3.34 m	71.2 (CH)	3.35 m
5'	77.6 (CH)	3.30 m	74.7 (CH)	3.45 m
6′	62.5 (CH ₂)	3.69 dd (5.1, 12.0)	64.5 (CH ₂)	4.23 dd (6.3, 12.0)
		3.86 dd (2.1, 12.0)		4.37 dd (2.4, 12.0)
1″			172.2 (C)	
2″			20.9 (CH ₃)	2.04 s

Table 1. ¹H and ¹³C NMR data for compounds 1 and 2.^a

^a In a mixture of 50% CDCl₃ in MeOD; Chemical shifts in ppm relative to TMS; coupling constant (\mathcal{J}) in Hz. Assignments were made in the basis of ¹H-¹H COSY, HMBC, and HMQC data.

-21ab; *C-18*: H-14, -17; *C-20*: H-17, H-21ab; *C-21*: H-17, -20; *C-1'*: H-3, -2'; *C-2'*: H-3', -4'; *C-3'*: H-2', -4'; *C-5'*: H-6'a; *C-1''*: H-6'ab, -2''; ESIMS *m*/*z* 527 [M + Na]⁺, 505 [M + H], 283 [M – acetyl glucose]⁺, 230 (4).

Pregna-5-ene-3 β ,20 α ,21-triol (3)

White needles; mp. 223–225 °C [lit.: 222–223 °C (acetone) (Kirk & Rowell, 1970)]; $[\alpha]^{24}$ D -35° (*c* 0.85, 10% MeOH in dioxane) [lit.: -34° (*c* 0.6, 10% MeOH in dioxane) (Kirk & Rowell, 1970)]; IR (KBr) λ_{max} 3408, 2931, 1638, 1465, 1371, 1095, 1047, 802 cm⁻¹; ¹H NMR (50% CDCl₃ in CH₃OH, 300 MHz) δ 1.08 (1H, m, H-1a), 1.83 (1H, m, H-1b), 1.52 (1H, m, H-2a), 1.84 (1H, m, H-2b), 3.42 (1H, m, H-3), 2.23 (1H, m, H-4a), 2.35 (1H, m, H-4b), 5.34 (1H, d, J = 5 Hz, H-6), 1.58 (1H, m, H-7a), 1.96 (1H, m, H-7b), 1.50 (1H, m, H-8), 0.96 (1H, m, H-9), 1.46 (1H, m, H-1a), 1.56 (1H, m,

H-11b), 1.16 (1H, m, H-12a), 1.86 (1H, m, H-12b), 1.10 (1H, m, H-14), 1.18 (1H, m, H-15a), 1.67 (1H, m, H-15b), 1.54 (1H, m, H-16a), 1.79 (1H, m, H-16b), 1.47 (1H, m, H-17), 0.74 (3H, s, H-18), 1.02 (3H, s, H-19), 3.58 (1H, ddd, J = 2.4, 7.8, 15.6 Hz, H-20), 3.35 (1H, dd, J = 7.8, 10.8 Hz, H-21a), 3.67 (1H, dd, J = 2.4, 10.8 Hz, H-21b). ESIMS m/z 357 [M + Na]⁺, 335 [M + H], 317 (19), 299 (14), 281 (8), 129 (2).

Acetylation of 1 and 2

Compounds 1 and 2 (3 mg) were individually treated with pyridine (50 μ L) and acetic anhydride (50 μ L) overnight at room temperature. The reaction mixture was evaporated to dryness to give 4 as a white amorphous powder, mp 190–192 °C. Compound 4 showed the following spectral features: ¹H NMR (50% CDCl₃ in CD₃OD, 300 MHz) δ 5.75 (1H,

br m, H-20), 5.39 (1H, d, J = 4.5 Hz, H-6), 5.25 (1H, dd, J = 9.3, 9.9 Hz, H-3'), 5.03 (1H, dd, J = 9.6, 9.9 Hz, H-4'), 4.97 (1H, m, H-21b), 4.93 (1H, m, H-21a), 4.89 (1H, dd, J = 8.4, 9.3 Hz, H-2'), 4.74 (1H, d, J = 8.4 Hz, H-1'), 4.29 (1H, dd, J = 4.8, 12.3 Hz, H-6'b), 4.11 (1H, dd, J = 2.1, 12.3, H-6'a), 3.83 (1H, ddd, J = 2.1, 4.8, 9.6 Hz, H-5'), 3.52 (1H, m, H-3), 2.07, 2.05, 2.02, 1.99 (each 3H, s, OCOCH3), 1.01 (3H, s, methyl in C-10), 0.62 (3H, s, methyl in C-13).

Biological testing

Extracts, fractions, and the isolated compounds were tested for growth inhibition against human colon carcinoma cells (HCT-116) cultured in 96-well microtiter plates in McCoys 5A medium, supplemented with 10% fetal bovine serum. Antibacterial activities were determined against *Bacillus subtilis* and *Staphylococcus aureus* using the standard agar disk-diffusion method (Encarnación et al., 2000).

Results and discussion

The specimens of *M. austera* used in this study were collected in Bahia de La Paz, Gulf of California, in April 1999. The animals were stored frozen and later extracted at room temperature with 30% MeOH in CH_2Cl_2 The solvent was then removed under vacuum to yield the crude extract. The extract (17% dry wt animal) was next fractionated by silica gel chromatography, using cytotoxicity and antibacterial assays as guides, to give **3** (0.09% based on dry wt). The more polar fractions from the chromatography were found to contain smaller amounts of two new sterol glycosides. Continued purification by repetitive silica gel chromatography yielded compounds **1** and **2**, at 0.0017% and 0.0016% dry wt of the animal.

Compound 1 with a pseudomolecular ion at m/z 463 $[M + H]^+$ was analyzed for the molecular formula $C_{27}H_{42}O_6$ by mass spectral analysis and by interpretation of ¹H and ¹³C NMR spectral data. The IR spectrum of 1 showed absorption for the presence of a hydroxyl and a vinyl group (3429, 1638 cm-1). The seven double-bond equivalents calculated from the molecular formula of 1 were ascribed by NMR to two double bonds (δ 141.3, 122.3, 140.2, 114.7), and the remaining double bond equivalents were assumed to be from five carbocyclic rings. A group of methine resonances between δ 3.19 and 4.40 (7H), in conjunction with their corresponding ¹³C NMR bands (δ 102.0, 74.6, 77.3, 71.2, 77.6, and 62.5) and the loss of a fragment of 179 amu by electrospray ionization, suggested the presence of a cyclized hexose functional group. The steroidal nature of 1 was shown by resonances in the ¹H NMR spectrum, showing the presence of two methyl singlets (δ 0.62, 1.03), a methine proton [δ 3.60 (1H, m)], and a methylene envelope between δ 2.4 and 1.2. By comprehensive analysis of 2D NMR data, in particular COSY, HMBC, and HMQC correlation data, the tetracyclic (aglycone) portion of 1 was readily identified as pregna-5,20dien-3β-ol. The vinyl group at C-17 was assigned by homonuclear correlation of the protons attached to C-17 and C-20, as well as H-20 and H-21ab. In the same way, heteronuclear correlation between the C-5 and the protons H-4b and those of the methyl at C-19 were observed by HMBC. The position of the second double bond at C-5 was confirmed by correlation between the vinylic proton at C-6 and H-7ab. The same aglycone has been isolated from the sponge *Damiriana hawaiiana* (Delseth et al., 1978), and the octocorals *Gersemia rubiformis* (Kingston et al., 1979), *Muricea fructicosa* (Bandurraga & Fenical, 1985), *Pseudoplexaura wagenaari* (Wasylyk et al., 1989), *Pieterfaurea unilobata* (Beukes et al., 1997), and *Eunicea* sp. (Cóbar et al., 1997).

The assignment of the hexose stereochemistry of this glycoside was difficult because of the considerable overlap of proton signals in the ¹H NMR spectrum of **1**. Acetylation of 1, however, cleanly provided the tetracetate derivative 4, which was more amenable to NMR analysis. The proton at the acetal carbon (δ 102.0) yielded a doublet with a typical axial coupling -constant ($J_{H-1' H-2'} = 8.4$ Hz), confirming that the sugar was in a hexopyranose configuration with an equatorial or β -glycoside linkage. The methine protons of the sugar at C-2', C-3', C-4', and C-5' showed coupling constants consistent with an all axial methine proton configuration, hence the sugar was confirmed as glucose. The D configuration for glucose was assumed to be the most commonly encountered in nature. A long-range HMBC correlation between C-3 of the aglycone and H-1' of the sugar indicated the linkage of the sugar moiety at C-3. These spectral data established 1 as a new natural product, pregna-5,20-diene-3-O- β -glucopyranoside.

Compound 2 was assigned the molecular formula $C_{29}H_{44}O_7$ on the basis of ESIMS data in conjunction with combined ¹H and ¹³C NMR spectral data (Table 1). The molecular ions of 1 and 2 showed a difference of 42 units corresponding to one acetyl group. When 1 was used as an NMR reference compound in the analysis of 2, the same aglycone was found in both metabolites. For the sugar moiety, close similarities were observed between with just an additional acetyl group [δ_C 172.2, 20.9, and δ_H 2.04 (3H, s)] being present in 2. The lower field shifts of the protons at C-6' [δ 4.23, dd (6.3, 12.0), and 4.37, dd (2.4, 12.0)] and the C-6' carbon (δ 64.5) indicated that acetylation had occurred at C-6' in this metabolite. This assignment was also confirmed by interpretation of HMBC data, in which correlation was observed between C-1" and H-6'ab and H-2". On the basis of these experiments, the structure of 2 was established as the new pregna-5,20-diene-3-O-\beta-(6'-O-acetyl) glucopyranoside. Acetylation of compound 2 gave the same peracetylated product, 4, as that derived from 1.

Compound 3, ultimately identified as the known pregna-5-ene-3 β ,20 α ,21-triol, was isolated as white needles, mp. 223–225 °C. The ESIMS for 3 showed a pseudomolecular ion at *m/z* 335 [M + H]⁺, which in combination with the ¹³C NMR spectral data, suggested the molecular formula C₂₁H₃₄O₃. On the basis of comprehensive 2D NMR spectral analysis, and by comparison of the physical data reported in the literature (Kirk & Rowell, 1970), we established the structure of compound **3** as pregna-5-ene- 3β ,20 α ,21-triol. This compound is a constituent of human newborn and infant body fluids (Gustafsson et al., 1969; Shackleton et al., 1971). Compound **3** was first isolated from a Gulf of California collection of *M. austera* in 1990 (Encarnación & Rios, 1990) and this gorgonian is the only-known marine source of this compound.

The crude extract, fractions, and pure compounds reported herein were examined for their in vitro cytotoxic and antibacterial properties. Though compound **1** was inactive for antibacterial and cytotoxic activity, the sterol glycoside **2** and its peracetylated derivative **4** showed moderate cytotoxicity toward HCT-116 human colon carcinoma in in vitro testing, with IC₅₀ values of 17.3 and 14.8 µg/mL. Compound **2** was present in fractions 5 and 6, and was responsible for the cytotoxicity initially shown by them. The trihydroxy sterol **3** was the major metabolite isolated. Biologically, **3** was the active compound against of the pathogenic bacteria *S. aureus* and *B. subtilis* at 250 µg/disc, but it did not show cytotoxic activity.

Acknowledgements

We thank the Mexican Science Council (CONACYT Grant No 43333100-5-34984-E) for their generous financial support for this work. We thank Dr. Lik Tan Tong, Scripps Institution of Oceanography, University of California in San Diego, for spectra acquisition and discussions. Diana Medina-Hernández and Sara Kelly are acknowledged for assistance in biological testing. Thanks to Dr. Ellis Glazier for editing the English-language text.

References

- Bandurraga M, Fenical W (1985): Isolation of muricins. Evidence of chemical adaptation against fouling in the marine octocoral *Muricea fructicosa* (Gorgonacea). *Tetrahedron* 41: 1057–1065.
- Benito-Pruna L, Llerena-Martinez E, Izquierdo-Lozano JC, Meras-Ceper M, Henriques RD, (1983): Chemical studies of Cuban gorgonians. IX. 24-methylenecholesterol in *Muricea muricata* gorgonia. *Rev Cubana Farm.* 17: 23–27.
- Beukes DR, Davies-Coleman MT, Eggleston DS, Haltiwanger RC, Tomkowicz B (1997): New polyhydroxylated pregnadienes from the South African soft coral *Pieterfaurea unilobata*. J Nat Prod 60: 573–577.

- Block JH (1974): Marine sterols from some gorgonians. *Steroids* 23: 421–424.
- Cóbar OM, Rodríguez AD, Padilla OL (1997): A new steroidal glycoside from a Caribbean gorgonian, *Eunicea* sp. J Nat Prod 60: 1186–1188.
- Delseth C, Carlson RMK, Djerassi C, Erdman TR, Scheuer PJ (1978): Identification of short side chain sterols in sponge Damiriana hawaiiana. Helv Chim Acta 61: 1470–1476.
- Encarnación R, Rios T (1990): Unpublished data.
- Encarnación-Dimayuga R, Franzblau SG, Tapia CA, Cedillo-Rivera R (2000): Screening of marine organisms for antimicrobial and antiprotozoal activity. *Pharm Biology 38*: 379–384.
- Faulkner J (1999): Marine natural products. *Nat Prod Rep 16*: 155–198, and papers in this series.
- Gustafsson JA, Shackleton CHL, Sjovall J (1969): Steroids in newborns and infants. C19 and C21 steroids in feces from infants. *Eur J Biochem 10*: 302–311.
- Izac RR, Bandurraga MM, Wasylyk JM, Dunn FW, Fenical W (1982): Germacrene derivatives from diverse marine softcorals (Octocorallia). *Tetrahedron 38*: 301–304.
- Jeffs PW, Lytle LT (1974): Isolation of (–)-α-curcumene, (–)-βcurcumene and (+)-β-bisabolene from Gorgonian corals. Absolute configuration of (–)-β-curcumene. *Lloydia 37*: 315–317.
- Kingston JF, Gregory B, Fallis AG (1979): Marine natural products: Novel C21 pregnanes from the sea raspberry (*Gersemia rubiformis*) J Chem Soc Perkin Trans 1: 2064–2068.
- Kirk DN, Rowell FJ (1970): Urinary steroids. VIII. Exploration of routes to pregnane-20α,21-diol derivatives. J Chem Soc (C): 1498–1502.
- Ortega MJ, Zubía E, Rodríguez S, Carballo JL, Salvá J (2002): Muricenones A and B: Degraded pregnanes from a Gorgonian of the genus *Muricea*. Eur J Org Chem 19: 3250–3253.
- Popov S, Carlson RMK, Djerassi C (1983): Occurrence and seasonal variation of 19-norcholest-4-en-3-one and 3βmonohydroxy sterols in the Californian gorgonian, *Muricea californica. Steroids* 41: 537–548.
- Shackleton CHL, Gustafsson JA, Sjovall J (1971): Steroids in newborns and infants. Identification of steroids in urine from newborn infants. *Steroids* 17: 265–280.
- Verrill AE (1868): Notes on Radiata in the Museum of Yale College. Review of the corals and polyps of the west coast of America. *Trans. Connecticut Acad 1*: 377–422.
- Wasylyk JM, Martin GE, Weinheimer AJ, Alam M (1989): Isolation and identification of a new pregnene glycoside from the gorgonian *Pseudoplexaura wagenaari*. J Nat Prod 52: 391–394.