Muricenones A and B: New Degraded Pregnanes From a Gorgonian of the Genus *Muricea*

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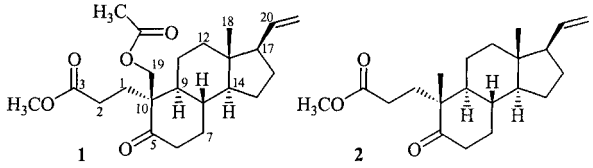
Keywords: Natural products / Octocorals / Steroids / Structure elucidation / Cytotoxicity

The gorgonian *Muricea* sp. contains two new degraded pregnanes, muricenones A (**1**) and B (**2**), whose structures have been defined by spectroscopic analysis. The uncommon carbon framework of muricenones may be biosynthetically derived by oxidative cleavage and loss of one carbon atom of the A-ring of the steroidal nucleus. The new compounds, **1** and **2**, isolated from *Muricea* sp., selectively inhibit the growth of the A-549 human lung carcinoma cell line.

Introduction

Marine invertebrates are widely recognized as possessing a vast array of steroidal metabolites with unusual structures. Among marine steroids, those displaying a C_{21} pregnane skeleton constitute a minor group of metabolites that have been isolated from sponges,[1−4] echinoderms,[5−6] and octocorals.[7−19] In particular, this latter group of invertebrates has been the main source of marine pregnanes, all of which are structurally characterized as possessing an uncommon vinyl side chain.

As a part of our project directed towards the search for pharmacologically active metabolites from marine octocorals, we examined specimens of a gorgonian of the genus *Muricea,* collected in the Bay of Mazatlán (Mexico). Previous chemical studies of this genus of octocorals led to the isolation of germacrane sesquiterpenes from *Muricea austera* and *M. fungifera*,[20] while *M. fruticosa* was found to contain the new pregnane glycosides muricins-1 through -4, which seem to play an important role in reducing fouling on *M. fruticosa*.[13] Finally, ergosterol peroxide was the only secondary metabolite isolated from *M. californica*.[13] In this paper, we report the isolation, structure, and cytotoxicity of two new degraded pregnanes, muricenone A (**1**) and muricenone B (**2**), from the gorgonian *Muricea* sp. collected at Mazatlán (Mexico).



Results and Discussion

Specimens of *Muricea* sp. were collected by hand and immediately frozen. The frozen material was freeze-dried and subsequently extracted with acetone. After evaporation of the solvent, the residue was partitioned between H_2O and Et_2O. Column chromatography of the organic extract, followed by HPLC separation of selected fractions, allowed for isolation of the new compounds muricenone A (**1**) and muricenone B (**2**).[21]

Muricenone A (**1**) was isolated as an optically active oil. The molecular formula $C_{23}H_{34}O_5$, obtained from the high resolution mass spectrum, indicated seven degrees of unsaturation.

The ^{13}C NMR spectrum (Table 1) exhibits three carbonyl signals at δ = 211.1 (s), 174.1 (s) and 170.9 ppm (s), attributable to one ketone and two ester groups, respectively, as well as two olefinic carbon signals at δ = 139.1 (d) and 115.1 ppm (t) assigned to a monosubstituted double bond. As these functional groups account for all the oxygen atoms of the molecular formula and for four degrees of unsaturation, it was concluded that compound **1** was tricarbocyclic.

The ester functions present in **1** were identified as one acetoxyl group and a methoxycarbonyl group upon observation of singlets in the ^1H NMR spectrum (Table 1) at δ = 2.00 (s, 3 H) and 3.66 ppm (s, 3 H), which are correlated in the HMBC spectrum with the carbonyl signals at δ = 170.9

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Table 1. NMR spectroscopic data for muricenones A (**1**) and B (**2**) in $CDCl_3$ [a]

Carbon atom.	**1**		**2**	
	δ_C (mult.)	δ_H [mult., *J* (Hz)]	δ_C (mult.)	δ_H [mult., *J* (Hz)]
1	26.1 (t)	2.41 (m), 1.60 (m)	29.5 (t)	1.30 (m), 1.60 (m)
2	28.9 (t)	2.37 (m) 2.19 (ddd, 16.6, 11.7, 4.8)	29.1 (t)	2.32 (m), 2.19 (m)
3	174.1(s)	–	174.4 (s)	–
5	211.1(s)	–	214.7 (s)	–
6	39.0 (s)	2.52 (ddd, 14.3, 14.3, 6.1)β 2.33 (m)α	38.1 (t)	2.55 (ddd, 14.5, 14.5, 6.3)β 2.27 (m)α
7	31.4 (t)	2.03 (m), 1.20 (m)	31.3 (t)	1.96 (m), 1.20 (m)
8	35.4 (d)	1.84 (m)β	34.9 (d)	1.74 (m)β
9	48.2 (d)	1.32 (ddd, 12.0, 11.2, 10.8)α	48.1 (d)	1.25 (m)α
10	53.8 (s)	–	50.5 (s)	–
11	21.9 (t)	1.45 (dddd, 12.4, 12.4, 12.4, 3.8)β 1.54 (m)α	21.0 (t)	1.48 (m), 2.10 (m)
12	37.1 (t)	1.02 (m)α, 1.72 (bd, 12.8)β	36.9 (t)	1.06 (m), 1.73 (m)
13	43.5 (s)	–	43.5 (s)	–
14	55.2 (d)	1.02 (m)	55.1 (d)	1.06 (m)
15	24.6 (t)	1.72 (m)α, 1.27 (m)β	24.8 (t)	1.27 (m), 1.72 (m)
16	26.9 (t)	1.58 (m)β, 1.82 (m)α	27.0 (t)	1.81 (m), 1.60 (m)
17	55.0 (d)	1.96 (bq, 7.7)	54.9 (d)	2.00 (m)
18	12.9 (q)	0.65 (s)	12.8 (q)	0.66 (s)
19	67.3 (t)	4.63 (d, 11.3),4.05 (d, 11.3)	20.5 (q)	1.12 (s)
20	139.1(d)	5.73 (ddd, 17.0, 10.4, 7.7)	139.4 (d)	5.75 (ddd, 16.9, 10.6, 7.6)
21	115.1 (t)	4.96 (ddd, 17.0, 2.1, 1.1) 4.99 (ddd, 10.4, 2.1, 1.3)	114.9 (t)	5.00 (ddd, 10.6, 2.2, 1.0) 4.97 (ddd, 16.9, 2.2, 1.2)
$OCOCH_3$	20.8 (q)	2.00 (s)	–	–
$OCOCH_3$	170.9 (s)	–	–	–
OCH_3	51.6 (q)	3.66 (s)	51.5 (q)	3.66 (s)

[a] Assignments were aided by COSY, HMQC and HMBC experiments.

(s) and 174.1 ppm (s) respectively. Furthermore, the ^1H NMR spectrum shows two signals of an AB system at δ = 4.63 (d, *J* = 11.3 Hz, 1 H) and 4.05 ppm (d, *J* = 11.3 Hz, 1 H) that were assigned to a methylene linked to the acetoxyl group and to a quaternary carbon.

A careful analysis of the COSY, HMQC and HMBC spectra allowed us to determine the presence of the subunit A (Figure 1) in the structure of **1**. The correlations exhibited in the HMBC spectrum are especially diagnostic and in particular the cross peaks observed between the signal of the proton geminal to the acetoxyl group at δ = 4.05 ppm with the ketone carbonyl signal at δ = 211.1 ppm (s) and with the sp^3 carbon signal of a methylene at δ = 26.1 ppm (t). This latter signal is, in addition, correlated with the methylene proton signals at δ = 2.37 (m, 1 H) and 2.19 ppm (ddd, *J* = 16.6, 11.7, 4.8 Hz, 1 H) which, in turn, show

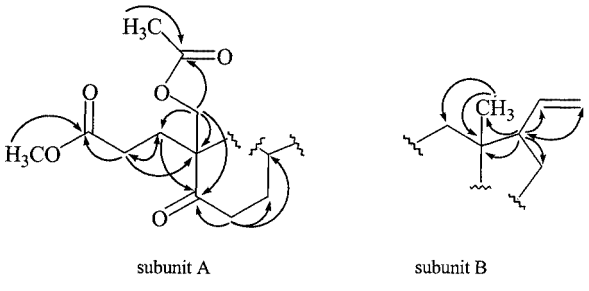


Figure 1. HMBC correlations for subunits A and B of compound **1**

cross peaks with the signal of the methoxycarbonyl group at δ = 174.1 ppm (s), defining the presence of a $-CH_2CH_2COOCH_3$ chain. Furthermore, the ^1H NMR signals at δ = 2.52 (ddd, *J* = 14.3, 14.3, 6.1 Hz, 1 H) and 2.33 ppm (m, 1 H), assigned to a methylene adjacent to the ketone carbonyl, are exclusively correlated in the COSY spectrum with the signals of another methylene at δ = 2.03 (m, 1 H) and 1.20 ppm (m, 1 H), while in the HMBC spectrum the signal at δ = 2.52 ppm shows a three-bond correlation with a methyne carbon signal at δ = 35.4 ppm (d).

The ^1H NMR signals at δ = 5.73 (ddd, *J* = 17.0, 10.4, 7.7 Hz, 1 H), 4.99 (ddd, *J* = 10.4, 2.1, 1.3 Hz, 1 H) and 4.96 ppm (ddd, *J* = 17.0, 2.1, 1.1 Hz, 1 H) were assigned to the monosubstituted double bond located on a methyne, whose proton signal at δ = 1.96 ppm (br. q, *J* = 7.7 Hz, 1 H) is correlated in the COSY spectrum both with the olefinic proton signal at δ = 5.73 ppm and with the methylene proton signals at δ = 1.82 (m, 1 H) and 1.58 ppm (m, 1 H). In addition, the upfield region of the ^1H NMR spectrum shows a singlet at δ = 0.65 ppm (s, 3 H) assigned to a methyl group linked to a quaternary carbon. The two- and three-bond correlations observed in the HMBC spectrum for the signals of the allylic methine and the methyl group mentioned above are in agreement with the presence of the subunit B (Figure 1) in the structure of compound **1**.

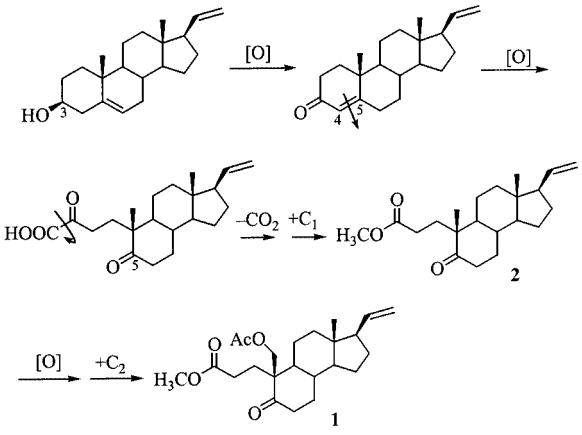
At this point the four remaining carbon signals in the ^{13}C NMR spectrum at δ = 21.9 (t), 24.6 (t), 48.2 (d) and

55.2 ppm (d) are due to two aliphatic methylenes and two aliphatic methynes respectively, that must link subunits A and B (Figure 1). In fact, in the HMQC spectrum, the carbon signal at δ = 21.9 ppm (t) is correlated with the proton signals at δ = 1.45 (dddd, *J* = 12.4, 12.4, 12.4, 3.8 Hz, 1 H) and 1.54 ppm (m, 1 H).This latter signal shows correlations in the HMBC spectrum with the signals at δ = 35.4 (d) and 43.5 ppm (s), assigned to a methyne and a quaternary carbon in subunits A and B, respectively. Furthermore, the carbon signal at δ = 48.2 ppm (d) is correlated in the HMQC spectrum with the signal at δ = 1.32 ppm (ddd, *J* = 12.0, 11.2, and 10.8 Hz, 1 H), which in the HMBC spectrum shows cross peaks with the carbon signal assigned to the methylene carbon of the acetoxymethyl group in subunit A at δ = 67.3 ppm (t) and with the methylene carbon signal at δ = 21.9 ppm (t). These data, together with the additional correlations observed in the COSY, HMQC and HMBC spectra, and considering the tricarbocyclic nature of compound **1**, allowed us to define completely the planar structure of muricenone A (**1**).

Finally, a series of NOE experiments defined the relative stereochemistry of compound **1**. Thus, irradiation of the Me-18 signal at δ = 0.65 ppm caused, among others, enhancements of the H-8 and H-20 signals, while irradiation of the signal at δ = 4.63 ppm of a methylene proton adjacent to the acetoxyl group caused enhancements of the H-6β (axial) and H-8 signals. Finally, irradiation of the H-2 signal at δ = 2.19 ppm caused an NOE enhancement of the H-9 signal. All these data indicate a β orientation of the vinyl side chain, H-8, Me-18 and the acetoxymethyl group on C-10, while the H-9 and the chain on C-10 are α oriented.

Muricenone B (**2**) was obtained as an optically active oil with a molecular formula $C_{21}H_{32}O_3$, as indicated by the high resolution mass measurement. The NMR spectra of **2** are closely similar to those of compound **1**, except for the absence of the signals due to the acetoxymethyl group and the presence of signals for a methyl group at δ_H = 1.12 (s, 3 H) and δ_C = 20.5 (q). It was therefore proposed that muricenone B (**2**) is the 19-deacetoxy derivative of muricenone A (**1**). A careful study of the COSY, HMQC and HMBC spectra of **2** allowed us to confirm the proposed structure and to fully assign the ^1H and ^{13}C NMR spectra of compound **2** (Table 1). Furthermore, the relative stereochemistry of **2** was secured by a series of NOE experiments. Thus, irradiation of H-6β (axial) at δ = 2.55 ppm caused an enhancement of the Me-19 singlet at δ = 1.12 ppm, while irradiation of the Me-18 signal at δ = 0.66 ppm caused enhancements of the H-8 and H-20 signals.

The new metabolites muricenones A (**1**) and B (**2**) are degraded pregnanes characterized by the cleavage and loss of one carbon atom from the A-ring of the steroidal nucleus. From a biosynthetic point of view, compounds **1** and **2** could be biosynthesized from a pregnane such as that shown in Scheme 1 by oxidation of the hydroxyl group at C-3 and double bond isomerization to give an α,β-unsaturated ketone, followed by oxidative cleavage of ring A and decarboxylation of the resulting α-ketoacid. Although the starting pregnane of Scheme 1 was not found in the extracts of *Muricea* sp., it is a known metabolite found in the octocoral *Gersemia rubiformis*[9] and as the aglycon of the muricins isolated from *Muricea fruticosa*.[13] The carbon skeleton of muricenones A (**1**) and B (**2**) has not been found among natural products. Recently, however, in a novel partial synthesis developed for the preparation of (2,3,4−$^{13}C_3$)-testosterone and progesterone, synthetic intermediates displaying this skeleton have been obtained by oxidative cleavage of the steroid A-ring.[22]



Scheme 1. Proposed biosynthetic pathway for muricenones A (**1**) and B (**2**)

The new compounds isolated from *Muricea* sp. were tested in bioassays to detect in vitro activity against A-549 human lung carcinoma and HT-29 human colon carcinoma cell lines. The parameters measured were: GI_{50} (concentration that causes 50% growth inhibition), TGI (concentration that causes total growth inhibition; a cytostatic effect) and LC_{50} (concentration that causes 50% cell killing; a cytotoxic effect).

Compounds **1** and **2** show a significant and selective activity as inhibitors of the growth of A-549 cells with GI_{50} values of 2 and 3 μg/mL, respectively. Furthermore, both compounds show a mild cytostatic effect on the A-549 cell line, with a TGI of 8 μg/mL, while only compound **1** shows a cytotoxic effect against this line, with an LC_{50} of 10 μg/mL.

Conclusion

The new metabolites muricenones A (**1**) and B (**2**), obtained from a Pacific gorgonian of the genus *Muricea*, are degraded pregnanes which inhibit the growth of the A-549 human lung carcinoma cell line. Although several examples of highly degraded steroids are known in the marine environment,[23−26] muricenones A (**1**) and B (**2**) display an uncommon carbon framework most likely derived by oxidative cleavage and loss of one carbon atom from the A-ring of the steroidal nucleus of a pregnane precursor.

Experimental Section

General Remarks: Optical rotations were measured on a Perkin−Elmer 241 polarimeter, and IR spectra were recorded on a Genesis Series FT IR Mattson spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz respectively, on a Varian Unity 400 spectrometer, using $CDCl_3$ as solvent. Proton chemical shifts were referenced to the residual $CHCl_3$ signal at δ = 7.26 ppm, and ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at δ = 77.0 ppm. NOE experiments, ^1H-^1H correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond coherence (HMBC) were performed using standard Varian pulse sequences. Low resolution mass spectra were recorded on a Finnigan Voyager GC8000^{top} spectrometer. High resolution mass spectra (HRMS) were obtained on a VG Autospec spectrometer. Column chromatography was carried out on Merck Silica gel 60 (70−230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrosorb RP-18 (Merck) and LiChrosorb Si 60 (Merck) columns using a differential refractometer RI-71. All solvents were spectral grade or were distilled prior to use.

Biological Material: Specimens of *Muricea* sp. were collected by hand by means of scuba diving in the Bay of Mazatlán (Sea of Cortez, Mexico) and immediately frozen. A voucher specimen (M-28−61−68) has been deposited at the Laboratorio de Ecología del Bentos, Instituto de Ciencias del Mar y Limnología, UNAM (Mexico). Taxonomy: Phylum Cnidaria, Class Anthozoa, Subclass Octocorallia, Order Gorgonacea, Family Plexauridae. Description: *Muricea* sp. is a branched yellow gorgonian, 10−15 cm high and 20−25 cm wide, with branches usually in only one plane; the branches are up to about 3.5 mm in diameter; sclerites are yellow, and the longer ones are tuberculated spindles up to 1.7 mm in length.

Extraction and Isolation: Frozen specimens of *Muricea* sp. were lyophilized, and the freeze-dried material (159.8 g) was extracted with acetone (1.5 L). After filtration, the acetone solution was evaporated under reduced pressure to obtain a residue that was partitioned between H_2O and Et_2O. The organic layer was concentrated under reduced pressure to give an orange oil (1.8 g), that was chromatographed on a SiO_2 column using solvents of increasing polarity, from hexane to Et_2O, then EtOAc, and finally mixtures of increasing polarity from $CHCl_3$ to MeOH. Fractions eluted with hexane/Et_2O (7:3) were further separated on a SiO_2 column eluting with $CHCl_3$/MeOH (99:1). Selected fractions were subjected to normal-phase HPLC eluting with hexane/EtOAc (9:1) and finally purified by repeated reversed-phase HPLC eluting with MeOH/H_2O mixtures (93:7 and 9:1) to yield muricenone B (**2**, 6.5 mg, 0.004% yield). Fractions of the general chromatography eluted with hexane/Et_2O (1:1) were further separated on a SiO_2 column eluting with $CHCl_3$/MeOH (99:1). Selected fractions were purified by reversed-phase HPLC with MeOH/H_2O (9:1) as eluent to obtain muricenone A (**1**, 24.8 mg, 0.016% yield).

Muricenone A (1): Yellow oil. $[\alpha]_D^{25}$ = +24.0 (c = 0.1, $CHCl_3$). IR (film): $\tilde{\nu}$ = 1740, 1712 cm^{-1}. EIMS (70 eV): m/z (%) = 390 (1) [M^+], 330 (17) [M^+ − AcOH]. HREIMS m/z = 390.2404, calculated for $C_{23}H_{34}O_5$ $(M)^+$ 390.2406. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are listed in Table 1.

Muricenone B (2): Colorless oil. $[\alpha]_D^{25}$ = +15.0 (c = 0.1, $CHCl_3$). IR (film): $\tilde{\nu}$ = 1740, 1705 cm^{-1}. EIMS (70 eV): m/z (%) = 332 (7) [M^+], 317 (17) [$M^+ - CH_3$], 245 (100) [$M - C_4H_7O_2$]. HREIMS m/z = 332.2347, calculated for $C_{21}H_{32}O_3$ $(M)^+$ 332.2351. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are listed in Table 1.

Acknowledgments

This research was supported by grants from MCYT (research project PPQ2001-0020) and from the Junta de Andalucía (FQM-285). Cytotoxicity assays were performed through a cooperation agreement with PharmaMar S.A., which includes a gratefully acknowledged fellowship awarded to S. R.

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Received April 18, 2002
[O02215]