Muriceanol, a 24(28)-Epoxide Sterol Link in the Carbon Flux Toward Side-Chain Dealkylation of Sterols

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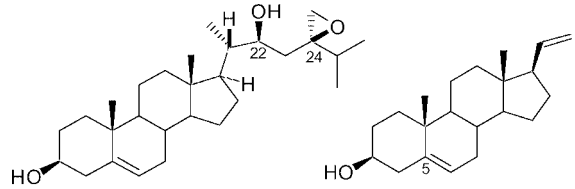
Side-chain-oxidized C-28-sterol **1** and one new pregnane metabolite **2** were isolated from eastern Pacific *Muricea* spp. The C-24(28)-epoxide functionality is a key intermediate in the C-24-dealkylation mechanism of the conversion of phytosterol to cholesterol by phytophagous insects. Certain marine invertebrates share this dealkylation pathway; however, such a key epoxide feature has not yet been found in a naturally occurring sterol from marine invertebrates. The unusual oxidation pattern of the side chain of **1** encourages speculation about its biogenesis and converts this non-zooxanthellate gorgonia into an interesting candidate organism for biosynthetic studies on C-24-dealkylation of phytosterols in octocorals. The (22*S*)-22-hydroxy group, after 24-dealkylation of **1**, may be an advantageous functionalization in the side-chain cleavage to C-21-pregnane steroids in *Muricea* spp.

Introduction

The mechanism of dealkylation of sterols is an important biochemical transformation that involves the oxidation of the unsaturation at C-24 to a key 24(28)-epoxide intermediate in the carbon flux toward the essential end product cholesterol.[1] Although the dealkylation of the sterol side chain is unusual in the marine environment, it is known that certain mollusc arthropods, coelenterates and sponges utilize this pathway.[2] This reaction proceeds through the same epoxide intermediate operative in insects.[3] Such a biosynthetic intermediate has been detected in a cell-free extract of sponges.[4] We herein provide the first report of a naturally occurring 24(28)-epoxidized side-chain-containing sterol in marine invertebrates.

Muriceanol (**1**) is the C-24(28)-epoxide derivative of 24-methylenecholesterol, hydroxylated at C-22. This metabolite, together with the pregnanes **2** and **3**, was obtained from the crude extract of the octocoral *Muricea* spp, collected at Pacheca Island, Gulf of Panama, after flash chromatography, followed by HPLC. Structure **2** was shown to be identical to a synthetic compound obtained from 3β-hydroxy-5α-pregnan-20-one.[5] Compound **3** has been previously isolated from the soft coral *Gersemina rubiformis* (Figure 1).[6]



1 **2**: Δ^5-dihydro **3**

Figure 1. Muriceanol and pregnane structures.

Muricea spp (Anthozoa, Octocorallina, Alcyonacea, Plexauridae) was mostly found in crevices and lateral cracks where sunlight is considerably reduced, and very rarely attached to the upper surface of rocks directly exposed to sunlight. This distribution pattern is in agreement with non-zooxanthellate corals. Microscopic examination confirmed the absence of endosymbiotic algae in tissues from the collected specimens.

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Results and Discussion

Compound **1** was isolated as an oil. NMR spectroscopic data coupled with a molecular ion peak at *m/z* = 430 (HREIMS) suggest a molecular formula of $C_{28}H_{46}O_3$, indicating six degrees of unsaturation. The ^{13}C NMR spectroscopic data and a DEPT NMR experiment are consistent for a Δ^5-C-28 sterol having two hydroxy groups, when compared with the reported values for a tetracyclic cholestane nucleus.[7] For example, the resonance at δ = 5.34 ppm in its ^1H NMR spectrum is characteristic of a Δ^5-vinylic proton; the ^{13}C NMR signals at δ = 140.8 and 121.6 ppm are also in agreement with those of C-5/C-6, respectively, of a 3β-hydroxy-Δ^5 moiety; the ^1H NMR singlets at δ = 0.68 and 1.00 ppm agree with those expected for the C-18/C-19 angular methyl groups of a Δ^5-sterol, thus confirming the identity of a tetracyclic cholestane. The corresponding peak at *m/z* = 271 in the mass spectrum corroborats such a steroidal structure.

Having established the basic structure, in the absence of other unsaturation besides the Δ^5-olefin, an oxirane ring must be placed on the side chain, since the remaining oxygen atom is part of a hydroxy group and a signal for a methylene group bearing an oxygen atom is present at δ = 51.4 ppm in the ^{13}C NMR spectrum of **1**. The HMBC correlation between one quaternary carbon atom bearing an oxygen atom and the COSY-correlated secondary methyl groups, Me-26 and Me-27, indicates that the 24(28)-methyl group, typical of a phytosterol side chain, was epoxidized. The HMBC correlation of the remaining C-21 atom with a proton (22-H) geminal to a secondary hydroxy group allowed the regiochemistry of the oxygen atoms in the side chain to be established.

The relative stereochemistry of the substituents on the side chain of **1** was assigned on the basis of a 2D NOESY experiment, molecular mechanics calculations and coupling constants. In order to obtain an energy-minimized conformation of **1** to justify the observed NOE between 22-H and one proton of 28-H_2, molecular mechanics energy minimization was performed.[8] The minimized structures **1**–**1c** and the corresponding calculated energy values ΔE, are depicted in Figure 2. Whereas all of them show a hydrogen bond between the hydroxy proton and the oxirane ring, **1** and **1a** place one of the oxymethylene protons at suitable interatomic distance to allow the observed NOE with 22-H. The theoretical coupling constants (**1**: *J* = 1.1, 1.8, 11.5 Hz; **1a**: *J* = 2.7, 9.3, 11.7 Hz) given by the program for these compounds indicate that the configuration (20*S*,22*S*,24*R*) represented in **1** (Figure 2) is consistent with the measured *J* values (**1**: *J* = 1.9, 1.9, 9.4 Hz; Table 1). Although the conformation **1c** possesses a similar calculated energy value to **1** (ΔE = 0.202 kcal/mol) and also similar theoretical coupling constants, the orientation of its oxymethylene protons does not match the observed NOE with 22-H. Thus, the stereoisomer depicted in **1** is consistent with the measured *J* values of the selected protons and the observed NOEs. The occurrence of only one C-24 stereoisomer indicates that the formation of the epoxide proceeds, unlike in insects,[9] with a high degree of stereospecificity.

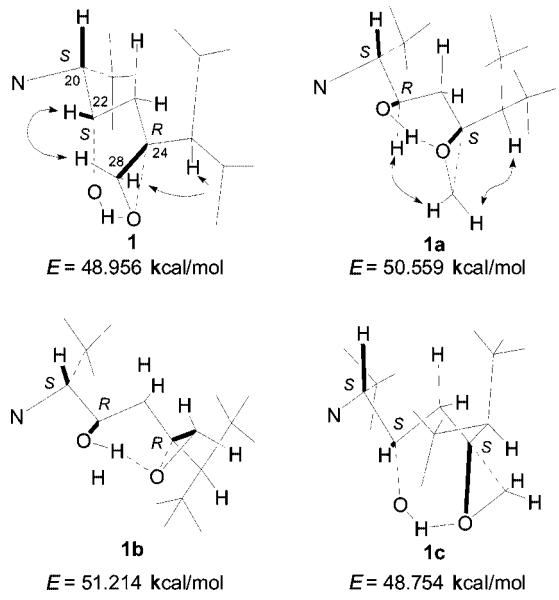


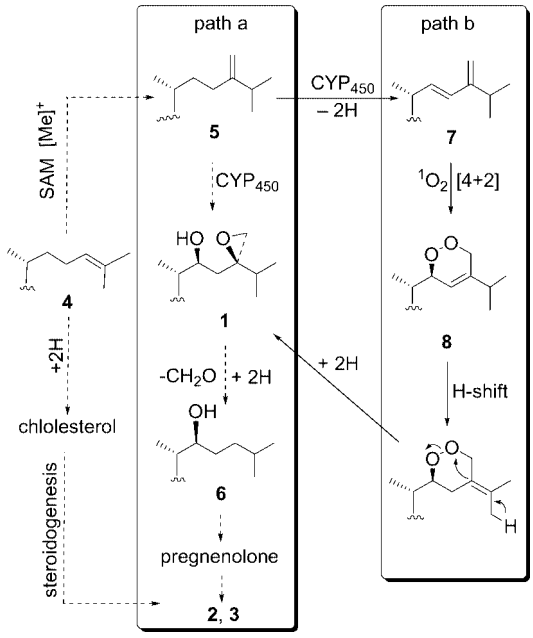
Figure 2. Selected NOEs of muriceanol (**1**).

Table 1. ^1H and ^{13}C NMR data of compound **1**; 500 MHz, $CDCl_3$, δ [ppm] (*J* [Hz]).

#	δ_H	δ_C
1	1.10 m, 1.80 m	37.2
2	1.50 m, 1.83 m	31.6
3	3.50 dddd (4.6, 4.6, 11.0, 11.0)	71.7
4	2.27 m, 2.30 m	42.3
5	–	140.8
6	5.34 m	121.6
7	1.50 m, 1.95 m	31.6
8	1.50 m	32.5
9	0.94 ddd (4.8, 12.0, 12.0)	50.1
10	–	36.5
11	1.53 m	21.1
12	1.98 m, 1.20 ddd (4.8, 13.4, 13.4)	39.8
13	–	42.2
14	1.10 m	56.6
15	1.30 m, 1.80 m	27.7
16	1.10 m, 1.70 m	24.2
17	1.46 m	52.5
18	0.68 s	11.7
19	1.00 s	19.4
20	1.33 m	41.8
21	0.90 d (6.7)	12.1
22	3.93 ddd (1.9, 1.9, 9.4)	69.9
23	1.62 m, 1.70 m	36.4
24	–	62.3
25	1.82 m	31.9
26	0.88 d (6.9)	17.4
27	0.98 d (6.8)	18.6
28	2.64 d (4.5), 2.74 d (4.5)	51.4

Compound **1** has a unique and intriguing oxygenated side chain. The C-24(28)-epoxide functionality suggests it is a biosynthetic intermediate (Scheme 1, path a) in the carbon flux toward cholestane-based sterol **6** and then to steroidogenic end products, for instance **2**, **3**. A mechanism for C-24 dealkylation in insects was proposed by Ikekawa,[9] and it is believed to involve the loss of the epoxidized C-24 methyl group as formaldehyde (**1** → **6**). This was later

experimentally confirmed in the dealkylation of the sterol side chain in sponges.[10] The (22*S*)-22-hydroxy group of **1** (path a) appears to be an advantageous functionalization for steroidogenic side-chain cleavage when compared to cholesterol since the conversion of **6** proceeds at twice the rate of that of cholesterol.[11]



Scheme 1. Possible biogenetic pathways for **1**–**3**.

The sterol content of *Muricea* spp might have de novo, dietary, dietary-modified but not symbiotic origin because *Muricea* spp is a non-zooxanthellate gorgonia.

The C-24 alkyl group containing phytosterols come from *S*-adenosyl-L-methionine[12] and *C*-methylation is an energy-expensive process costing the cell about 14 ATP per methyl group.[13] Therefore, it would be a big paradox that *Muricea* spp biosynthesizes 24-methylenecholesterol (**5**) from desmosterol (**4**; Scheme 1) and dealkylates 24-methylenecholesterol (**5**) via epoxide intermediate **1** as an extra pathway to obtain **6**. Thus, dietary microalgae rather than *Muricea* spp seem the most likely C-24 alkylating producer. However, since sterols with hydroxylated side chain are unusual in microalgae,[14,15] coral should be responsible for the 22-hydroxylation, but whether the oxygen atoms of the 22-hydroxy group and the oxirane ring of **1** arise from the same oxygen molecule or from separate molecules is an open question[16] and encourages biosynthetic speculations.

Insertion of biologically excited singlet oxygen O_2 ($^1\Delta g$) in a 1,4-diene **7** leading to **8** (path b) in an overall 1,4-addition, followed by stereospecific endoperoxide cleavage should also be considered as an alternative pathway[17] for muriceanol (**1**). The regioselectivity of the oxygenated side-chain substituents appears to be compatible with this mechanism and its stereospecificity suggests the addition of singlet oxygen is enzymatically catalyzed rather than photochemically produced.

It is interesting to observe that path b involves two key oxidation sites in one cycloaddition step and may represent an evolutionary advantage in both shortening the pathway toward a steroidogenic end product and quenching damaging reactive oxygen species (ROS), thus enhancing the fitness of *Muricea* spp. The occurrence of the C-22-hydroxylated 24(28)-epoxide **1** in the non-filter feeders and non-zooxanthellate *Muricea* spp converts this organism into a very good candidate for biological and biosynthetic studies on the C-24-dealkylation mechanism of phytosterol in corals as well as on steroidogenesis.

Experimental Section

Biological Material: *Muricea* sp. was collected by SCUBA diving off Pacheca Island (Panama) at –10 m.

Extraction and Isolation: Air-dried samples (450 g) were extracted with acetone at room temperature, and were concentrated to give a dark residue (56.9 g), which was partitioned between EtOAc (3 × 500 mL) and water (500 mL). The EtOAc extracts were combined to obtain a brown oil (6.5 g) that was chromatographed on an LH-20 column and further separated by HPLC to give compounds **1** (10.3 mg, 0.003% dry sample), **2** (4.0 mg, 0.0008% dry sample) and **3** (15.0 mg, 0.0033% dry sample).

Compound 1: Colorless oil. $[a]_D^{25}$ = –9 (*c* = 0.51, CH_2Cl_2). IR (film): $\tilde{\nu}_{max}$ = 3407, 2934 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. EIMS: *m*/*z* (%) = 430 (<1) [M^+], 412 (20) [$M^+ - H_2O$], 400 (12) [$M^+ - CH_2O$], 369 (44) [M^+ – 2 Me – CH_2 – OH], 271 (70) [$C_{19}H_{27}O^+$], 107 (100) [$C_8H_{11}^+$]. HREIMS: *m*/*z* = 430.3541 (calcd. for $C_{28}H_{46}O_3$ 430.3447), 412.3410 (calcd. for $C_{28}H_{44}O_2$ 412.3341), 400.3439 (calcd. for $C_{27}H_{44}O_2$ 400.3341), 369.2761 (calcd. for $C_{25}H_{37}O_2$ 369.2794), 271.2107 (calcd. for $C_{19}H_{27}O_2$ 271.2062), 107.0875 (calcd. for C_8H_{11} 107.0861).

Compound 2: White powder. $[a]_D^{25}$ = –20 (*c* = 0.20, CH_2Cl_2). IR (film): $\tilde{\nu}_{max}$ = 3303, 2928 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.57 (s), 0.79 (s), 3.57 (dddd, 4.8, 4.8, 11.1, 11.1), 4.93 (m), 4.95 (m), 5.74 (ddd, 7.7, 11.1, 16.4) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ =12.4 (CH_3), 12.9 (CH_3), 20.9 (CH_2), 24.8 (CH_2), 27.2 (CH_2), 28.7 (CH_2), 31.5 (CH_2), 32.2 (CH_2), 35.6 (C), 35.7 (CH), 37.0 (CH_2), 37.6 (CH_2), 38.2 (CH_2), 43.6 (C), 44.9 (CH), 54.7 (CH), 55.4 (CH), 55.7 (CH), 71.3 (CH), 114.4 (CH_2), 139.9 (CH) ppm. EIMS: *m*/*z* (%) = 302 (100) [M^+], 287 (52) [M^+ – Me], 269 (18) [M^+ – Me – H_2O], 233 (32), 215 (56). HREIMS: *m*/*z* = 302.2595 (calcd. for $C_{21}H_{34}O$ 302.2610), 287.2370 (calcd. for $C_{20}H_{31}O$ 287.2375), 269.2270 (calcd. for $C_{20}H_{29}$ 269.2269).

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