Antimalarial, Antiviral, and Antitoxoplasmosis Norsesterterpene Peroxide Acids from the Red Sea Sponge *Diacarnus erythraeanus*

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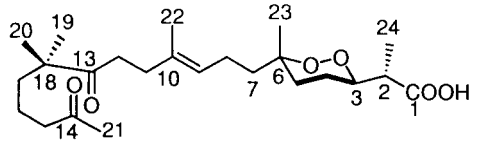
A new norsesterterpene acid, named muqubilone (**1**), along with the known sigmosceptrellin-B and muqubilin were isolated from the Red Sea sponge *Diacarnus erythraeanus*. The structure determination of **1** was based primarily on 1D and 2D NMR analyses. Sigmosceptrellin-B exhibits significant in vitro antimalarial activity against *Plasmodium falciparum* (D6 and W2 clones) with IC_{50} values of 1200 and 3400 ng/mL, respectively. Muqubilin and **1** show in vitro antiviral activity against herpes simplex type 1 (HSV-1) with ED_{50} values of 7.5 and 30 μg/mL, respectively. Muqubilin and sigmosceptrellin-B display potent in vitro activity against *Toxoplasma gondii* at a concentration of 0.1 μM without significant toxicity.

Norsesterterpene peroxide acids and their methyl esters are frequently isolated secondary metabolites from marine sponges of the genera *Prianos*,[1,2] *Sigmosceptrella*,[3,4] *Latrunculia*,[5−7] *Mycale*,[8−12] and *Diacarnus*.[13] They display a wide range of bioactivity including antimicrobial,[5] antiviral,[10] cytotoxicity,[10] and antimalarial activities.[13,14] In this paper, we describe the isolation of a new norsesterterpene peroxide acid (**1**), named muqubilone, from the Red Sea sponge *Diacarnus erythraeanus* Kelly-Borges & Vacelet (1995) (family Podospongiidae, order Poecilosclerida)[15−18] and the bioactivities of **1** as well as the known sigmosceptrellin-B and muqubillin, isolated from the same sponge species.

The lipophilic extract of the freshly collected Red Sea sponge *D. erythraeanus* was chromatographed on Si gel with a hexanes−EtOAc gradient. The intermediate polar fractions afforded **1**, sigmosceptrellin-B, and muqubilin after repeated RP C18 flash chromatography using a H_2O−CH_3CN gradient.



Muqubilone (1)

Compound **1** was obtained as a colorless oil. The HR-FTMS electrospray ionization data of **1** displayed molecular ion peaks at *m*/*z* 423.2763 $(M - H)^-$, suggesting the molecular formula $C_{24}H_{40}O_6$ and five degrees of unsaturation. The IR spectrum ($CHCl_3$) of **1** showed absorption bands at 3442 and 1695 cm^{-1}, suggesting the presence of carboxyl and ketone functionalities. The ^1H and ^{13}C NMR spectra of **1** (Table 1) indicate close structural homology with muqubilin[1,2] with the replacement of $\Delta^{13,14}$ with new ketones at C-13 and C-14. The quaternary ketone carbon resonating at δ 215.1 (Table 1) was assigned to C-13. This carbon signal showed HMBC coupling to the methylene protons resonating at δ 2.19 (H_2-11) and 1.42 (H_2-17), as well as the methyl singlet which resonated at δ 1.11, which was assigned as the C-19 and C-20 methyl groups (Figure 1). The methylene doublet of doublets H_2-12 resonating at δ 2.53 also showed HMBC coupling to C-13. The quaternary ketone carbon resonating at δ 208.7 (Table 1) was assigned C-14. Carbon C-14 displayed HMBC coupling to the proton methyl singlet absorbed at δ 2.12 (C-21) and the methylene doublet of doublets resonating at δ 2.40 (H_2-15). The conformation of segment C-13/C-18 was proved nonlinear, as indicated by the NOESY correlation between

Table 1. ^{13}C and ^1H NMR Data of **1**[a]

no.	^{13}C	^1H
1	179.1, s	
2	43.0, d	2.66, m
3	81.2, d	4.15, brs
4	23.4, t	1.76, 2H, m
5	32.0, t	1.65, m 1.44, m
6	80.1, s	
7	39.8, t	1.44, m 1.29, m
8	21.7, t	2.00, 2H, m
9	124.3, d	5.09, dd (6.7, 6.4)
10	134.5, s	
11	33.5, t	2.19, 2H, dd (7.7, 7.4)
12	35.6, t	2.53, 2H, dd (7.8, 7.4)
13	215.1, s	
14	208.7, s	
15	43.9, t	2.40, 2H, dd (6.6, 6.2)
16	19.0, t	1.44, 2H, m
17	39.1, t	1.42, 2H, m
18	47.5, s	
19	24.3, q	1.11, 3H, s
20	24.3, q	1.11, 3H, s
21	29.9, q	2.12, 3H, s
22	16.1, q	1.60, 3H, s
23	20.6, q	1.28, 3H, s
24	13.3, q	1.26, 3H, d (7.6)

[a] In $CDCl_3$, 400 MHz for ^1H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by DEPT135° experiments. s = quaternary, d = methine, t = methylene carbons. Coupling constants (*J*) are in Hz.

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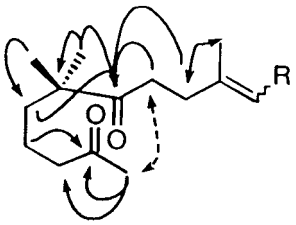

Figure 1. Important $^{1}H-^{13}C$ GHMBC (plain) and NOESY (dashed) correlations of **1**.

Table 2. In Vitro Antimalarial and Antiviral Activities of Muqubilone (**1**), Muqubilin, and Sigmosceptrellin-B[a]

	Plasmodium falciparium					
	D6 clone		W2 clone		HSV-1	cytotoxicity
compound	IC_{50} ng/mL	SI	IC_{50} ng/mL	SI	ED_{50} μg/mL	Vero cells IC_{50} μg/mL
muqubilone (**1**)	NA		NA		30.0	60.0
muqubilin	2900	>1.6	>4760	>1.0	7.5	30.0
sigmo-sceptrellin-B	1200	>2.7	3400	>1.0	C	2.5

[a] NA = No activity at a maximum concentration of 80 μg/mL. HSV-1 = Herpes simplex virus type-1. C = cytotoxicity obscured reading. SI (selectivity index) = IC_{50} (Vero cells)/IC_{50} (*P. falciparium*).

the C-21 methyl and C-12 methylene protons. The *E* configuration of $\Delta^{9,10}$ was justified based on the similarity of the carbon chemical shift values of C-9, C-10, and C-22, in addition to the *J* value of H-9 (Table 1), to those of muqubilin.[1,2,19] The assignments of the chiral centers C-2, C-3, and C-6 were based on the comparable optical rotation of **1** (+48.0°) with that reported for muqubilin (+31.6°) using the same solvent.[2] Comparison of the ^{13}C NMR data of **1** with those of muqubilin further supported these assignments.[2,19] The chemical shift value of the C-23 methyl (δ 20.6) in **1** indicates axial orientation. The equatorially oriented C-23 methyl has been shown to resonate in the range δ 32.5−24.0.[19] Comparing the chemical shift value of C-7 in **1** (δ 39.8) versus C-7 in compounds containing an equatorial C-23 methyl group (δ 34.2−34.8) further supported these assignments.[19] The prediction of the *threo* orientation of C-2 and C-3 in muqubilin using the chemical shift value of the C-24 methyl group (δ 1.24) is also reported.[19] In the case of related compounds containing the C-2/C-3 *erythro* configuration, the C-24 methyl group resonates at δ 1.13−1.14.[19] Since C-24 in **1** resonates at δ 1.26, the configuration of C-2 and C-3 of **1** is the same as muqubilin. The splitting pattern of the proton signal H-3 in muqubilin was reported unresolved.[1,2] The unresolved splitting pattern of H-3 in **1** will require reisolation of additional compound and further studies using variable-temperature and solvent conditions. The segment C-10/C-21 of **1** was structurally related to the ozonolysis product of muqubilin methyl ester generated at −40 °C (ozonolysis of $\Delta^{13,14}$ with the formation of ketone groups at C-13 and C-14);[1] however, compound **1** was clearly natural since it could be detected by TLC in the original fresh sponge extract.

Two additional compounds isolated were the known muqubilin[1,2] and sigmosceptrellin-B.[4,20] Identity is based on comparison with their previously reported NMR data.

Sigmosceptrellin-B shows in vitro antimalarial activity against *P. falciparium* (D6 and W2 clones) (Table 2) with IC_{50} 1200 and 3400 ng/mL, respectively. The C-3 epimer of sigmosceptrellin-B (sigmosceptrellin-A) previously showed better activity against the same clones of *P. falciparium* with IC_{50} 470 and 420 ng/mL, respectively, with better selectivity indices due to its low cytotoxicity.[14] Muqubilin shows less activity. The functional group homology of these compounds with the artemisinins suggests that the activity may be attributed to their peroxide moieties.

Muqubilin and muqubilone possess in vitro antiviral activity against HSV-1,[21,22] as shown by protecting a confluent nonproliferating monolayer of Vero African green monkey kidney cells from the cytopathic effect of the virus with ED_{50} of 7.5 and 30.0 μg/mL, respectively (Table 2). The cytotoxicity of sigmosceptrellin-B obscured its antiviral activity.

Muqubilin and sigmosceptrellin-B display potent in vitro activity against *T. gondii* at nontoxic concentrations (Table 3).[23] Muqubilin at a concentration of 0.1 μM inhibited 63−84% or 46−58% of the intracellular replication of the parasite in HFF or L929 cells. At 0.1 μM, sigmosceptrellin-B was more active (84−99% inhibition) in HFF cells but less active (19−43% inhibition) in L929 cells. Toxicity of both compounds markedly increased at higher concentrations.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, on Bruker AMX-NMR spectrometers operating at 400 or 500 MHz for ^{1}H NMR and 100 or 125 MHz for ^{13}C NMR. The HRMS spectra were measured using a Bioapex FT mass spectrometer with electrospray ionization. TLC analyses were carried out on precoated Si gel G_{254}, 500 μm plates, with the developing system

Table 3. Activity of Muqubilin and Sigmosceptrellin-B against *T. gondii*[a]

	in HFF cells								in L929 cells							
	24 h				48 h				24 h				48 h			
	toxicity		activity		toxicity		inhibition activity		toxicity		activity		toxicity		inhibition activity	
dose, μg/mL (μM)	% of cont.	Sc.	% of cont.	Sc.	% of cont.	Sc.	% of cont.	Sc.	% of cont.	Sc.	% of cont.	Sc.	% of cont.	Sc.	% of cont.	Sc.
							Muqubilin									
0.004 (0.010)	0.00	0	3.16	1	0.00	0	0.00	0	0.00	0	33.85	2	0.00	0	0.00	0
0.039 (0.1000)	7.15	1	84.00	4	0.94	1	62.88	3	0.00	0	58.35	3	0.00	0	46.30	3
0.392 (1.000)	86.15	4	86.18	4	85.93	4	87.94	4	66.30	3	84.03	4	83.36	4	96.77	5
3.920 (10.000)	85.85	4	91.72	5	84.73	4	100.00	5	93.00	5	87.84	4	94.90	5	100.00	5
							Sigmosceptrellin-B									
0.004 (0.010)	0.00	0	0.00	0	0.00	0	37.57	2	0.00	0	00.00	0	0.00	0	24.15	2
0.039 (0.1000)	0.00	0	84.72	4	5.56	1	98.32	5	0.00	0	18.61	1	0.00	0	35.17	3
0.392 (1.000)	86.81	4	96.49	5	64.37	3	100.00	5	60.48	3	91.88	5	91.21	5	98.82	5
3.920 (10.000)	87.41	4	94.91	5	94.65	5	100.00	5	92.96	5	96.62	5	91.05	5	100.00	5

[a] Inhibition of intracellular replication of *T. gondii* in vitro measured by [^{3}H]uracil incorporation. HFF = human foreskin fibroblast ATCC CRL1635. % of cont. = % of control. Sc. = score that represents the extent of toxicity or activity (in % of control) based on the following criterion: 0 = no reduction, 1 = ≤20%, 2 = >20 to ≤40%, 3 = >40 to ≤70%, 4 = >70 to ≤90%, 5 = >90%.

$CHCl_3$−MeOH (90:10) or on C_{18}-reversed-phase plates, 200 μm, using CH_3CN-H_2O (50:50). For column chromatography, Si gel 60, 40 μm, or LiChroprep RP-18, 25−40 μm, was used.

Animal Material. The sponge is ramose-digitate, forming an anastomosing mat, and was collected on the Egyptian Red Sea coast northeast of Hurghada at a depth of −10 m, in December 1997. The surface is covered in blunt spines, and the color is mottled dark reddish maroon and oak brown. The sponge is fleshy and compressible but difficult to cut. This sponge is characterized by huge primary fibers, which appear as "sinews" in the sponge, and large yolk-like larvae 1−2 mm in diameter. The sponge is *Diacarnus erythraenus* Kelly-Borges & Vacelet.[15−18] A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 2000.2.13.2). The sponge was stored frozen at −20 °C until extracted.

Isolation. Frozen sponge (220 g) was thawed, blended, and exhaustively extracted with 95% EtOH (0.5 L × 4). The freeze-dried ethanolic extract (13.0 g) was dissolved in 5% MeOH−$CHCl_3$ and filtered. The lipophilic extract (6.5 g) obtained after evaporating the filtrate under reduced pressure was subjected to Si gel flash chromatography, using *n*-hexane−EtOAc. The intermediate polar fractions were subjected to repeated RP C_{18} flash chromatography using a H_2O-CH_3CN gradient to afford **1** (13 mg, R_f 0.76, RP C_{18}, CH_3CN-H_2O, 1:1), sigmosceptrellin-B (46 mg, R_f 0.48), and muqubilin (59 mg, R_f 0.42).

Muqubilone (1): colorless oil, $[\alpha]_D^{25}$ +48.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 229 (2.72), 257 (2.41) nm; IR ($CHCl_3$) ν_{max} 3442 (OH), 2950−2850, 1695 (C=O), 1629, 1444, 1365 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFTMS *m/z* $(M - H)^-$ 423. 2763 (calcd for $C_{24}H_{39}O_6$ 423.2752).

Antiviral Assays. Antiviral assays were carried out on serial dilutions of test compounds with herpes simplex type 1 virus (R.G. Hughes, Roswell Park Memorial Institute, Buffalo, NY) in cultures of Vero African green monkey kidney cells (Viromed Laboratories, Minnetonka, MN) in 96-well culture trays using the simplified plaque reduction assay.[21] Cytotoxicity was estimated[22] in the same cultures as the concentrations of the test compounds causing half-maximal loss of uninfected Vero cells from the monolayers surrounding the plaques.

Anti-*Toxoplasma gondii* Assays. In vitro anti-*T. gondii* assays were carried out using [^{3}H]uracil incorporation in cultures of human foreskin fibroblast (HFF) or L929 cells infected with 4×10^4 tachyzoites/well in 96-well flat bottom tissue culture microtiter plates.[23] The toxicity of the tested compound for HFF and L929 cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay using Cell Titer 96 Kit (Promega Corporation, Madison, WI).[23]

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Supporting Information Available: Spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The genera *Diacarnus* and *Sigmosceptrella* are now considered to be separate from the genus *Latrunculia* (family Latrunculiidae, order Poecilosclerida), being contained in the family Podospongiidae (order Poecilosclerida).[16] Specimens of *Diacarnus* and *Sigmosceptrella* have been frequently mistakenly identified as *Latrunculia* due to the presence of spined microscleres termed discorhabds. Along with other features of the skeletal architecture, the microscleres of *Latrunculia* are quite different from those of *Diacarnus* and *Sigmosceptrella*, hence their separation into two families.[17] With the removal of peroxide-containing sponges from Latrunculiidae and discorhabdin-containing sponges from the *Diacarnus*−*Sigmosceptrella*−*Mycale* family group, these compounds become reliable chemotaxonomic markers for the two families.[18] The diagnostic character of the genus *Prianos* is the presence of strongyles, the type species of which is clearly a chalinid haplosclerid sponge (family Chalinidae, order Haplosclerida) as the case in the genus *Haliclona* or *Adocia*. Historically, any sponge identified with one size category of strongyles that is not a species of *Strongylophora* has been placed in the "catchall" genus *Prianos*. Several species of *Diacarnus*, particularly those from atoll locations, have reduced skeletal complements with microscleres so rudimentary as to be almost undetectable and their megascleres appear to be strongyles. Thus, it is extremely unlikely that the identification of the *Prianos*[1,2] is correct; these sponges are probably species of *Diacarnus* with a reduced skeletal complement.[18]
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