DOI: 10.1002/ejoc.200500404

Antimalarial Polyketide Cycloperoxides from the Marine Sponge *Plakortis simplex*

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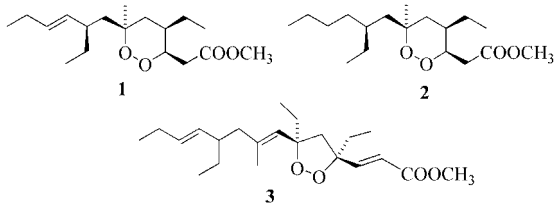
Keywords: Natural Products / Polyketides / Peroxides / Configuration determination / Antimalarial agents

Three new (**5**–**7**) and two known (**4** and **8**) polyketide derivatives have been isolated from the Caribbean sponge *Plakortis simplex*. Their stereostructures have been determined by spectroscopic methods and chemical transformations that include Zn-mediated reduction and Dess–Martin oxidation, and by application of Mosher's method. The cycloperoxide derivatives plakortide Q (**7**) and compound **8** were evaluated for their in vitro antimalarial activity against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. The lower potency of these molecules relative to plakortin indicates that the change of configuration at C-3 exerts negative effects on the antimalarial activity.

Introduction

Malaria, an infectious disease caused by protozoans of the genus *Plasmodium*, still continues to be a major cause of morbidity and mortality in the tropics: recent analyses estimate up to 3 million deaths each year from malaria, with unacceptably high percentages among children from Sub-Saharan Africa.[1,2] Part of the reason for the failure to control malaria in these areas is the spread of cross-resistance and multi-drug resistance to the first-line antimalarial drugs, such as chloroquine and antifolates.[3] A major breakthrough in antimalarial therapy came with the discovery that the cycloperoxide-containing sesquiterpene artemisinin, previously isolated from *Artemisia annua* (Compositae), possessed nanomolar activity against chloroquine-resistant *P. falciparum* strains.[4] Artemisinin derivatives are now used for treatment of severe malaria with the support of the World Health Organization, but, unfortunately, they still possess unfavorable side effects.[5] An essential requirement to design optimized artemisinin derivatives would be a perfect knowledge of the mechanism of its antimalarial activity. While the crucial importance of the peroxide pharmacophore is no longer questioned, unfortunately, the mechanism of action of this class of antimalarial agents is far from being fully understood. The isolation of different antimalarial cycloperoxides from natural sources could undoubtedly help in gaining more insight, which would afford information about the structural features required for the carbon backbone of a cycloperoxide-based antimalarial.

In this context, several research groups are currently engaged in the isolation of cycloperoxide-containing compounds from terrestrial plants and marine organisms. We have recently reported that plakortin (**1**), a cycloperoxide polyketide isolated from the Caribbean sponge *Plakortis simplex*,[6] shows considerable (μM range) in vitro antimalarial activity against *Plasmodium falciparum* chloroquine-resistant strains (W2).[7] The close analogue dihydroplakortin (**2**) proved to be almost equally active, while, interestingly, the five-membered cycloperoxide derivative plakortide E (**3**) was found to be inactive.[7]



1 **2** **3**

In order to gain additional information to establish structure–activity relationships, we decided to re-examine the apolar organic extract of *Plakortis simplex* by looking for additional analogues of the major secondary metabolite plakortin. This investigation resulted in the isolation of five molecules, the non-cycloperoxide polyketides **4**–**6** (**5** and **6** are new molecules) and the cycloperoxide derivatives **7** and **8**. While compound **8** had already been obtained from *P. halichondrioides*,[8] but with incomplete spectroscopic and stereostructural characterizations, compound **7** is a new

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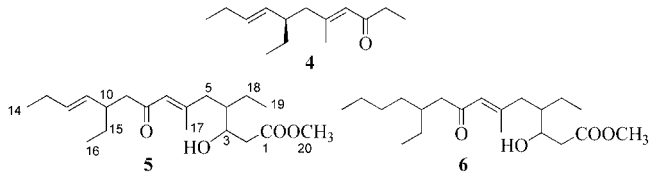
molecule, that we have named plakortide Q. Details on the structures of compounds **4**–**8** and the antimalarial activity of **7** and **8** are presented in this paper.



Results and Discussion

The sponge *Plakortis simplex* (order Homosclerophorida, family Plakinidae) was collected during the summer of 2002 from along the coasts of the Bahamas and was immediately frozen. After homogenization, the organism was exhaustively extracted, in sequence, with methanol and chloroform. The methanolic layer was partitioned between *n*BuOH and water, and the organic phase, combined with the $CHCl_3$ extract, was then subjected to chromatography over a reversed phase silica column. Selected fractions were combined and further chromatographed by MPLC (silica gel, gradient from *n*-hexane to MeOH). Fractions eluted with *n*-hexane/EtOAc, 9:1, were subjected to repeated column and HPLC chromatography to afford plakortin (**1**, 1.18 g), dihydroplakortin (**2**, 5.5 mg), compound **4** (3.0 mg), compound **8** (4.2 mg), and plakortide Q (**7**, 2.8 mg) in the pure form. Fractions eluted with *n*-hexane/EtOAc, 8:2, were re-chromatographed by HPLC to afford pure compounds **5** (1.8 mg) and **6** (0.9 mg). The structure of the known compound **4** was identified by comparison of its spectroscopic data with those reported in the literature.[9]

Compound **5** shows a pseudomolecular ion peak at *m*/*z* = 339 $[M + H]^+$ in the FAB (positive ion) mass spectrum. The molecular formula $C_{20}H_{34}O_4$, which suggests four degrees of unsaturation, was assigned to compound **5** on the basis of HR-FABMS. Analysis of ^1H- and ^{13}C NMR spectra of **5** ($CDCl_3$, Table 1), with the help of the 2D HMQC experiment, reveals the presence of three sp^3 methines (one of which is oxygenated: δ_H = 4.09 ppm, δ_C = 70.3 ppm), six sp^3 methylenes, two double bonds [one trisubstituted (δ_H = 6.03 ppm, δ_C = 125.1 ppm; δ_C = 158.5 ppm) and one disubstituted (δ_H = 5.06 ppm, δ_C = 134.4 ppm; δ_H = 5.39 ppm, δ_C = 131.2 ppm)], and five methyls (one of which is a methoxy group: δ_H = 3.72 ppm, δ_C = 52.1 ppm). The two remaining carbon atoms and the two remaining oxygen atoms and degrees of unsaturation, indicated by the molecular formula, were all accounted for by the presence of two C=O groups. Their ^{13}C NMR resonances at δ_C = 201.0 and 174.2 ppm suggests the presence of a conjugated ketone carbonyl (supported by UV absorption at λ_{max} = 235 nm and IR absorption band at $\tilde{\nu}_{max}$ = 1690 cm^{-1}) and of an ester group (IR absorption at $\tilde{\nu}_{max}$ = 1735 cm^{-1}), respectively.

Table 1. ^1H- and ^{13}C NMR spectroscopic data of compounds **5** and **6** (in $CDCl_3$).

Position	**5**		**6**	
	δ_C, multiplicity	δ_H, multiplicity, *J* [Hz]	δ_C, multiplicity	δ_H, multiplicity, *J* [Hz]
1	174.2, C		174.0, C	
2	37.4, CH_2	2.42[a]	37.4, CH_2	2.40[a]
3	70.3, CH	4.09, m	70.0, CH	4.11, m
3-OH		3.37, bd, 2.5		3.38, bd, 2.5
4	43.2, CH	2.05, m	43.2, CH	2.03[a]
5a	48.1, CH_2	2.17, dd, 10.5, 3.5	47.9, CH_2	2.09, dd, 10.5, 3.5
5b		2.02[a]		2.00[a]
6	158.5, C		158.2, C	
7	125.1, CH	6.03, s	125.5, CH	6.08, s
8	201.0, C		201.2, C	
9a	41.3, CH_2	2.63, dd, 14.5, 4.2	41.3, CH_2	2.61, dd, 14.5, 4.2
9b		2.40[a]		2.38[a]
10	39.4, CH	2.08, m	30.3, CH	1.43, m
11	134.4, CH	5.06, dd, 15.4, 7.3	34.2, CH_2	1.21[a]
12	131.2, CH	5.39, dt, 15.4, 6.6	29.0, CH_2	1.23[a]
13	22.2, CH_2	1.98[a]	21.9, CH_2	1.26[a]
14	14.0, CH_3	0.94, t, 7.3	13.1, CH_3	0.89, t, 7.3
15a	26.6, CH_2	1.40, m	26.6, CH_2	1.36, m
15b		1.30, m		1.27[a]
16	13.1, CH_3	0.90, t, 7.3	13.1, CH_3	0.87, t, 7.3
17	21.1, CH_3	2.09, s	21.0, CH_3	2.08, s
18a	28.1, CH_2	1.39, m	27.9, CH_2	1.36, m
18b		1.18, m		1.19[a]
19	12.2, CH_3	0.84, t, 7.3	12.2, CH_3	0.84, t, 7.3
20	52.1, CH_3	3.72, s	52.0, CH_3	3.70, s

[a] Overlapped with other signals.

The COSY spectrum of **5** discloses the sequential arrangement of proton resonances indicating the presence of two spin systems (depicted in bold in Figure 1). The first spin system involves a C_6 chain comprising a double bond and an ethyl branched methine, while the second connects two relatively downfield shifted methylenes spanning an ethyl branched methine and an OH-bearing methine. The 2D HMBC spectrum of compound **5** reveals that these two spin systems are actually separated by an α,β-unsaturated-β-methyl ketone group (key $^{2,3}J_{CH}$ correlations are depicted in Figure 1). In particular, the enone system is assigned on the basis of the correlation of the ketone carbon C-8 with 7-H and of the correlations of 17-H_3 with both C-7 (δ_C = 125.1 ppm) and the unprotonated C-6 (δ_C = 158.5 ppm). The connections between the above two spin systems and the enone moiety were established on the basis of the HMBC cross-peaks of both 9-H and 10-H with C-8, and those of 5-H_2 with both C-6 and C-7. Furthermore, the gross structure of compound **5** was completely defined by placing the ester carbonyl (δ_C = 174.2 ppm) at C-1 on the basis of its correlations with 2-H_2 and 3-H.

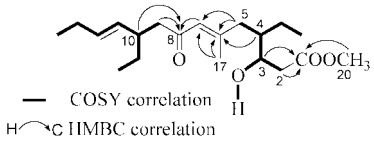


Figure 1. COSY and $^{2,3}J$ H→C HMBC correlations of compound **5**.

The *E* geometry of the double bond Δ^{11} was deduced from the large vicinal coupling constant $J_{11-H/12-H}$ = 15.4 Hz, while the spatial coupling (evidenced by a ROESY spectrum) between 7-H and 5a-H (δ_H = 2.17 ppm) indicates the *E* geometry of the double bond $\Delta^{6(7)}$. The determination of relative and absolute configurations at the three chiral centers C-3, C-4, and C-10 of **5** did not appear to be trivial, and thus, given the limited amount of material available, this issue was not addressed.

Together with compound **5**, the closely related compound **6** was isolated and identified as its 11,12-dihydroderivative by interpretation of the following evidence: i) the FAB (positive ion) mass spectrum of **6** exhibits a pseudomolecular ion peak at *m*/*z* = 341; this value is two mass units higher than that of **5**. The molecular formula $C_{20}H_{36}O_4$ of **6** is confirmed by HR-FABMS (found: *m*/*z* = 341.2679, calculated: *m*/*z* = 341.2692). ii) The ^{1}H NMR spectrum of **6** (Table 1) appears to be similar to that of **5**, the main differences are restricted to the absence of the olefin proton signals assigned to 11-H and 12-H (instead, two overlapping methylene signals at around δ_H = 1.20 ppm are observed) and to a significant upfield shift of 13-H_2 and 10-H signals. The structure of the new metabolite **6** was confirmed by 2D NMR spectroscopy, and, in particular, COSY, HMQC, and HMBC spectra were used to assign all its ^{1}H and ^{13}C NMR resonances (Table 1). As for compound **5**, the *E* geometry of the double bond $\Delta^{6(7)}$ is indicated by the NOE contact of 7-H with 5a-H.

The isolation of compounds **4**–**6** from *Plakortis simplex* appears particularly intriguing since it raises some questions about the biogenetic origin of this class of acyclic polyketide derivatives. Higgs and Faulkner postulated that compound **4** could be derived, through oxidative cleavage, from the same 1,3-diene precursor that, most likely, gives rise plakortin (**1**) through enzyme-mediated addition of oxygen (Figure 2).[9] Interestingly, in spite of the dozens of polyketides isolated from *Plakortis* sponges,[10] the carbon skeletons of compounds **5** and **6**, which contain an additional acetate unit than plakortin (Figure 2), are unprecedented; moreover, the oxidation pattern of **5** and **6** also appears to be unique.

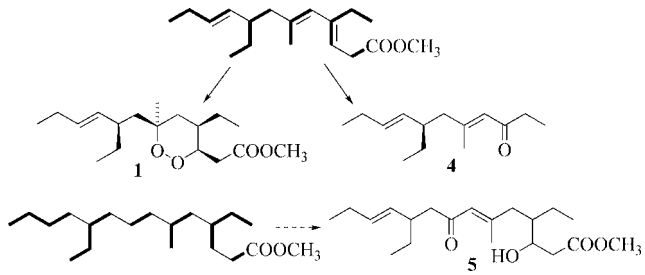
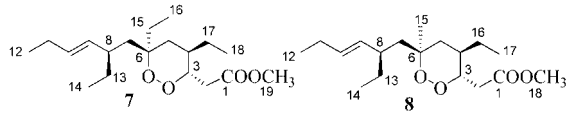


Figure 2. Postulated biogenetic origin for compounds **4** and **5**.



Plakortide Q (**7**), which is isolated as a colorless oil, shows HR-FABMS data that is in agreement with the molecular formula $C_{19}H_{34}O_4$. This implies three degrees of unsaturation, one of which can be assigned to an ester carbonyl on the basis of the IR absorption band at $\tilde{\nu}_{max}$ = 1741 cm^{-1}. The ^{13}C NMR spectrum of plakortide Q (**7**) (Table 2) contains 19 carbon resonances that, analyzed by the DEPT spectrum, are divided into those of two nonprotonated carbon atoms (one of these, at δ_C = 170.8 ppm, supports the presence of the ester functionality) and 17 proton-bearing carbon atoms. The latter resonances were associated with those of the relevant protons through inspection of the 2D HMQC spectrum (Table 2). In particular, the signals for three oxygen-bearing sp^3 carbon atoms are detected: δ_C = 83.2 (C) and 81.9 ppm (CH, δ_H = 4.11 ppm) appear to be quite similar to the values reported for carbon atoms flanking a cycloperoxide bond,[8–11] while the resonance at δ_C = 51.9 ppm can easily be attributed to an ester methoxy group (δ_H = 3.69 ppm). In addition, resonances of a disubstituted double bond (δ_C = 134.4 ppm, δ_H = 5.10 ppm; δ_C = 131.8 ppm, δ_H = 5.39 ppm) are also present and account for the remaining formal unsaturation indicated by the molecular formula.

The COSY spectrum of plakortide Q (**7**) indicates the presence of an isolated ethyl group (15-H_2/16-H_3) and of two larger spin systems (Figure 3). Inspection of the HMBC spectrum establishes that the oxygenated C-6 is the connection point for these three fragments (Figure 3). In particular, C-6 shows cross-peaks with 4-H, 5-H_2, 7-H_2, 8-

Table 2. ^{1}H- and ^{13}C NMR spectroscopic data of plakortide Q (**7**) and compound **8** (in $CDCl_3$).

Position	**7**		**8**	
	δ_C, multiplicity	δ_H, multiplicity, J [Hz]	δ_C, multiplicity	δ_H, multiplicity, J [Hz]
1	170.8, C		171.0, C	
2a	36.4, CH_2	2.64, dd, 15.5, 3.5	36.9, CH_2	2.66, dd, 15.5, 3.5
2b		2.37, dd, 15.5, 8.8		2.38, dd, 15.5, 8.8
3	81.9, CH	4.11, dt, 8.8, 8.8, 3.5	81.8, CH	4.15, dt, 8.8, 8.8, 3.5
4	35.6, CH	1.60, m	36.6, CH_2	1.62, m
5a	37.4, CH_2	1.46[a]	39.3, CH_2	1.49[a]
5b		1.23, dd, 13.8, 7.4		1.25, dd, 13.8, 7.4
6	83.2, C		82.5, C	
7a	45.8, CH_2	1.75, dd, 14.5, 3.5	46.8, CH_2	1.78, dd, 14.5, 3.5
7b		1.48[a]		1.46[a]
8	40.1, CH	1.96[a]	40.4, CH	2.01[a]
9	134.4, CH	5.10, dd, 15.6, 7.0	134.9, CH	5.10, dd, 15.6, 7.0
10	131.8, CH	5.39, dt, 15.6, 7.0	131.6, CH	5.37, dt, 15.6, 7.0
11	25.5, CH_2	1.99[a]	25.8, CH_2	1.99[a]
12	13.8, CH_3	0.96, t, 7.3	14.0, CH_3	0.97, t, 7.3
13a	29.6, CH_2	1.43[a]	29.8, CH_2	1.43[a]
13b		1.20, m		1.20, m
14	11.5, CH_3	0.82, t, 7.3	11.7, CH_3	0.80, t, 7.3
15a	29.8, CH_2	1.56, m	21.3, CH_3	1.32, s
15b		1.41[a]		
16a	7.4, CH_3	0.84, t, 7.3	24.0, CH_2	1.40[a]
16b				1.07, m
17a	23.8, CH_2	1.42[a]	10.6, CH_3	0.88, t, 7.3
17b		1.04, m		
18	10.6, CH_3	0.87, t, 7.3	51.5, CH_3	3.68, s
19	51.9, CH_3	3.69, s		

[a] Overlapped with other signals.

H, 15-H_2, and 16-H_3, while C-1 correlates with 2-H_2, 3-H, and 19-H_3 to give the planar structure of plakortide Q (**7**), a novel cycloperoxide polyketide of the plakortin family.

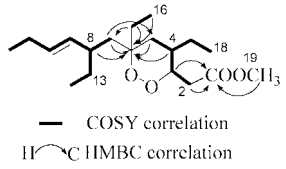
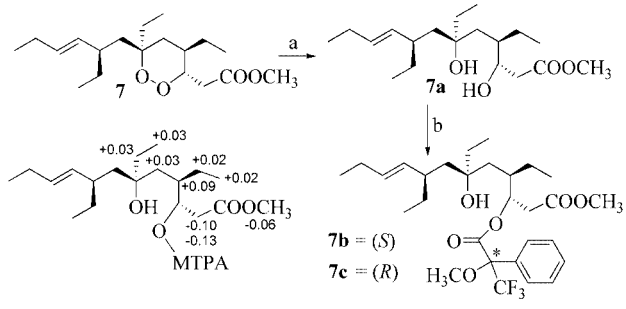


Figure 3. COSY and $^{2,3}J$ H→C HMBC correlations of plakortide Q (**7**).

The *trans* geometry of the double bond was deduced by the large coupling constant $J_{9\text{-H}/10\text{-H}}$ (15.6 Hz), while the relative orientation of the substituents around the dioxane ring of **7** was established by some spatial interactions, evidenced by a ROESY spectrum. In particular, the cross-peaks of 3-H with 17-H_2 and 18-H_3 indicate the 3,4-*trans* geometry that was also corroborated by the large coupling constant $J_{3\text{-H}/4\text{-H}}$ (8.8 Hz). This is in agreement with a *trans*-diaxial relationship. Furthermore, the spatial proximity between H-4 and H_2-15 suggests the *trans* orientation of the ethyl groups that are linked at C-4 and C-6, respectively.

The absolute configuration of the chiral centers belonging to the cycloperoxide ring of **7** was established with the aid of the reactions reported in Scheme 1. Treatment of **7** with Zn/AcOH furnished the diol **7a**, which was then esterified at C-3 by (*R*)- and (*S*)-MTPA chloride in dry pyridine (MTPA chloride = methoxytrifluoromethylphenylacetyl chloride). Analysis of the obtained MTPA derivatives, **7b** (*S*) and **7c** (*R*), respectively, according to the modified Mosher method[12] (Scheme 1), enabled us to assign the *S* configuration at C-3. Consequently, the absolute configurations at C-4 (*R*) and C-6 (*S*) were established on the basis of the above-deduced relative geometry. Given the limited amount of plakortide Q (**7**) available, which was also employed in biological tests, the absolute configuration at C-8 has not been determined. However, by comparing the ^{1}H and ^{13}C NMR spectroscopic signals for the fragment C-7/C-14 of **7** with the corresponding signals for plakortin (**1**) and compound **8** (see below) an *R* configuration can be confidently assigned at C-8.

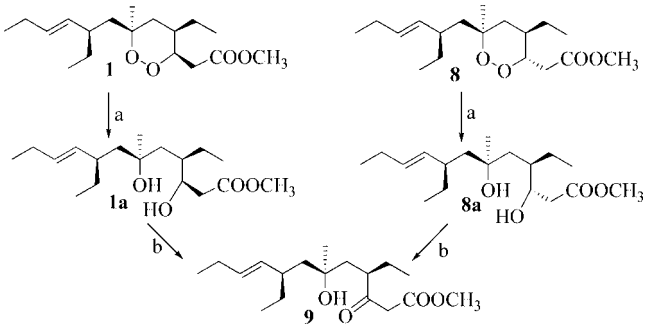


Scheme 1. Transformation of plakortide Q (**7**) to the diol **7a** and application of the modified Mosher's method for secondary alcohols. $\Delta\delta$ ($\delta_S - \delta_R$) are given in ppm. a) Zn/AcOH, b) (*S*)-MTPA chloride for **7c** and (*R*)-MTPA chloride for **7b**.

The HR-FABMS of compound **8** indicates a molecular formula of $C_{18}H_{32}O_4$, which indicates one less methylene unit than in plakortide Q (**7**). Inspection of the ^{1}H and ^{13}C NMR spectra of compound **8** (Table 2), which was completely assigned by interpretation of COSY, HMQC, and

HMBC data, led us to identify **8** as a close analogue of plakortide Q (**7**) – the ethyl group at C-6 is replaced by a methyl group. Accordingly, the only significant difference between the NMR spectra of **8** and those of **7** is the absence of the C/H signals assigned to the ethyl group C-15/C-16; instead methyl resonances are observed at δ_C = 21.3 ppm and δ_H = 1.32 ppm (singlet). The presence of this methyl unit at C-6 was confirmed by HMBC correlations of its protons (H_3-15) with C-5, C-6, and C-7.

The planar structure of compound **8** is identical to that of plakortin (**1**) –consequently, **8** must be a diastereomer. The spatial coupling (ROESY cross peaks) of 3-H with 16-H_2 and of 4-H with 15-H_3 and the pattern of the ring proton coupling constants suggest that compound **8** and plakortide Q (**7**) share the same relative configuration of the 1,2-dioxane chiral centers. To assign the absolute configuration of **8**, we designed the chemical transformations depicted in Scheme 2. Both plakortin (**1**) and compound **8** were treated with Zn/AcOH to afford the corresponding diols **1a** and **8a**, respectively. When compounds **1a** and **8a** were separately oxidized with the Dess–Martin periodinane,[13] the same ketone derivative **9** was obtained (NMR spectroscopic data and $[\alpha]_D$). This result unambiguously indicates that the only difference between plakortin and compound **8** is their configuration at C-3, and thus, the four chiral centers of **8** can be assigned as 3*S*, 4*R*, 6*S*, 8*R*.



Scheme 2. Transformation of both plakortin (**1**) and compound **8** to the ketone **9**. a) Zn/AcOH, b) periodinane in CH_2Cl_2.

Compound **8** had been previously isolated from *Plakortis halichondrioides*,[8] however, the absolute configuration at the four chiral centers was left undetermined, and the resonances of only a few proton and carbon signals were assigned.

Andersen and Kubanek demonstrated that some ethyl-branched polyketides of marine origin can be biosynthesized by incorporation of intact butyrate units, and they postulated that this could also apply to the biogenesis of plakortin (**1**).[14] The co-occurrence, in the same sponge, of polyketides with different lengths (**1** and **5**, see Figure 2) and of polyketide cycloperoxides with slightly different carbon skeletons (**7** and **8**) or with different stereochemistry (**1** and **8**) seems to indicate the existence of a rather flexible polyketide pathway that can assemble butyrate, propionate, and acetate substrates through different combinative arrangements. Accordingly, compounds **7** and **8** can be derived by alternative incorporation, as third acyl unit, of butyrate or propionate, respectively.

Compounds **7** and **8** are interesting metabolites from structural and biogenetic standpoints, but they are also valuable tools to test the effect of a single configurational change on the in vitro antimalarial activity of plakortin. Thus, using the pLDH assay, plakortin (**1**), plakortide Q (**7**), and compound **8** were assayed against D10 (chloroquine sensitive, CQ-S) and W2 (chloroquine resistant, CQ-R) strains of *Plasmodium falciparum*. The results are reported in Table 3. The three cycloperoxide derivatives **1**, **7**, and **8** exhibit consistent antimalarial activity against both CQ-R and CQ-S *P. falciparum* clones, but the IC_{50} values are lower (higher efficacy) for the CQ-R strain, as expected for artemisinin-like molecules. In addition, the activity of compounds **7** and **8** against W2 (CQ-R) is lower than that of plakortin (**1**), and thus, the change in configuration at C-3 appears to exert a negative effect on the antimalarial activity.

Table 3. In vitro antimalarial activity of plakortin (**1**), plakortide Q (**7**), and compound **8** against D10 (CQ-S) and W2 (CQ-R) strains of *Plasmodium falciparum*.

	D10 IC_{50} in μM	W2 IC_{50} in μM
1	0.87 ± 0.35	0.39 ± 0.13
7	1.00 ± 0.56	0.52 ± 0.16
8	1.03 ± 0.30	0.67 ± 0.18
Chloroquine	0.05 ± 0.02	0.81 ± 0.31
Artemisinin	0.013 ± 0.004	0.009 ± 0.005

Data are the means and SDs of four different experiments in triplicate.

Experimental Section

General Remarks: Optical rotations were measured in $CHCl_3$ on a Perkin–Elmer 192 polarimeter equipped with a sodium lamp (λ = 589 nm) and a 10-cm microcell. IR (KBr) spectra were recorded on a Bruker model IFS-48 spectrophotometer. UV spectra were obtained in CH_3CN using a Beckman DU70 spectrophotometer. HR-FABMS were performed on a FISONS Prospec mass spectrometer using a glycerol matrix. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Bruker AMX-500 spectrometer; chemical shifts are referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm; C_6D_6: δ_H = 7.15 ppm, δ_C = 128.0 ppm). The multiplicities of the ^{13}C resonances were determined by DEPT experiments. Homonuclear ^1H connectivities were determined by using COSY experiments. One-bond heteronuclear ^1H-^{13}C connectivities were determined with HSQC pulse sequence (interpulse delay set for $^1J_{CH}$ = 125 Hz). Two- and three-bond ^1H-^{13}C connectivities were determined by gradient-selected HMBC experiments optimized for a $^{2,3}J$ of 8.0 Hz. Medium-pressure liquid chromatography (MPLC) was performed using a Büchi 861 apparatus with RP18 and SiO_2 (230–400 mesh) stationary phases. High performance liquid chromatography (HPLC) separations were achieved in isocratic mode on a Beckmann apparatus equipped with RI detector and LUNA (Phenomenex) columns (SI60, 250 × 4 mm).

Animal Material, Extraction and Isolation: A specimen of *Plakortis simplex* was collected in July 2002 along the coasts of Bahamas. A

voucher specimen is deposited at the Dipartimento di Chimica delle Sostanze Naturali, Italy with the ref. number 02–10. The organism was immediately frozen after collection and kept frozen until extraction when the sponge (43 g, dry weight after extraction) was homogenized and extracted with methanol (4 × 500 mL) and with chloroform (4 × 500 mL). The methanol extract was initially partitioned between H_2O and *n*BuOH, and the organic phase was then combined with the $CHCl_3$ extract and concentrated in vacuo to afford a brown oil (22.1 g). This was subjected to chromatography on a column packed with RP18 silica gel and eluted with H_2O/MeOH, 9:1 (A_1), H_2O/MeOH, 7:3 (A_2), H_2O/MeOH, 4:6 (A_3), H_2O/MeOH, 2:8 (A_4), and MeOH (A_5). Fractions A_4 and A_5 were combined (6.5 g) and further chromatographed by MPLC (SiO_2 230–400 mesh; solvent gradient system with increasing polarity from *n*-hexane to MeOH). MPLC fractions eluted with *n*-hexane/EtOAc (9:1) were re-chromatographed using an SiO_2 column, with a solvent gradient system from *n*-hexane to *n*-hexane/EtOAc (8:2) to afford plakortin (**1**, 1.18 g). The other fractions were combined and purified by HPLC (eluent *n*-hexane/EtOAc, 97:3, flow 0.8 mL/min) to give dihydroplakortin (**2**, 5.5 mg), compound **4** (3.0 mg), compound **8** (4.2 mg), and plakortide Q (**7**, 2.8 mg) in the pure form. MPLC fractions eluted with *n*-hexane/EtOAc (8:2) were re-chromatographed by HPLC (eluent *n*-hexane/EtOAc, 85:15, flow 1.0 mL/min) to afford pure compounds **5** (1.8 mg) and **6** (0.9 mg).

Compound 5: Colorless oil. $[\alpha]_D^{25}$ = +25.1 (*c* = 1.5 mg/mL in $CHCl_3$). IR (KBr): $\tilde{\nu}_{max}$ = 3345, 1735, 1690 cm^{-1}. UV(CH_3CN): λ_{max} = 235 nm (ε = 18500). ^1H and ^{13}C NMR ($CDCl_3$): see Table 1. LR-FABMS: *m*/*z* = 339 $[M + H]^+$. HR-FABMS: observed *m*/*z* = 339.2547 $[M + H]^+$; calcd. for $C_{20}H_{35}O_4$, 339.2535; Δ = 3.5 ppm.

Compound 6: Colorless oil. $[\alpha]_D^{25}$ = +23.3 (*c* = 0.9 mg/mL in $CHCl_3$). IR (KBr): $\tilde{\nu}_{max}$ = 3345, 1737, 1692 cm^{-1}. UV(CH_3CN): λ_{max} = 235 nm (ε = 18500). ^1H and ^{13}C NMR ($CDCl_3$): see Table 1. LR-FABMS: *m*/*z* = 341 $[M + H]^+$. HR-FABMS: observed *m*/*z* = 341.2677 $[M + H]^+$; calcd. for $C_{20}H_{37}O_4$, 341.2692; Δ = 4.3 ppm.

Plakortide Q (7): Colorless oil. $[\alpha]_D^{25}$ = +10.3 (*c* = 2.5 mg/mL in $CHCl_3$). IR (KBr): $\tilde{\nu}_{max}$ = 1741, 1449, 970 cm^{-1}. ^1H and ^{13}C NMR ($CDCl_3$): see Table 2. LR-FABMS: *m*/*z* = 327 $[M + H]^+$. HR-FABMS: observed *m*/*z* = 327.2549 $[M + H]^+$; calcd. for $C_{19}H_{35}O_4$, 327.2535; Δ = 4.2 ppm.

Compound 8: Colorless oil. $[\alpha]_D^{25}$ = +22.3 (*c* = 3.5 mg/mL in $CHCl_3$). IR (KBr): $\tilde{\nu}_{max}$ = 1740, 1450, 970 cm^{-1}. ^1H and ^{13}C NMR ($CDCl_3$): see Table 2. LR-FABMS: *m*/*z* = 313 $[M + H]^+$. HR-FABMS: observed *m*/*z* = 313.2383 $[M + H]^+$; calcd. for $C_{18}H_{33}O_4$, 313.2379; Δ = 1.3 ppm.

Reduction of Plakortide Q (7), Plakortin (1), and Compound 8: Plakortide Q (**1**, 1.6 mg, 5×10^{-3} mmol) in dry ether (100 μL) was treated with acetic acid (40 μL) and excess Zn dust (10 mg). The mixture was then stirred vigorously for 24 h at room temperature. After it was confirmed by TLC that the starting material had disappeard, the solution was neutralized with Na_2CO_3 and the solid removed by filtration. The solvent was then evaporated, and the obtained product was partitioned between H_2O and $CHCl_3$. The organic phase contained compound **7a** (1.4 mg) in the pure form. The same procedure was applied to compound **8** (2.0 mg, 6.4×10^{-3} mmol) to afford 1.6 mg of compound **8a** and to plakortin (**1**, 4.0 mg, 1.2×10^{-2} mmol) to afford 3.6 mg of compound **1a**. Spectroscopic data of compound **1a** have already been reported.[15]

Compound 7a: Colorless oil. $[\alpha]_D^{25}$ –18 (*c* = 1.0 mg/mL in $CHCl_3$). IR (KBr): $\tilde{\nu}_{max}$ = 3350, 2975 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 5.48 (dt, *J* = 15.2, 6.2 Hz, 1 H, 10-H), 5.16 (dd, *J* = 15.2, 9.0 Hz, 1 H, 9-H), 3.90 (dt, *J* = 9.8, 2.1 Hz, 1 H, 3-H), 3.69 (s, 3 H, 19-H_3), 2.59 (dd, *J* = 15.9, 2.1 Hz, 1 H, 2a-H), 2.45 (dd, *J* = 15.9, 9.8 Hz, 1 H, 2b-H), 2.06 (overlapped, 8-H), 2.04 (overlapped, 11-H_2),.80 (dd, *J* = 14.5, 3.5 Hz, 1 H, 7a-H), 1.70 (m, 1 H, 4-H), 1.52 (overlapped, 15a-H), 1.50 (overlapped, 7b-H), 1.48 (overlapped, 15b-H), 1.45 (overlapped, 5a-H), 1.44 (overlapped, 17a-H), 1.38 (overlapped, 13a-H), 1.25 (dd, *J* = 13.8, 6.4 Hz, 1 H, 5b-H), 1.18 (overlapped, 13b-H), 1.18 (overlapped, 17b-H), 0.97 (t, *J* = 6.2 Hz, 3 H, 12-H_3), 0.87 (t, *J* = 7.3 Hz, 3 H, 14-H_3), 0.85 (t, *J* = 6.3 Hz, 3 H, 18-H_3), 0.82 (t, *J* = 6.3 Hz, 3 H, 16-H_3) ppm. FABMS: *m*/*z* = 329 $[M + H]^+$.

Preparation of MTPA Esters of Compound 7a: Compound **7a** (0.5 mg, 1.6×10^{-3} mmol) was dissolved in dry pyridine (0.3 mL), treated with (*R*)-MTPA chloride (10 μL), and the mixture was maintained at room temperature whilst stirring overnight. After removal of the solvent, the reaction mixture was purified by HPLC with a SI60 column (eluent *n*-hexane/EtOAc, 95:5) to afford the (*S*)-MTPA ester **7b** in the pure form (0.6 mg). The same procedure with (*S*)-MTPA chloride afforded the (*R*)-MTPA ester **7c** in the same yield.

(*S*)-MTPA Ester (7b): Amorphous solid. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.35 and 7.45 (MTPA phenyl protons), 5.71 (dt, *J* = 9.8, 2.1 Hz, 1 H, 3-H), 5.46 (dt, *J* = 15.2, 6.2 Hz, 1 H, 10-H), 5.18 (dd, *J* = 15.2, 9.0 Hz, 1 H, 9-H), 3.59 (s, MTPA OCH_3), 3.50 (s, 3 H, 19-H_3), 2.52 (dd, *J* = 15.9, 2.1 Hz, 1 H, 2a-H), 2.46 (dd, *J* = 15.9, 9.8 Hz, 1 H, 2b-H), 2.00 (q, *J* = 7.0 Hz, 2 H, 11-H_2), 1.92 (m, 1 H, 8-H), 1.85 (overlapped, 7a-H), 1.84 (overlapped, 4-H), 1.56 (overlapped, 15a-H), 1.55 (overlapped, 7b-H), 1.48 (overlapped, 15b-H), 1.48 (overlapped, 5a-H), 1.45 (overlapped, 17a-H), 1.35 (m, 1 H, 13a-H), 1.28 (overlapped, 5b-H), 1.22 (overlapped, 17b-H), 1.15 (m, 1 H, 13b-H), 0.97 (t, *J* = 7.0 Hz, 3 H, 12-H_3), 0.87 (t, *J* = 7.3 Hz, 3 H, 14-H_3), 0.87 (t, *J* = 6.3 Hz, 3 H, 18-H_3), 0.84 (t, *J* = 6.3 Hz, 3 H, 16-H_3) ppm. FABMS (glycerol matrix, positive ion): *m*/*z* = 545 $[M + H]^+$.

(*R*)-MTPA Ester (7c): Amorphous solid. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.32 and 7.55 (MTPA phenyl protons), 5.71 (dt, *J* = 9.8, 2.1 Hz, 1 H, 3-H), 5.46 (dt, *J* = 15.2, 6.2 Hz, 1 H, 10-H), 5.18 (dd, *J* = 15.2, 9.0 Hz, 1 H, 9-H), 3.64 (s, MTPA OCH_3), 3.56 (s, 3 H, 19-H_3), 2.62 (dd, *J* = 15.9, 2.1 Hz, 1 H, 2a-H), 2.59 (dd, *J* = 15.9, 9.8 Hz, 1 H, 2b-H), 2.00 (q, *J* = 7.0 Hz, 2 H, 11-H_2), 1.91 (m, 1 H, 8-H), 1.82 (overlapped, 7a-H), 1.75 (overlapped, 4-H), 1.55 (overlapped, 15a-H), 1.52 (overlapped, 7b-H), 1.45 (overlapped, 15b-H), 1.45 (overlapped, 5a-H), 1.43 (overlapped, 17a-H), 1.35 (m, 1 H, 13a-H), 1.25 (overlapped, 5b-H), 1.20 (overlapped, 17b-H), 1.15 (m, 1 H, 13b-H), 0.97 (t, *J* = 7.0 Hz, 1 H, 12-H_3), 0.87 (t, *J* = 7.3 Hz, 3 H, 14-H_3), 0.85 (t, *J* = 6.3 Hz, 3 H, 18-H_3), 0.82 (t, *J* = 6.3 Hz, 3 H, 16-H_3) ppm. FABMS (glycerol matrix, positive ion): *m*/*z* = 545 $[M + H]^+$.

Compound 8a: Colorless oil. $[\alpha]_D^{25}$ –9 (*c* = 1.5 mg/mL in $CHCl_3$). IR (KBr): $\tilde{\nu}_{max}$ = 3350, 2975 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 5.50 (dt, *J* = 15.2, 7.0 Hz, 1 H, 10-H), 5.21 (dd, *J* = 15.2, 9.0 Hz, 1 H, 9-H), 3.89 (dt, *J* = 9.8, 2.1 Hz, 1 H, 3-H), 3.70 (s, 3 H, 18-H_3), 2.59 (dd, *J* = 15.9, 2.1 Hz, 1 H, 2a-H), 2.40 (dd, *J* = 15.9, 9.8 Hz, 1 H, 2b-H), 2.14 (m, 1 H, 8-H), 2.03 (q, *J* = 7.0 Hz, 11-H_2), 1.76 (dd, *J* = 13.8, 5.0 Hz, 1 H, 5a-H), 1.64 (m, 1 H, 4-H), 1.56 (overlapped, 5b-H), 1.54 (overlapped, 7a-H), 1.45 (overlapped, 16a-H), 1.43 (overlapped, 7b-H), 1.35 (m, 1 H, 13a-H), 1.19 (overlapped, 13b-H), 1.19 (overlapped, 16b-H), 1.17 (s, 3 H, 15-H_3), 0.96 (t, *J* = 7.0 Hz, 3 H, 12-H_3), 0.89 (t, *J* = 7.3 Hz, 3 H, 17-H_3), 0.82 (t, *J* = 6.3 Hz, 3 H, 14-H_3) ppm. FABMS: *m*/*z* = 315 $[M + H]^+$.

Oxidation of Compounds 1a and 8a: Compound **1a** (3.0 mg, 1×10^{-2} mmol) was stirred at room temperature in CH_2Cl_2 (600 μL) under an inert atmosphere and periodinane (8 mg, 1.9×10^{-2} mmol)

was added in one portion. The reaction mixture was stirred to completion (TLC, about 2 h). The reaction was then quenched with sodium thiosulfate (saturated solution) and extracted four times with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The product was purified by HPLC (eluent *n*-hexane/EtOAc, 96:4) to yield 2.4 mg of compound **9**. Following the same procedure, compound **8a** (1.4 mg, 5×10^{-3} mmol) was treated with periodinane (5 mg, 1×10^{-2} mmol) to afford 1.0 mg of compound **9**.

Compound 9: Colorless oil. $[a]_D^{25} = +9$ ($c = 2$ mg/mL in $CHCl_3$). IR (KBr): $\tilde{\nu}_{max} = 3350, 1713\ cm^{-1}$. ^1H NMR (500 MHz, C_6D_6): $\delta =$ 5.19 (dt, $J = 14.5, 7.0$ Hz, 1 H, 10-H), 5.04 (dd, $J = 14.5, 9.0$ Hz, 1 H, 9-H), 4.90 (s, 1 H, 6-OH), 3.57 (s, 3 H, 18-H_3), 3.42 (s, 2 H, 2-H_2), 2.50 (m, 1 H, 4-H), 1.87 (overlapped, 8-H), 1.87 (overlapped, 11-H_2), 1.51 (dd, $J = 13.0, 3.5$ Hz, 1 H, 7a-H), 1.47 (overlapped, 5a-H), 1.45 (overlapped, 7b-H), 1.37 (m, 1 H, 13a-H), 1.36 (m, 1 H, 16a-H), 1.16 (overlapped, 13b-H), 1.11 (overlapped, 5b-H), 1.05 (s, 3 H, 15-H_3), 1.03 (overlapped, 16b-H), 0.87 (t, $J = 7.0$ Hz, 3 H, 12-H_3), 0.81 (t, $J = 6.3$ Hz, 3 H, 14-H_3), 0.57 (t, $J = 7.3$ Hz, 3 H, 17-H_3) ppm. FABMS: $m/z = 313\ [M + H]^+$.

Antimalarial Tests: *Plasmodium falciparum* cultures were prepared according to Trager and Jensen's method,[16] with slight modifications. The CQ-sensitive, moderately mefloquine-resistant clone D10 and the CQ-resistant, mefloquine-susceptible clone W2 were maintained at 5% haematocrit (human type A-positive red blood cells) in the complete culture medium at 37 °C. The complete medium contained RPMI 1640 medium (Gibco BRL, $NaHCO_3$ 24 mM) with the addition of 10% heat-inactivated A-positive human plasma, 20 mM Hepes (Biological Industries, Kibbutz, Israel), and 2 mM Glutammine (Biological Industries, Kibbutz, Israel). All the cultures were maintained in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. When parasitemia exceeded 5%, subcultures were taken; the culture medium was changed every second day.

The compounds were dissolved in either water (chloroquine) or DMSO (compounds **1**, **7**, **8**, and artemisinin), and then diluted with medium to achieve the required concentrations (in all cases the final concentration contained <1% DMSO, which was found to be non-toxic to the parasite). The drugs were placed in 96-wells flat-bottomed microplates (Costar # 3596), and serial dilution was made. Asynchronous cultures with parasitemia of 1–1.5% and 1% final haematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically (OD_{650}) by measuring the activity of the parasite lactate dehydrogenase (LDH), according to a modified version of the method of Makler,[17,18] in control and drug-treated cultures. Antimalarial activity was expressed as the 50% inhibitory concentrations (IC_{50}, μM); each IC_{50} value presented in Table 3 is the mean and standard deviation of four separate experiments performed in triplicate.

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Received: June 07, 2005
Published Online: October 12, 2005

