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New antimalarial polyketide endoperoxides from the marine sponge *Plakinastrella mamillaris* collected at Fiji Islands

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ABSTRACT

Plakortides R–U, four new polyketide endoperoxides, have been isolated from the marine sponge *Plakinastrella mamillaris*. Their structures were elucidated on the basis of extensive NMR spectroscopic (^{1}H and ^{13}C, COSY, HSQC, HMBC, and ROESY) and MS analyses and by chemical methods. In addition, a new method for the unambiguous stereochemical elucidation of 3,6-disubstituted 1,2-dioxines, frequently isolated from Plakinidae sponges, is reported. Pharmacological analysis demonstrated that plakortide U is endowed with in vitro antiplasmodial activity against a chloroquine-resistant strain.

1. Introduction

Six-membered cyclic peroxides are a large family of oxygenated polyketides characteristic of marine sponges of the family Plakinidae. After the discovery in 1978 of plakortin from *Plakortis halichondroides*,[1] several new derivatives, differing in the length of the side chain attached at C-6, by the presence of methyl- and/or ethyl branching, and in the number and position of double bonds have been isolated and found to exhibit a wide spectrum of biological activities, including antiparasitic and cytotoxic properties.[2–12]

In the frame of a project aimed at the investigation of marine invertebrates of the South Pacific Ocean, we had the opportunity to study the sponge *Plakinastrella mamillaris* collected at Fiji Islands. Previous investigation of the same organism by our research group resulted in the identification of several mono- and polycyclic oxygenated polyketides,[13,14] some of which displayed PPARγ agonistic activity.[13] We now report the results of investigations of the *n*-hexane extract, obtained from a solvent partitioning[15] of the crude methanol extract, which led to the isolation of four new cyclic polyketide peroxides (Fig. 1), which we named plakortides R–U (**1**–**4**).

In particular, plakortide R (**1**) was shown to be the methyl ester of a diastereomer of haterumadioxin A (**5**), previously isolated from the Okinawan sponge *Plakortis lita*.[8] In this paper we report the isolation and the structural elucidation of all new compounds, including a detailed stereochemical analysis of plakortide R (**1**) and the evaluation of their antimalarial activity.

2. Results and discussion

Plakortide R (**1**) has the molecular formula $C_{19}H_{32}O_4$ as determined by HRESIMS (*m*/*z* 347.2188 $[M+Na]^+$, calcd 347.2198), which requires 4° of unsaturation.

A carbomethoxy group was indicated by one methoxy signal (δ_H 3.71, δ_C 52.2) and one acyl carbon at δ_C 172.2. The ^{13}C NMR spectrum also contained 4 olefinic carbon signals attributable to a trisubstituted and a disubstituted double bond [δ_C 138.2 (s) and 124.8 (d), 134.6 (d) and 131.8 (d)], 2 signals at δ_C 83.3 (s) and 77.0 (d) assigned to oxygen-bearing carbon atoms, and 11 aliphatic carbon signals ($4CH_3$, $6CH_2$, and 1CH). Analysis of the 2D NMR spectra, including DQF-COSY, HSQC and HMBC spectra allowed the assignment of the structure of plakortide R (**1**). This was identified as the methyl ester of a compound sharing the entire molecular framework with the known haterumadioxin A (**5**) (Fig. 1), isolated by Uemura and co-workers from an Okinawan *P. lita*.[8] The availability in our laboratories of an original sample of haterumadioxin A,

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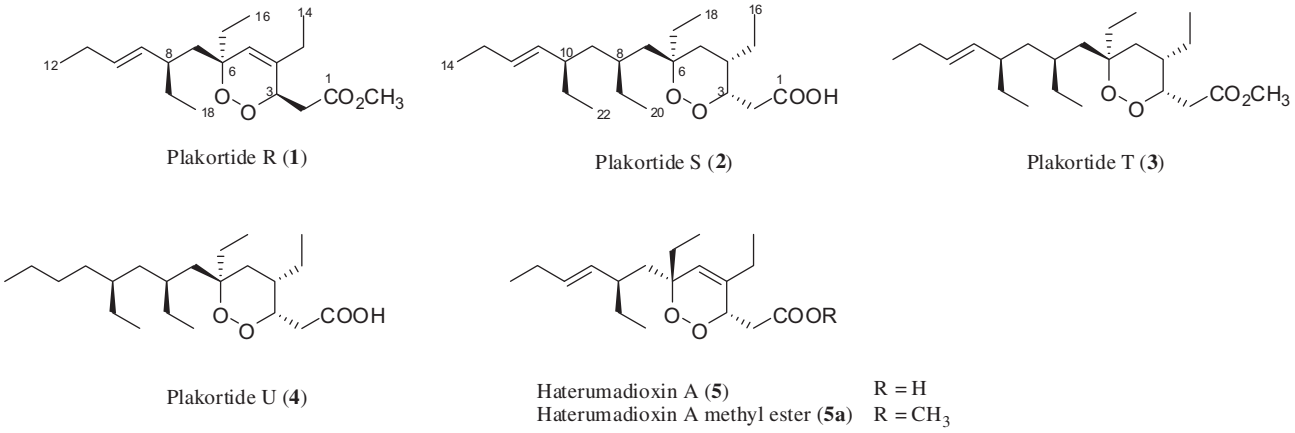

Fig. 1. New cyclic polyketide endoperoxides (**1**–**4**) from *Plakinastrella mamillaris*, the previously reported haterumadioxin A (**5**) and its methyl ester derivative (**5a**).

obtained from our previous investigations on Plakinidae sponges, allowed the preparation of its methyl ester derivative (**5a**) (Fig. 1), whose ^{1}H NMR spectroscopic chemical shifts were carefully compared to those of plakortide R (Table 1 and Experimental section). This comparison evidenced several small but significant differences between the two sets of signals, especially in the resonances of the nuclei attached to the peroxide ring and of the diastereotopic CH_2-7 protons. For example, these latter signals resonated as well-separated double doublets at δ_H 1.59 and 1.86 in plakortide R and as closer signals at δ_H 1.49 and 1.60 in **5a**. Thus, we concluded that plakortide R is a diastereomer of haterumadioxin A methyl ester (**5a**). Having assigned the trans configuration at $\Delta^{9,10}$ of **1** on the basis of the large value of $J_{H\text{-}9/H\text{-}10}$ (15.3 Hz), we concluded that **1** and **5a** should differ in the configuration at one (or two) of the stereogenic centres at C-3, C-6 and C-8.

Table 1
^{1}H and ^{13}C NMR data (500 and 125 MHz, $CDCl_3$) of plakortide R (**1**)

Position	δ_H[a]	δ_C
1	—	172.2
2	2.57 dd (3.0, 15.1), 2.85 dd (8.8, 15.1)	37.2
3	4.62 br d (8.8)	77.0
4	—	138.2
5	5.48 s	124.8
6	—	83.3
7	1.59 ovl, 1.86 dd (3.5, 14.2)	43.1
8	1.98 ovl	40.9
9	5.13 dd (9.2, 15.3)	134.6
10	5.35 dt (6.5, 15.3)	131.8
11	2.01 ovl	25.6
12	0.97 t (7.5)	14.2
13	1.99 ovl	25.6
14	1.08 t (7.3)	11.9
15	1.58 ovl	30.2
16	0.79 t (7.5)	8.2
17	1.13–1.21 m, 1.32–1.40 m	30.1
18	0.80 t (7.3)	11.8
OMe	3.71 s	52.2

[a] Coupling constants are in parentheses and given in hertz. ^{1}H and ^{13}C assignments aided by COSY, HSQC and HMBC experiments. ovl: overlapped with other signals.

To determine the absolute configuration at C-3, plakortide R (**1**) was reduced by treatment with acetic acid and Zn dust in dry ether to give the diol **1a**, which was in turn esterified at C-3 with *R*-(−)- or *S*-(+)-MTPA chloride in dry CH_2Cl_2 (Fig. 2). Analysis of the obtained MTPA derivatives, **1b**, *S*-(−)- and **1c** *R*-(+)-, respectively, according to the modified Mosher method,[16] established the 3*R* configuration, opposite to that reported for haterumadioxin A (**5**).

The absolute configuration at C-8 was determined using a procedure based on the ^{1}H NMR spectroscopic chemical shift difference of the hydroxymethylene protons in diastereomeric *R*-(+)- and *S*-(−)-MTPA esters,[17] a method frequently used to assign the absolute configuration at C-2 of primary β-alkyl-substituted alcohols.[9,18] Accordingly, plakortide R (**1**) was converted into the alcohol derivative **1d** by mild ozonolysis[2] followed by reduction with $NaBH_4$ as shown in Fig. 3. Compound **1d** was treated with *R*-(−)- and *S*-(+)-MTPA chloride to give the *S*-(−)-MTPA (**1e**) and *R*-(+)-MTPA (**1f**) esters, respectively (Fig. 3). In the ^{1}H NMR spectra, the protons at C-9 of the *R*-(+)-MTPA derivative (**1f**) appeared as two well-separated double doublets at δ_H 4.52 and 4.21, whereas those of the *S*-(−)-MTPA ester (**1e**) were closer at δ_H 4.40 and 4.30. These data suggested the *R* configuration at C-8 of **1**, the same as in haterumadioxin A (**5**).

The analysis of spatial couplings did not allow an unambiguous definition of the relative stereochemistry around the unsaturated six-membered ring. In haterumadioxin A,[8] a strong ROE effect observed between the remote H-2a and H-9, and justified by the folding of the side chain due to π−π stacking between C-4 and C-9 double bonds, has been used to suggest a *cis* relationship between the carbomethoxy methyl substituent at C-3 and the side chain at C-6. Although we observed this ROE contact also for **1**, we reasoned that, given the remote nature of the involved protons, the same contact could also be feasible in the case of trans oriented carbomethoxy and C-6 side chains. Thus, for the sake of an unambiguous assignment of the relative configuration at C-6, we envisaged that saturation of the C-4 olefin could offer a better opportunity to analyze the relative spatial arrangement of the substituents around the 1,2-dioxane ring. Since the chemoselective hydrogenation of double bond in monocyclic 1,2-dioxines has been demonstrated to be practically unfeasible under different experimental conditions,[19] we subjected the side chain truncated monounsaturated derivative **1d** to epoxidation with *m*-CPBA in $CHCl_3$ (Fig. 4). Epoxidation was found to proceed stereoselectively, affording a 3:1 mixture of two diastereomeric epoxide derivatives **1g** and **1h**, which were separated through HPLC and characterized by 1D and 2D NMR spectroscopy.

Analysis of the ROESY spectrum of the major epoxide derivative **1g** clearly evidenced the β-configuration of the epoxide ring and gave definitive information on the stereochemistry of the six-membered ring. In particular ROESY correlations (Fig. 4) H-3/H-5, H-10 and H-5/H-12, H-13 indicated that H-3 and the two ethyl groups on C-4 and C-6 were on the same face of the molecule and, consequently, the carbomethoxy methyl substituent at C-3 and the side chain at C-6 should be cis-oriented.

On the basis of the overall stereochemical analysis, the absolute stereochemistry of plakortide R (**1**) was determined to be 3*R*,6*S*,8*R*.

The same sequence of derivatization reactions was applied to haterumadioxin A methyl ester (**5a**) (Fig. 4). The stereoselective