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Plakorin, a potent Ca^{2+}-ATPase activator from the Okinawan marine sponge *Plakortis* sp.

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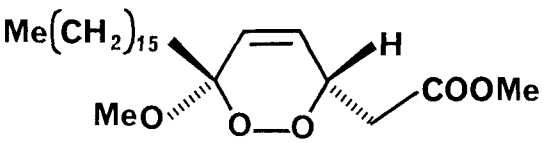
Summary. A new cyclic peroxide, plakorin, which is a potent sarcoplasmic reticulum (SR) Ca^{a+}-ATPase activator has been isolated from the Okinawan marine sponge *Plakortis* sp., its structure was elucidated on the basis of spectral data.
Key words. Sponge; plakorin; *Plakortis* sp.; cyclic peroxide; Ca^{2+}-ATPase activator.

The Ca^{2+}-ATPase in sarcoplasmic reticulum (SR) membrane plays a key role in muscle relaxation by energized Ca^{2+}-pumping from the cytoplasm into the lumen of SR[2]. In our continuing studies on pharmacological tools[3,4] for resolving the molecular mechanism of excitation-contraction coupling in skeletal and cardiac muscle, and on other bioactive compounds[5–8] from Okinawan marine organisms, we encountered extracts of the Okinawan marine sponge *Plakortis* sp. which exhibited remarkable activation of SR Ca^{2+}-ATPase activity. In this paper we describe the isolation and structure elucidation of a new cyclic peroxide, plakorin (**1**), as a powerful Ca^{2+}-ATPase activator.

The SR Ca^{2+}-ATPase was prepared from rabbit skeletal white muscle by the method of Meissner et al.[9]. The technique of measurement of the Ca^{2+}-ATPase activity was carried out as previously described[10]. The sponge *Plakortis* sp. was collected at Kerama Islands, Okinawa, and kept frozen until used. The methanol extracts of the sponge were partitioned between ethyl acetate and water. The ethyl acetate soluble fraction was subjected to silica gel column chromatography (hexane/ethyl acetate, 4:1) followed by preparative silica gel TLC (hexane/ethyl acetate, 3:1). Plakorin (**1**)[11] was obtained as a colorless oil, $[\alpha]_D^{27}$ + 44.3° (C = 0.2, $CHCl_3$), in 0.007% yield (wet wt) together with but-3-enolide (**2**, 0.003%).



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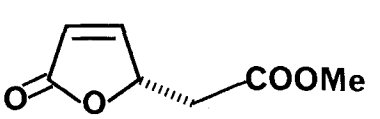

2

The FABMS spectrum of **1** showed a pseudomolecular ion at *m/z* 413 ($M^+ + H$), while the EIMS data revealed a characteristic M-O_2 peak at *m/z* 380. The molecular formula $C_{24}H_{44}O_5$ of **1** was established by the high-resolution FABMS (*m/z* 413.3273, $M^+ + H$, $\triangle$0.6 nm). The IR spectrum of **1** provided evidence for an ester carbonyl group (1740 cm^{-1}).

The ^1H NMR data indicated the presence of a long aliphatic chain (δ 1.26 brs, 28 H) with only one terminal methyl group (δ 0.89 t, H-22), two methoxy groups (δ 3.73 s, CO_2CH_3; δ 3.40 s, OCH_3), and a *Z*-double bond (δ 6.12, dd, J = 10.2 and 1.5 Hz, H-4; δ 5.86, dd, J = 10.2 and 2.1 Hz, H-5). The partial structure CH_2-CH(O)$-$CH = CH (C-2 to C-5) was deduced from analysis of the COSY spectrum in which the following cross peaks were observed: H_2-2 (δ 2.51 and 2.62)/H-3 (δ 5.01); H-3/H-4; H-3/H-5; H-4/H-5. The unsaturation number (3) in addition to these ^1H NMR data indicated the presence of a dioxene ring in **1**. This was further substantiated by the ^{13}C NMR data for the ring carbons (δ 73.5 d, C-3; δ 127.1 d, C-4 or C-5; δ 130.0 d, C-5 or C-4; 101.2 s, C-6). The ester carbonyl (δ 170.0 s, C-1) was connected to C-2, as shown by the chemical shifts (δ 2.51 and 2.62) and ABX coupling patterns of H_2-2. Both the remaining alkyl chain C-7 $\sim$ C-22) and the methoxy group (δ 3.40 s) were attached to C-6 (101.2 s) to complete the structure of plakorin (**1**).

The spectral data of **1** were all very similar to those of chondrillin[12] isolated from the sponge *Chondrilla* sp. However, the proton signals for H-2, H-3, and H-4 of **1** were almost identical to those of cyclic peroxides[13] (δ 2.62 and 2.52, H-2; δ 5.01, H-3; δ 6.12, H-4) isolated from the sponge *Plakortis lita* but significantly different from those of chondrillin (δ 2.93 and 2.26, H-2; δ 4.78, H-3; δ 6.18, H-4), indicating a stereochemical difference in the dioxene ring between **1** and chondrillin. This was also supported by the ^{13}C NMR data for the ring carbons which were identical for **1** and the cyclic peroxides from *Plakortis lita* (δ 73.5, C-3; δ 127.0 $\sim$ 127.1, C-4 or C-5; δ 130.0 $\sim$ 130.4, C-5 or C-4; δ 101.1 $\sim$ 101.2, C-6) but slightly different for chondrillin (δ 73.7, 126.4, 129.2, and 100.5). These results suggest that plakorin (**1**) as well as the cyclic peroxides from *Plakortis lita* have a configuration epimeric to chondrillin. The absolute configuration of the latter at C-3 has been assigned as S but that at C-6 has remained unknown[12]. The stereochemical assignment for **1** is coincident with those of the relative configuration of homologous cyclic peroxides based on proton couplings (^3J and ^4J) and conformational analysis[14].

The spectroscopic data of **2** were all identical to those of (5R)-but-3-enolide[14] isolated from the sponge *Xestospongia* sp. except for the value of $[\alpha]_D^{25}$ $-$ 51.3° (C = 1.3, $CHCl_3$), the sign of which was opposite to that of the latter ($[\alpha]_D^{20}$ + 80.3 °), but was the same as that of (5S)-2-oxo-4-hydroxy-2,5-dihydrofuran-5-acetic acid ($[\alpha]_D^{20}$ $-$ 41.6°)[14]. This indicates that **2** is (5S)-2-oxo-2,5-dihydrofuran-5-acetic acid methyl ester, the enantiomer of the but-3-enolide from *Xestospongia* sp.

Plakorin **1** (10^{-5} M) activated SR Ca^{2+}-ATPase activity by 30%, and was about ten times more potent than symbioramide[15] isolated from the symbiotic marine dinoflagellate *Symbiodinium* sp. in this assay. Plakorin (**1**) may provide a valuable chemical tool for studying the molecular mechanism of Ca^{2+} transport by Ca^{2+}-ATPase in the SR[10]. Plakorin (**1**) also exhibited antineoplastic activity[16] against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro, with IC_{50} values of 0.85 and 1.8 µg/ml, respectively. On the other hand, but-3-enolide (**2**) showed inhibitory activity on calmodulin-activated brain phosphodiesterase ($IC_{50} = 3 \times 10^{-5}$ M), although **2** also exhibited mild potentiation of SR Ca^{2+}-ATPase activity (17% activation at 3×10^{-5} M).

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