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Diana M. Kushlan, and D. John Faulkner

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A NOVEL PERLACTONE FROM THE CARIBBEAN SPONGE *PLAKORTIS ANGULOSPICULATUS*

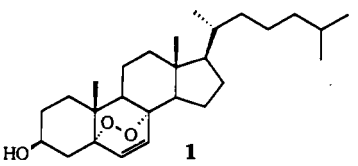
DIANA M. KUSHLAN and D. JOHN FAULKNER*

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0212

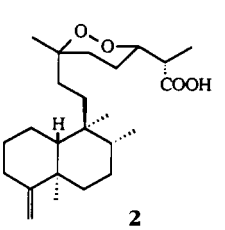
ABSTRACT.—The Caribbean sponge *Plakortis angulospiculatus* contains a perlactone, plakinidone [**5**], that was identified by interpretation of spectral data. Plakinidone is the first natural product that incorporates a six-membered perlactone ring.

There are three classes of cyclic peroxides that are commonly associated with marine sponges: steroidal peroxides, norsesesterterpene and norditerpene peroxides, and polyketide peroxides. The steroidal peroxides, such as 5α,8α-epidioxycholest-6-en-3β-ol [**1**], are encountered relatively frequently (1), and they have recently been shown to deter potential fish predators (2). The norterpene peroxides, exemplified by sigmosceptrillin A [**2**] from *Sigmosceptrella laevis* (3), are based on a variety of terpenoid carbon skeletons (4–11). A number of polyketide peroxides, of which plakortin [**3**] from *Plakortis halichondrioides* was the first example (12), have been described (12–21), but in most cases the relative and absolute stereochemistries have not been defined. There is one previous report of cyclic peroxides from *Plakortis angulospiculatus* Carter (order Homosclerophorida, family Plakinidae) (20), but the metabolites reported are not closely related to those that we encountered. The polyketide peroxides are associated primarily with sponges of the family Plakinidae, most of which give crude extracts that show exceptional antimicrobial and antifungal properties. However, the compounds that have been isolated from these extracts are usually esters or lactones that do not possess the activity of the crude extracts, which is thought to be due to peroxide acids such as plakortic acid [**4**] (13). In this paper we report an unusual perlactone, plakinidone [**5**], that was isolated from *P. angulospiculatus* while attempting to obtain the compounds responsible for the antimicrobial and antiviral properties of the crude extract.

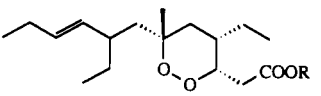
During a research cruise on R/V Columbus Iselin to Horse Shoe Reef, Tobago Cays, a small specimen of *P. angulospiculatus* was obtained. The crude MeOH extract of the sponge inhibited the growth of the bacteria *Staphylococcus aureus* and *Bacillus subtilis*, the yeast *Candida albicans*, and the *Herpes simplex* I virus, and inhibited cell division in the fertilized sea urchin egg assay. The antimicrobial activity was concentrated in the CH_2Cl_2-solubles from the MeOH extract, and this fraction was chromato-



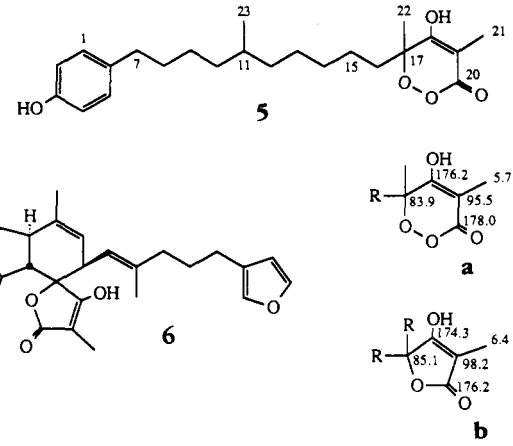
1



2



3 R=Me
4 R=H



graphed on Si gel to obtain plakinidone [**5**] (10 mg, 0.17% dry wt). Most of the biological activity was lost during Si gel chromatography.

Plakinidone [**5**] was obtained as a colorless oil of molecular formula $C_{23}H_{34}O_5$. The ^{13}C-nmr spectrum contained 21 signals, two of which were assigned to the symmetrically located carbons in a para-disubstituted aromatic ring. A DEPT experiment indicated 32 hydrogens attached to carbon atoms, leaving two hydrogens that must be on hydroxyl groups. The ir spectrum contained a broad hydroxyl band at 3100–3500 cm^{-1} together with bands at 1800 (weak), 1760, and 1670 cm^{-1} in the carbonyl region. The *p*-hydroxyphenethyl terminus of the molecule could be elucidated from ^{1}H-nmr signals at δ 6.95 (d, 2H, $J=8$ Hz), 6.69 (d, 2H, $J=8$ Hz), and 2.46 (t, 2H, $J=7$ Hz) and ^{13}C nmr signals at δ 154.0 (s), 134.3 (s), 129.3 (d, 2C), 115.0 (d, 2C), and 36.9 (t). The *p*-hydroxyphenyl group was joined through a hydrocarbon chain to a highly oxygenated moiety that contained four oxygen atoms, one of which must be incorporated in a hydroxyl group, and that accounted for three unsaturation equivalents. The remaining downfield ^{13}C-nmr signals at δ 178.0 (s), 176.2 (s), 95.5 (s), and 83.9 (s) were initially assigned to two ester (lactone) carbonyl carbons and two quarternary carbons bearing one or two oxygens, but no rational structure containing these groups could be devised. These signals were instead assigned to a perlactone ring system **a** analogous to the five-membered system **b** found in ircinianin [**6**] (22,23). The proposed structure **a** not only allows the ^{13}C-nmr signals of the ring carbons to be assigned but also provides a rationale for the unusual upfield shift of the vinyl methyl signal at δ 5.7 (q). The extreme chemical shift values for the olefinic carbon signals can only be found in β-hydroxy α,β-unsaturated carbonyl systems. The presence of the peroxide ring was supported by the facile loss of oxygen in the mass spectrum resulting in a prominent peak at *m/z* 374 $[M-16]^+$. A second prominent peak at *m/z* 290 $[M-C_4H_4O_3]^+$ was assigned to the methyl ketone that results from cleavage of the peroxide bond and the bond between C-17 and C-18. The ^{1}H-nmr spectrum contains three methyl signals at δ 1.62 (s, 3H, H-21), 1.36 (s, 3H, H-22), and 0.76 (d, 3H, $J=6$ Hz, H-23). The secondary methyl group must be located on the C_{10} hydrocarbon chain linking the peroxide and aromatic rings, and the ^{13}C-nmr signals at δ 19.6 (q) and 32.5 (d), assigned to C-23 and the adjacent methine carbon, indicate that the methyl group is located between C-9 and C-14. The decision to place the secondary methyl group at C-11 was based solely on mass spectral evidence: the series of peaks re-

sulting from sequential cleavage of the alkyl chain (sometimes referred to as CH_2 peeling) skips from *m/z* 149 $[C_{10}H_{13}O]^+$ (1.2%), a peak resulting from cleavage between C-10 and C-11, to *m/z* 177 $[C_{12}H_{17}O]^+$ (1.4%) due to cleavage between C-11 and C-12. The corresponding peaks for the perlactone end of the molecule, less oxygen, were observed at $m/z = 225$ $[M-16-149]^+$ and 197 $[M-16-177]^+$.

Plakinidone [**5**] showed mild antimicrobial activity against *S. aureus* and *B. subtilis* at 100 μg/disk in a standard disk assay but was inactive against *C. albicans*. An analysis of bioassay results indicated that most of the antimicrobial activity was lost during the Si gel chromatography.

EXPERIMENTAL

ISOLATION PROCEDURE.—The dark-olive-colored sponge *P. angulospiculatus* (5.9 g dry wt) was collected by hand using SCUBA at Horse Shoe Reef, Tobago Cays, at a depth of 17 m. The specimen, which has now been deposited in the SIO Benthic Invertebrate Collection (# P1128), was stored in MeOH (250 ml) for several months. The MeOH was decanted and filtered to obtain a crude extract that inhibited the growth of the bacteria *S. aureus* (ATCC 29213) and *B. subtilis* (ATCC 6633), the yeast *C. albicans* (ATCC 32354), and the *H. simplex* I virus, and inhibited cell division in the fertilized sea urchin egg assay. The MeOH was evaporated and the aqueous residue was diluted with H_2O (50 ml) and extracted sequentially with hexane (3 × 150 ml), CH_2Cl_2 (3 × 150 ml), and EtOAc (3 × 150 ml). The aqueous phase was lyophilized, and the residue was triturated with MeOH (50 ml). The hexane (161 mg), CH_2Cl_2 (244 mg), EtOAc (68 mg), and MeOH (45 mg) extracts were assayed against the microorganisms listed above, and most of the antimicrobial activity was found in the CH_2Cl_2 extract. The CH_2Cl_2 extract was chromatographed on Si gel to obtain two fractions having antimicrobial activity. The more polar of these fractions (64 mg) was further purified by preparative hplc on a Partisil column using 40% hexane in EtOAc as eluent to obtain plakinidone [**5**] (10 mg, 0.17% dry wt).

PLAKINIDONE [**5**].—Clear oil: $[\alpha]_D$ +7.9° ($c = 0.61$, MeOH); ir ($CHCl_3$) 3100–3500 (br), 1800 (weak), 1760, 1670 cm^{-1}; uv (MeOH) 224 nm (ε 6740), 279 nm (ε 1710); ^1H nmr ($CDCl_3$, 360 MHz) δ 0.76 (d, 3H, $J = 6$ Hz), 1.17 (br s, 11H), 1.36 (s, 3H), 1.40–1.51 (m, 4H), 1.62 (s, 3H), 2.46 (t, 2H, $J = 7$ Hz), 3.34 (br s, OH), 6.69 (d, 2H, $J = 8$ Hz), 6.95 (d, 2H, $J = 8$ Hz); ^{13}C nmr ($CDCl_3$, 50 MHz) δ 5.7 (q), 19.8 (q), 23.0 (q), 23.1 (t), 26.1 (t), 26.7 (t), 29.4 (t), 29.9 (t), 31.7 (t), 32.5 (d), 34.7 (t), 36.6 (t), 36.9 (t), 83.1 (s), 95.5 (s), 115.0 (d, 2C), 129.3 (d, 2C), 134.4 (s), 154.0 (s), 176.2 (s), 178.0 (s); hrms *m/z* 390.2431 ($C_{23}H_{34}O_5$ requires 390.2406); ms *m/z* (% int) 390 (2.6), 374 (14), 290 (5.6), 225 (1), 197 (1.3), 177 (1.4), 149 (1.2), 135 (1.8), 121 (4), 107 (100).

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