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METABOLITES OF THE PALAUAN SPONGE *DACTYLOSPONGIA* SP.

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Abstract: A Palauan sponge of the genus *Dactylospongia* contained four new diastereoisomeric sesquiterpene cyclopentenones, dactylospongenones A-D (**3-6**) that are related through a ring-contraction to ilimaquinone (**2**), which was also isolated from the sponge. The same sponge also contained the known metabolite, dictyoceratin-A (**7**), and a new derivative, dictyoceratin-C (**8**).

As a result of their intrinsic antimicrobial properties, hydroquinones and quinones have been isolated fairly frequently from marine sponges.[1] A recent report suggesting that avarol (**1**) and the corresponding quinone, avarone, "may prove to be useful in the treatment of patients with AIDS and AIDS-related complex"[2] has led to heightened interest in this class of marine natural products. We had previously observed significant pharmacological activity,[3] such as inhibition of chemically-induced inflammation, inhibition of cell division in the fertilized sea urchin egg assay and antimicrobial activity, in ilimaquinone (**2**), a metabolite that was first described from *Hippospongia metachromia*[4] and subsequently encountered in several other sponges.[5] While evaluating sponges as sources of ilimaquinone (**2**) for HIV screening, we encountered a specimen of *Dactylospongia* sp. that contained five new compounds in addition to ilimaquinone (**2**) and dictyoceratin-A (**7**), which is a metabolite of *Hippospongia* sp.[6] Four of the new metabolites, dactylospongenones A-D (**3-6**), are ring-contracted derivatives of ilimaquinone (**2**) and the remaining compound, dictyoceratin-C (**8**) is related to dictyoceratin-A (**7**).

A small specimen of *Dactylospongia* sp. was collected at Bairakaseru reef, Palau (-15 m) and was stored in methanol at 4°C until workup. The hexane and dichloromethane soluble fractions from the methanolic extract of the sponge were combined and chromatographed on silica gel using eluants of increasing polarity from hexane to ethyl acetate. Fractions having interesting ^{1}H NMR spectra were further purified by LC on Partisil

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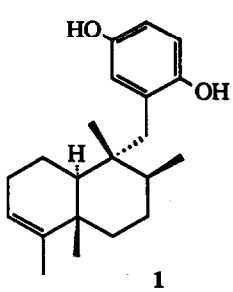
using 1:3 hexane-diethyl ether as eluant to obtain ilimaquinone (**2**, 30 mg, 0.024% dry weight), dactylospongenone A (**3**, 26 mg, 0.021% dry weight), dactylospongenone B (**4**, 3.9 mg, 0.003% dry weight), dactylospongenone C (**5**, 2.3 mg. 0.002% dry weight), dactylospongenone D (**6**, 2.4 mg, 0.002% dry weight), dictyoceratin-A (**7**, 9.7 mg, 0.008% dry weight), and dictyoceratin-C (**8**, 7.1 mg, 0.006% dry weight).

The dactylospongenones **3-6** all had the identical molecular formula, $C_{23}H_{34}O_5$, and possessed very similar IR and UV spectra. The ^1H and ^{13}C NMR spectra of the dactylospongenones were so similar that it could reasonably be assumed that the compounds were diastereomers. Comparison of the NMR data with those of ilimaquinone[7] revealed that all five compounds possessed the same bicyclic sesquiterpene skeleton with an exocyclic methylene group, two tertiary methyl groups and a secondary methyl group. The molecular formula of the dactylospongenones **3-6** could formally be derived by addition of methanol to ilimaquinone but the addition must involve the generation of two new chiral centers in order to obtain four diastereoisomers. A known ring contraction reaction of 2-hydroxy-1,4-quinones to produce cyclopentenones[8] met these requirements and allowed the generation of four diastereoisomeric structures for the dactylospongenones **3-6** that were consistent with the spectral data. Using dactylospongenone A (**3**) as an example, the ^1H NMR signals at δ 3.76 (s, 3 H) and 3.74 (s, 3 H) were assigned to the ester and ether methoxyl groups, the signal at 5.94 (s, 1 H) corresponds to the β-proton on the α,β-unsaturated ketone and the signals at 2.63 (t, 1 H, J = 4 Hz), 1.87 (dd, 1 H, J = 15, 4 Hz) and 0.97 (dd, 1 H, J = 15, 4 Hz) constitute the ABX system for the H11 and H16 protons. The ^{13}C NMR spectrum contains signals at δ 198.2 (s), 174.8 (s), 160.5 (s), 121.6 (d), 80.7 (s), 57.2 (q), 54.8 (d) and 53.5 (q) that are compatible with the carbons of the cyclopentenone ring and its substituents.



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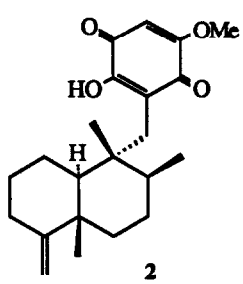

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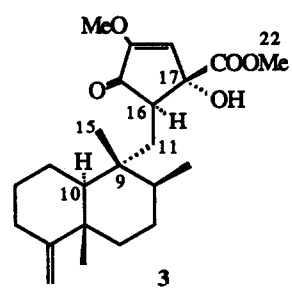

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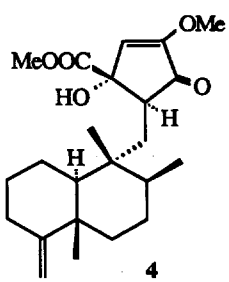

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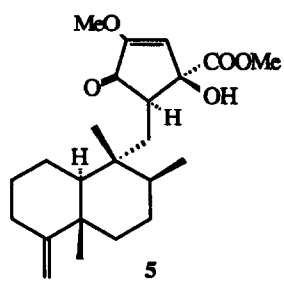

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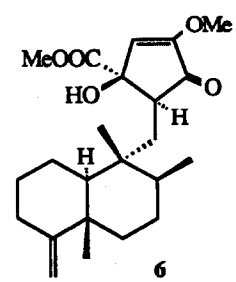

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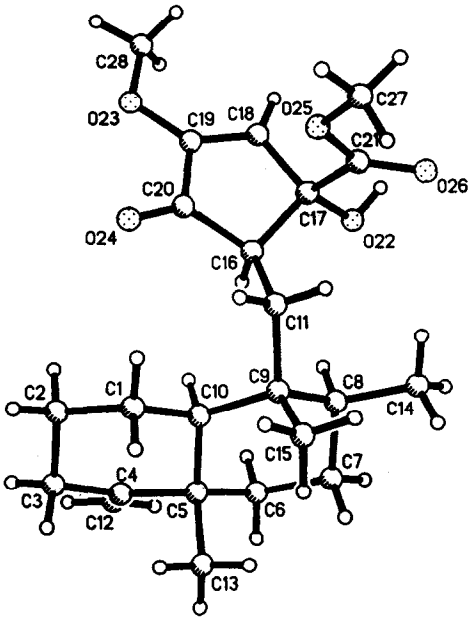


Figure 1. A computer generated perspective drawing of dactylospongenone A (3) with hydrogens. No absolute configuration is implied.

The spectral data alone were insufficient to define the stereochemistry of the dactylospongenones 3-6. However, the major diastereoisomer, dactylospongenone A (3) was nicely crystalline and its structure was determined by a single crystal X-ray diffraction study. A computer generated perspective drawing of dactylospongenone A (3) is given in Figure 1. The X-ray diffraction analysis did not define the absolute configuration, only the relative configuration, and the configuration shown was selected to conform with the assignment of Capon and MacLeod.[9] Both of the six-membered rings are in chair conformations, and the cyclopentenone ring is best described as having a shallow envelope conformation with C16 as the flap.

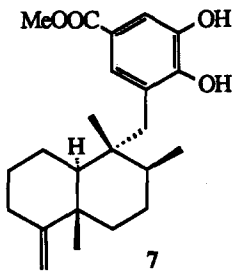
Having determined the relative stereochemistry of one diastereoisomer by X-ray analysis, the remaining three structures were assigned on the basis of comparison of spectral data. The chemical shifts of the H16, OCH_3(22) and OH protons (Table 1) are primarily dependent on the relative stereochemistry at C16 and C17. Dactylospongenones A(3) and B (4) were therefore assigned the same relative stereochemistry with the 17-hydroxyl group *cis* to the hydrogen at C16 while dactylospongenones C (5) and D (6) were assigned the opposite relative stereochemistry (OH (17) *trans* to H16). The X-ray model of dactylospongenone A (3) indicated that the molecule adopts a conformation in which the C11-C16 bond is approximately *trans*-anti-planar to the C9-C15 bond (θ = 178.3°) and the cyclopentenone ring points away from C9. Assuming that this is the most stable conformation, it follows that inversion of the stereochemistry at C16 will require a 180° rotation of the cyclopentenone ring so that in dactylospongenone B (4) the C20 carbonyl is nearer C8 than C10. The chemical shift of H10 is therefore the best guide to the stereochemistry at C16 relative to the bicyclic ring system. In both 3 and 5, H10 appears at δ 0.88, indicating the same configuration at C16. The H10 chemical shifts of δ 1.09 in 4 and 0.64 in 6 reflect the very different shielding effects of the adjacent hydroxyl or carbomethoxyl groups.

Table 1. Selected ^{1}H NMR chemical shift data and optical rotations of dactylospongenones A-D (3-6).

Compound	H10	H16	OMe(22)	OH	$[\alpha]_D$
3	0.88	2.63	3.75	4.00	-167°
4	1.09	2.65	3.75	3.83	+96°
5	0.88	2.74	3.84	3.59	+26°
6	0.64	2.86	3.81	3.61	-121°

Dictyoceratin-C (**8**) was obtained as a white microcrystalline solid. The mass spectrum showed a molecular ion at *m/z* = 356.2355 corresponding to the molecular formula $C_{23}H_{32}O_3$. The infrared bands at 3300 (broad) and 1710 cm^{-1} were assigned to hydroxyl and aromatic ester groups respectively. The UV spectrum exhibited a strong bathochromic shift (261 → 311 nm) on base treatment that is typical of a *p*-hydroxybenzoate. The ^1H NMR spectrum contained signals at δ 7.75 (m, 2 H) and 6.74 (d, 1 H, *J* = 8.8 Hz), that were assigned to two hydrogens *ortho* to the ester group and one hydrogen *ortho* to the hydroxyl group, and at 3.87 (s, 3 H), assigned to a methyl ester. Dictyoceratin-C (**8**) was therefore a sesquiterpene phenol with a 5-carbomethoxy-2-hydroxy substituted aromatic ring. Comparison of the ^1H and ^{13}C NMR spectra of dictyoceratin-C (**8**) with those of dictyoceratin-A (**7**) and ilimaquinone (**2**) indicated that all three compounds contained the same bicyclic sesquiterpene moiety.

The hypothesis that the dactylospongenones **3-6** might be artefacts formed by addition of the extraction solvent, methanol, to ilimaquinone (**2**), was tested by using mild acids, mild bases and cyanide ion as potential catalysts for this conversion. No reaction was observed with any catalyst even when the solutions were heated to 65°C. The conclusion that the dactylospongenones are indeed natural products is supported by the observation that much older sponge extracts contained ilimaquinone but no trace of the dactylospongenones.



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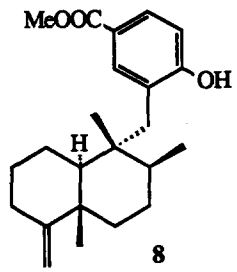

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Experimental Section

Collection, Extraction and Purification.

Dactylospongia sp. was collected by hand using SCUBA at Bairakaseru reef, Palau (-15 m) in January, 1985. The sponge (85-039, 124.7 g dry weight) was stored in methanol at 4°C for approximately 2 years. The methanol was decanted from the sponge and evaporated *in vacuo*. The resultant aqueous portion was sequentially extracted with hexane (3x 100 mL), dichloromethane (3x 100 mL) and ethyl acetate (3x 150 mL). The organic extracts were dried over sodium sulfate and the solvent evaporated. The compounds of interest were contained in the hexane extract (142.7 mg, 0.11% dry wt.) and the dichloromethane extract (313.7 mg, 0.25% dry wt.).

The combined hexane and dichloromethane extracts were chromatographed on silica gel using eluants of increasing polarity from hexane to ethyl acetate. The fractions of interest were then purified by repeated high performance liquid chromatography on Partisil using 25% hexane in diethyl ether as eluant. The LC fractions were combined, as indicated by the ^1H NMR spectra, to obtain ilimaquinone (**2**), dictyoceratin-A (**7**) and the following new compounds.

Dactylospongenone A (3): $[\alpha]_{589}$ -167.7 (c 0.062, MeOH); UV (MeOH) 248 nm (ε 7620); IR ($CHCl_3$) 3500 br, 1730, 1640, 1440, 1230, 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.73 (d, 3 H, *J* = 6.5 Hz), 0.74 (s, 3 H), 0.87 (d, 1

H, J = 12 Hz), 0.96 (dd, 1 H, J = 16, 4 Hz), 1.04 (s, 3 H), 1.86 (m, 1 H), 2.29 (m, 1 H), 2.63 (t, 1 H, J = 4 Hz), 3.74 (s, 3 H), 3.76 (s, 3 H), 3.93 (s, 1 H), 4.46 (br s, 2 H), 5.94 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 198.2 (s), 174.8 (s), 160.5 (s), 158.9 (s), 121.6 (d), 102.5 (t), 80.7 (s), 57.2 (q), 54.8 (d), 53.5 (q), 48.9 (d), 40.2 (s), 39.5 (s), 36.9 (d), 36.9 (t), 33.8 (t), 33.0 (t), 28.4 (t), 27.6 (t), 22.0 (t), 20.5 (q), 17.6 (q), 16.3 (q); HRMS, obsd. *m/z* 390.2433, $C_{23}H_{34}O_5$ requires 390.2406.

Dactylospongenone B (4): $[\alpha]_{589}$ 96.4 (c 0.22, MeOH); UV (MeOH) 249 nm (ε 6751); IR ($CHCl_3$) 3510 br, 1735, 1640, 1440, 1230, 1090 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.74 (s, 3 H), 1.00 (d, 3 H, J = 6 Hz), 1.03 (s, 3 H), 1.06 (m, 1 H), 1.09 (dd, 1 H, J = 8, 3 Hz), 1.45 (m, 4 H), 1.86 (m, 2 H), 2.09 (m, 1 H), 2.29 (m, 1 H), 2.65 (dd, 1 H, J = 5, 3 Hz), 3.74 (s, 3 H), 3.75 (s, 3 H), 3.83 (s, 1 H), 4.48 (s, 2 H), 5.92 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 198.3 (s), 174.9 (s), 160.0 (s), 158.9 (s), 121.4 (d), 102.8 (t), 80.6 (s), 57.2 (q), 54.2 (d), 53.4 (q), 48.4 (d), 40.0 (s), 39.7 (s), 37.2 (d), 36.9 (t), 33.7 (t), 32.9 (t), 28.1 (t), 27.7 (t), 22.1 (t), 20.3 (q), 17.5 (q), 16.7 (q); HRMS, obsd. *m/z* 390.2376, $C_{23}H_{34}O_5$ requires 390.2406.

Dactylospongenone C (5): $[\alpha]_{589}$ 25.5 (c 0.20, MeOH); UV (MeOH) 251 nm (ε 4845), IR ($CHCl_3$) 3510 br, 1730, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) 0.76 (s, 3 H), 0.78 (d, 3 H, J = 6 Hz), 1.05 (s, 3 H), 1.25 (br s, 1 H), 1.78 (d, 2 H, J = 5 Hz), 1.89 (m, 2 H), 2.10 (m, 1 H), 2.30 (m, 1 H), 2.74 (t, 1 H, J = 5 Hz), 3.58 (s, 1 H), 3.74 (s, 3 H), 3.84 (s, 3 H), 4.47 (d, 2 H, J = 5 Hz), 6.03 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 199.4 (s), 175.9 (s), 160.8 (s), 157.8 (s), 123.3 (d), 102.4 (t), 57.3 (q), 53.7 (q), 49.7 (d), 49.1 (d), 40.2 (s), 39.5 (s), 38.3 (d), 37.3 (t), 33.1 (t), 32.6 (t), 28.4 (t), 27.7 (t), 21.9 (t), 20.4 (q), 17.6 (q), 16.6 (q) (one signal obscured by solvent); HRMS, obsd. *m/z* 390.2430, $C_{23}H_{34}O_5$ requires 390.2406.

Dactylospongenone D (6): $[\alpha]_{589}$ -121.7 (c 0.14, MeOH); UV (MeOH) 249 nm (ε 5594); IR ($CHCl_3$) 3510 br, 1730, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.64 (dd, 1 H, J = 3, 12 Hz), 0.76 (s, 3 H), 0.94 (m, 4 H), 1.03 (s, 3 H), 1.25 (s, 1 H), 1.93 (dd, 1 H, J = 5, 16 Hz), 2.06 (m, 1 H), 2.29 (m, 1 H), 2.87 (t, 1 H, J = 5 Hz), 3.61 (s, 1 H), 3.74 (s, 3 H), 3.81 (s, 3 H), 4.46 (m, 2 H), 6.00 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 201.7 (s), 175.6 (s), 160.6 (s), 157.9 (s), 123.6 (d), 102.3 (t), 57.4 (q), 53.8 (q), 49.9 (d), 48.2 (d), 40.3 (s), 39.9 (s), 37.7 (d), 37.0 (t), 33.0 (t), 32.8 (t), 27.8 (t), 27.6 (t), 21.9 (t), 20.4 (q), 17.7 (q), 16.4 (q) (one signal obscured by solvent); HRMS, obsd. *m/z* 390.2417, $C_{23}H_{34}O_5$ requires 390.2406.

Dictyoceratin-C (8): IR ($CHCl_3$) 3300, 1710, 1285 cm^{-1}; UV (MeOH) 261 nm (ε 11490), (MeOH+NaOH) 311 nm (ε 15940); ^{1}H NMR ($CDCl_3$) δ 7.76 (m, 2 H), 6.74 (d, 1 H, J = 8.8 Hz), 5.56 (s, 1 H), 4.41 (br s, 1 H), 4.36 (br s, 1 H), 3.87 (s, 3 H), 2.65 (s, 2 H), 2.34 (td, 1 H, J = 13, 5 Hz), 1.06 (s, 3 H), 1.03 (d, 3 H, J = 6 Hz), 0.88 (s, 3 H), (acetone-d_6) δ 7.73 (d, 1 H, J = 2 Hz), 7.67 (dd, 1 H, J = 8.4, 2 Hz), 6.89 (d, 1 H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 159.9 (s), 158.8 (s), 138.0 (d), 129.3 (d), 125.1 (s), 122.2 (s), 115.4 (d), 102.9 (t), 51.8 (q), 48.3 (d), 42.2 (s), 40.3 (s), 37.3 (t), 36.6 (t), 36.5 (d), 33.0 (t), 27.9 (t), 27.8 (t), 23.3 (t), 20.6 (q), 17.6 (q), 17.5 (q) (C=0 signal not observed); HRMS, obsd. 356.2355, $C_{23}H_{32}O_3$ requires 356.2351.

Single crystal x-ray diffraction analysis of dactylospongenone A (3). A crystal of approximate dimensions 0.6x0.2x0.2 mm was used for all of the diffraction studies. Preliminary photographs displayed orthorhombic symmetry, and accurate lattice constants of **a** = 12.941(2), **b** = 14.261(3), and **c** = 11.731(2) Å were determined by a least-squares fit of fifteen diffractometer measured 2θ-values. Systematic extinctions and the known optical activity uniquely determined space group $P2_12_12_1$ with one molecule of composition $C_{23}H_{34}O_5$ forming the asymmetric unit ($\rho_{calc.}$ = 1.20 g/cm^3).

All unique diffraction maxima with 2θ ≤114° were collected using variable speed 1° w-scans and backgrounds of 50% of the peak collection time at the beginning and end of the scan. Three periodically monitored check reflections showed no crystal or instrumental instability. A total of 1676 reflections were measured in this fashion and, after correction by Lorentz, background, and polarization effects, 1515 (90%) were judged observed ($|F_o| \geq 3\ \sigma(F_o)$).[10]

A phasing model was found uneventfully using direct methods, and tangent formula recycling of plausible molecular fragments revealed the entire nonhydrogen atom structure. Hydrogen atoms were positioned at idealized locations and fixed in subsequent refinements. Block-diagonal least-squares refinement using the observed data and anisotropic nonhydrogen atoms has converged at a crystallographic residual of 0.058. Additional crystallographic details can be found in the Supplementary Material described at the conclusion of this paper.

Acknowledgement

We thank Dr. Rob van Soest for identification of the sponge and Professor Harold W. Moore for useful discussions concerning the rearrangements of quinones. We thank the Government of the Republic of Palau for a scientific research permit. This research was supported by grants from the Sea Grant College Programs of California (NA85AA-D-SG140, R/MP40 to DJF) and New York (to JC) and by the National Institutes of Health (CA24487 to JC and AI11969 to DJF).

Supplementary Material Available

Tables of fractional coordinates, thermal parameters, interatomic distances, interatomic and torsional angles for dactylospongenone A (5 pages) are available on request from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.

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3. These data were kindly provided by Dr. Robert S. Jacobs, University of California at Santa Barbara.

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7. Our ^{13}C NMR data suggests that the C4 signal of ilimaquinone (**2**) and related compounds is δ 160-162 (cf. references 4 and 6).

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