Structure assignments of two new C-18-oxygenated steroidal ketals isolated from a pacific soft coral of the genus *Sinularia*

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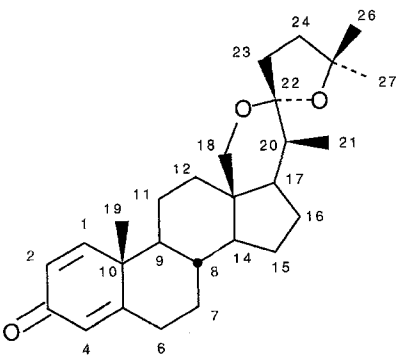
Summary. Two new C-18-oxygenated sterols possessing a spiroketal function (**1** and **2**) have been isolated from a soft coral of the genus *Sinularia*. The proposed structures are based on spectral data.

Key words. Sterols; ketals; soft coral; *Sinularia* sp.

As part of our continuing study of the secondary metabolites of marine soft corals (Cnidaria, Octocorallia), we have investigated the steroidal metabolites of an apparently undescribed species of the alcyonacean soft coral *Sinularia*. In this paper we report the structures of two new cholestadienone derivatives, **1** and **2**, which possess epimeric cyclic ketals at the C-22 side-chain position involving the oxidation of the C-18 methyl group. Similar steroidal compounds have been isolated from the gorgonian *Isis hippuris*[1-3] and the soft coral *Lobophytum depressum*[4].

The soft coral was collected at −130 m, by dredging, in the vicinity of Guam, and was immediately frozen. Extraction with chloroform : acetone (1 : 1), removal of solvents, and chromatographic purification involving silica and reversed phase HPLC, yielded ketals **1** and **2** as optically active, viscous oils.



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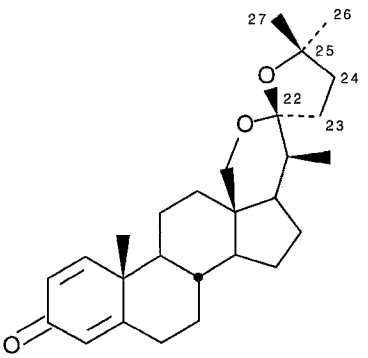

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The molecular formulae for **1** and **2** were determined as $C_{27}H_{38}O_3$ through interpretation of their respective high resolution mass spectra (M^+ at m/z 410.2813 for **1** and 410.2815 for **2**, calc. 410.2822). The carbon count was confirmed by ^{13}C-NMR data which showed that all 38 protons in **1** and **2** are attached to carbons, hence eliminating the possibility of alcohol functionalities in these molecules (table 1).

The combined spectral features of **1** and **2** made clear that these metabolites are cholesta-1,4-dienone derivatives[5], since the observed infrared absorption at 1660 cm^{-1} and UV absorption at 244 nm (ε = 13,500) are typical for a cross-conjugated cyclohexadienone functionality. The assignments of the ^{1}H-NMR features for ring A in **1** were made by comparison with similar model compounds. A doublet at δ 7.08 (J = 10 Hz) was

Table 1. ^{13}C-NMR assignments for ketals **1** and **2***.

C#	**1** δ	mult.	**1** $^3J_{C,H}$ observed with H-C(β) $^2J_{C,H}$ observed with H-C(α)	**2** δ
1	155.7	d	β = 19-H_3	155.9
2	127.5	d	β = 1, α = 4	127.5
3	186.3	s	α = 4	186.3
4	123.9	d		123.9
5	168.8	s	β = 19-H_3	169.1
6	32.8	t	β = 4	32.8
7	29.9[a]	t		28.6[a]
8	35.5	d		36.0
9	54.0[b]	d		54.1[b]
10	42.9	s	α = 19-H_3, β = 4	42.5
11	22.7[a]	t		22.9[a]
12	33.6	t	β = 18	33.8
13	43.6	s		43.7
14	52.6[b]	d		52.6[b]
15	23.4[a]	t		23.0[a]
16	24.9[a]	t		24.3[a]
17	49.3	d		49.4
18	62.1	t	β = 12	57.5
19	18.6	q		18.6
20	35.3	d	α = 21-CH_3	35.5
21	14.8	q		14.2
22	110.7	s	β = 18	109.3
23	33.8	t		33.8
24	37.6	t	β = 26-H_3, β = 27-H_3	36.1
25	79.9	s	α = 26-H_3, α = 27-H_3	82.9
26	30.0	q	β = 27-H_3	30.1
27	28.2	q	β = 26-H_3	28.6

*Assignments based on 2D XHCORR and comparison of chemical shifts with model compounds[8]. [a, b]These assignments may be interchanged. [c]Assignments made by comparison with ketal **1**.

assigned to H-C(1). This proton is coupled to the H-C(2) ($\delta = 6.22$) which appeared as a double doublet ($J = 10$ and 2 Hz), the small coupling arising from a 'W' coupling with the highly broadened C-4 proton ($\delta = 6.02$). In the ^{13}C-NMR spectrum of **1**, C(3) shows up at 186.3 ppm. The above assignments were confirmed by ^{13}C-NMR long-range heteronuclear correlation experiments (COLOC) (see table 1).

Conspicuously absent from the ^{1}H-NMR spectra of these steroid derivatives were the C-18 methyl groups which generally appear at exceptionally high field. However, the expected bands were replaced by an oxygenated methylene group which, in **1**, was observed as an AB double doublet ($J = 12.5$ Hz) at $\delta = 3.40$ and 3.69 ppm, respectively. The ^{13}C-NMR spectrum of **1** also showed a ketal carbon at 110.7 ppm and an additional quaternary, oxygen-bearing carbon atom at 79.9 ppm. The latter signal was assigned to C(25) since two deshielded methyl groups ($\delta = 1.38$ and 1.19, each singlets) were observed in the ^{1}H-NMR spectrum of **1.**

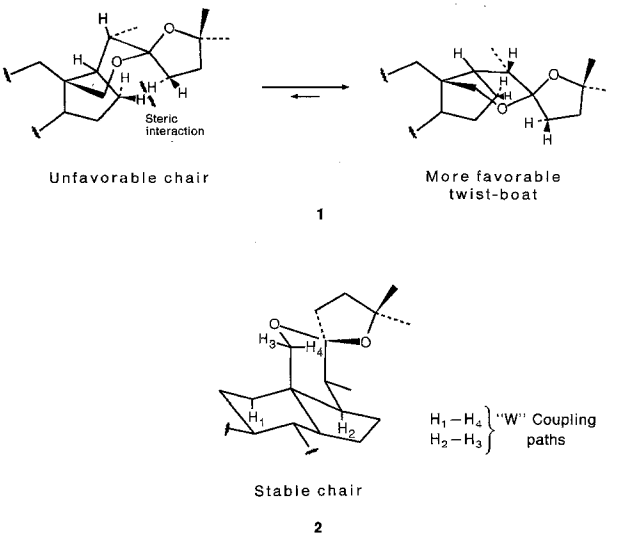
On the basis of these data, steroids **1** and **2** were formulated as epimeric spiro-bicyclic ketals with C-22 as the ketal carbon.

The disposition of the spiroketal function as depicted in the figure was established on the basis of the NMR analysis and the observation that the C-20, methyl-bearing carbon was not involved in ketal formation. An alternative and less likely possibility is that the ketal center be positioned at C-23, thus yielding an oxetane ketal. This was considered and eliminated after extensive 500 MHz ^{1}H-NMR analysis of both ketal isomers.

The configurations of C-20 and C-22 in **1** and **2** were assigned as follows: ^{1}H-NMR data indicated that the 6-membered ketal ring in **1** exists in a twist boat configuration, whereas the ketal ring in **2** is in the more stable chair form.

The C-18 methylene protons were particularly diagnostic of the conformation of the six-membered ketal ring. In d_6-benzene, the C-18 protons of ketal **2** showed additional small 'W' couplings to distant protons at C-12 and C-14. The C-18 α proton (H_3 below) is coupled to the α proton at C-14 (H_2), while the C-18 β proton (H_4) forms a 'W' coupled arrangement with the C-12 β proton (H_1). Molecular models confirm that 'W' coupling can only occur when the ketal ring is in a chair conformation (fig.).

Analogous NMR studies of ketal **1** led to the assignment of this isomer as the alternative epimer with the six-membered ketal ring in a twist boat conformation. In this conformation, the C-18 methylene protons are twisted in such a way that 'W' coupling can not occur. The rationale for the variation in ring conformation between ketals **1** and **2** lies in the stereochemistry of the ketal carbon (C-22). In the chair form (ketal **2**), with the oxygen of the five-membered ring in an axial position, models show no unfavorable steric interactions. However, inversion of this center, while maintaining the chair form, forces the



Proposed conformations of **1** and **2.**

C-23 methylene into a position of severe steric crowding with the protons at C-18 and with those at C-16 on the D ring of the steroid nucleus. To eliminate this unfavorable interaction the 6-membered ketal ring is forced into a twist-boat conformation. Evidence to support this contention was obtained by analysis of the 500 MHz ^{1}H-NMR shifts of the C-20 methine proton in d_6-benzene. Assuming the normal sterol configuration for this carbon, the methine proton in the chair form (ketal **2**) is *trans*-diaxial to the 5-membered ring oxygen. This proton resonates at a normal position of δ 1.83. However, in the twist-boat conformation of ketal **1**, the C-20 methine proton is *cis* to the 5-membered ring oxygen and experiences a significant low-field shift to δ 2.36 (dq, $J = 7, 2$).

The CD spectra of the two steroids are virtually superimposable, with a negative Cotton effect at 232 nm and a positive one at 263 nm for **1** and at 264 nm for **2.** Therefore, we conclude that the two dienones have the same absolute configuration. Hence, the absolute configurations at the side chain for ketal **1** are C-20 = R and C-22 = S and for ketal **2** C-20 = R and C-22 = R.

Experimental section

General methods. High resolution mass spectra were obtained by the mass spectrometry service laboratory, University of California, Riverside. IR spectra were recorded on a Perkin-Elmer model 137 spectrophotometer and UV spectra were obtained in MeOH on a Beckman MK IV instrument. ^{1}H and ^{13}C-NMR experiments were performed on a Bruker-MW-500 and 200 MHz spectrometers in $CDCl_3$ and C_6D_6 solutions. Proton chemical shifts are referenced to the residual chloroform ($\delta = 7.26$) and benzene ($\delta = 7.15$) signals in $CDCl_3$ and C_6D_6 solutions, respectively. Carbon-13 chemical shifts are referenced to the solvent ($\delta = 77$ ppm). The multiplicity of ^{13}C resonances was determined by DEPT ex-

Table 2. Selected ^{1}H-NMR assignments for steroidal ketals **1** and **2**.

H#	δ ($CDCl_3$, m, J in Hz)[a]	δ (C_6D_6, m, J in Hz)[b]	δ ($CDCl_3$, m, J in Hz)[a]	δ (C_6D_6, m, J in Hz)[b]
1	7.08 (d, J = 10)	6.34 (d, J = 10)	7.08 (d, J = 10)	6.40 (d, J = 10)
2	6.22 (dd, J = 10, 2)	6.27 (dd, J = 10, 2)	6.23 (dd, J = 10, 2)	6.29 (dd, J = 10, 2)
4	6.02 (m)	6.17 (m)	6.08 (m)	6.17 (m)
	3.69 (d, J = 12.5)	3.53 (d, J = 12.5)	3.71 (d, J = 12.5)	3.72 (dd, J = 12.5, 3)
18				
	3.40 (d, J = 12.5)	3.20 (d, J = 12.5)	3.41 (d, J = 12.5)	3.37 (dd, J = 12.5, 2)
19	1.20 (s)	0.66 (s)	1.21 (s)	0.67 (s)
20	2.15 (dq, J = 7, 2)	2.36 (dq, J = 7, 2)	2.05 (dq, J = 7, 2)	1.82 (m)
21	0.93 (d, J = 7)	0.95 (d, J = 7)	0.89 (d, J = 7)	0.94 (d, J = 7)
26[c]	1.38 (s)	1.49 (s)	1.33 (s)	1.43 (s)
27[c]	1.19 (s)	1.16 (s)	1.17 (s)	1.13 (s)

[a] 200 MHz. [b] 500 MHz. [c] Assignments may be reversed.

periments which were performed using polarization transfer pulses of 90° and 135°, obtaining in the first case only signals for -CH groups and in the other case positive signals for -CH and $-CH_3$ and negative ones for $-CH_2$ groups. Polarization transfer delays were adjusted to an average C-H coupling of 135 Hz. The shift correlations with polarization transfer via ^1J coupling[6] were carried out adjusting fixed delays to give maximum polarization for $J_{C-H} = 135$ Hz. The long range heteronuclear correlations[7] were performed with maximum polarization for $J_{C-H} = 8$ Hz, leading to ^2J and ^3J spots in the same spectrum.

Extraction and purification. Sinularia sp. was collected in November 1986 by dredging at −130 m in the vicinity of Guam. The frozen animal (ca 100 g wet) was blended in $CHCl_3$: acetone (1 : 1), the material was filtered and the solvents were removed in vacuo to leave 250 mg of crude extract. The extract was chromatographed over Sephadex LH-20, eluting with increasing proportions of CH_2Cl_2 in hexane. Fractions eluting with CH_2Cl_2 : hexane (3 : 1) were combined and further fractioned by Silica HPLC with 20 % EtOAc in isooctane. Final purification of ketals **1** and **2** was achieved by C-18 Silica reversed phase HPLC (15 % aqueous methanol) to yield **1** (15 mg, 6 %) and **2** (14 mg, 5.6 %) as viscous oils. Ketal **1** showed the following physical and spectral features: HRMS (EI) M^+, m/z = 410.2813, calc. for $C_{27}H_{38}O_3$, 410.2822 UV (MeOH) 244 nm (ε = 13,500), IR 1660, 1615 cm^{-1}. ^{1}H and ^{13}C-NMR data are reported in tables 1 and 2.

For ketal **2:** HRMS (EI) M^+ m/z = 410.2815, calc. for $C_{27}H_{38}O_3$, 410.2822, UV (MeOH) 244 nm (ε = 13,500), IR 1660, 1615 cm^{-1}. ^{1}H and ^{13}C-NMR data are reported in tables 1 and 2.

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Fijiensin, the first phytotoxin from *Mycosphaerella fijiensis*, the causative agent of Black Sigatoka disease

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Summary. Fijiensin, a novel phytotoxic metabolite, was isolated from a culture of the fungus *Mycosphaerella fijiensis*, the causal agent of Black Sigatoka disease of banana. Fijiensin is phytotoxic on various banana cultivars, but not toward non-host plants. The structure of fijiensin was determined by X-ray analysis.
Key words. Fijiensin; *Mycosphaerella fijiensis;* Black Sigatoka; fungal phytotoxin.

Bananas and plantains are important basic food crops on three continents, and they are valuable export products in many countries of Central and South America. These plants are currently being ravaged by the Sigatoka leaf spot disease complex. The disease complex involves three very closely related fungal pathogens: *Mycosphaerella*