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Three Pregnane-10,2-Carbolactones from a Sponge, *Strongylophora* sp.

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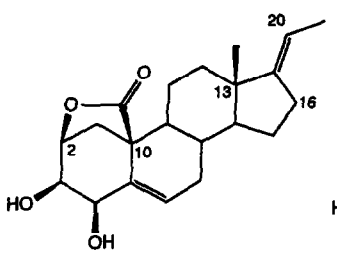
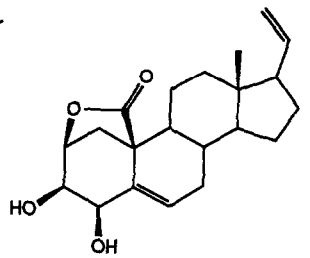
Abstract: Three pregnanes, 3,4-dihydroxypregna-5,17-diene-10,2-carbolactone (**1**), 3,4-dihydroxypregna-5,20-diene-10,2-carbolactone (**2**) and 3,4-dihydroxypregna-5,15-dien-20-one-10,2-carbolactone (**3**) were isolated from a sponge, *Strongylophora* sp. The structures were assigned on the basis of spectral data and confirmed by X-ray crystallography of **1**.

Marine sponges have become recognized for their prolific production of sterols, many of them with unprecedented side-chains. Conversely, relatively few pregnanes have been isolated so far from marine sources, none of them with uncommon structural features.[1] We now report three new pregnanes, 10,2-carbolactones derived from 2,3,4-trihydroxy-5-pregnenes differing in Ring D and/or their side-chains. 3,4-Dihydroxy-pregnanes have been encountered previously,[2b,h,l] but no 2,3,4 - trihydroxy pregnane, or an oxidized C-19.

A sponge, *Strongylophora* sp.[3] was collected using SCUBA at Puako, West Hawai'i. Extraction with ethanol, concentration of the extract, partitioning with methylene chloride, and chromatography of the methylene chloride solution led to the isolation of two pregnanes, **1** and **2**. A third congener **3** was present in the precipitate that had formed when the methylene chloride phase was concentrated. 3,4-Dihydroxy-5,17-pregnadiene-10,2-carbolactone (**1**) was initially isolated as a white powder of composition $C_{21}H_{28}O_4$ determined by HREIMS. One carbonyl (176 ppm) and four olefinic carbons (151.0, 136.0, 132.0 110.6 ppm) in the ^{13}C NMR spectrum of **1** pointed to a pentacyclic compound. The carbonyl frequency was compatible with an ester and an IR band at 1775 cm^{-1} pointed to a 5-membered lactone.[4]



1



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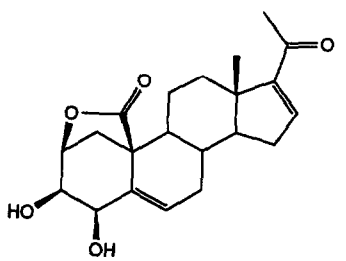
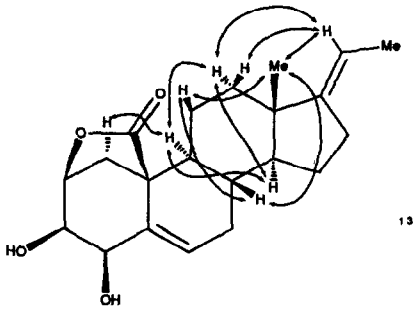
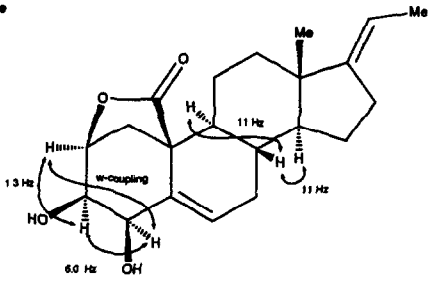


3

^{1}H NMR data (Table I) defined the tetracyclic steroid nucleus of the ring A spin system (H_2-1, H-2, H-3, H-4). A second system, defined by a COSY NMR experiment, encompassed the remaining protons (H-6, H_2-7, H-8, H-9, H_2-11, H_2-12, H-14, H_2-15, H_2-16) except those of the C-18 methyl and the side chain. An HMBC NMR experiment located the lactone by correlating the carbonyl carbon (176 ppm) with H-2 (4.73 ppm) and H-9 (1.25 ppm). HMBC also established the olefin in ring B by correlating H-1a (2.70 ppm) and H-1b (1.86 ppm) to C-5 (136.0 ppm), and H-4 (4.48 ppm) to C-6 (132.0 ppm). The C-17 double bond was located by long range COSY of H-20 (5.06 ppm) with H-16a (2.35 ppm) and H-16b (2.26 ppm), and by HMBC correlations of H-20 with C-16 and C-13 (26.3, 43.7 ppm). Relative stereochemistry was determined by NOE experiments (partial structure **A**) and by ^{1}H coupling constants. (partial structure **B**).



A



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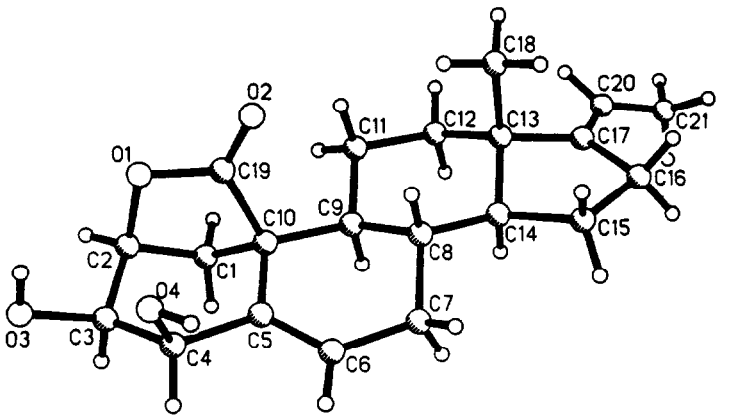


Fig. 1. A 3-dimensional representation of **1**

Table 1. ^{13}C and ^{1}H Chemical Shifts for **1**, **2** and **3**

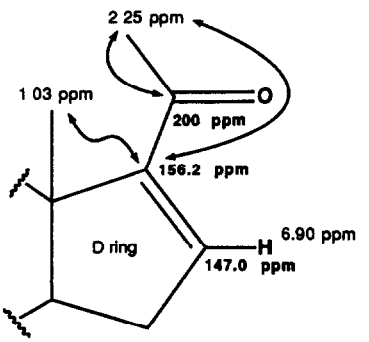
	Pregnane 1*		Pregnane 2*		Pregnane 3**	
Position	^{13}C δ	^{1}H δ (m, *j* Hz)	^{13}C δ	^{1}H δ (m, *j* Hz)	^{13}C δ	^{1}H δ (m, *j* Hz)
1a	37.6	2.70 dd (12.1, 6.7)	37.4	2.68 dd (11.2, 4.0)	38.9	2.67 dd (12.1, 6.8)
b		1.86 m		1.56 m		1.61 d (12.1)
2	79.4	4.73 dt (6.7, 1.3)	79.8	4.73 br d (4.0)	81.3	4.65 dd (6.8,1.2)
3a	69 8	3.80 ddd (9.6, 6.0, 1.3)	69.7	3.82 s	70 6	3.82 dd (5.8, 1.2)
b		3.42 br d OH (9.6)				
4a	71.2	4.48 br d (6.0)	71.8	4.49 br d (1.9)	72.4	4 36 br d (5.8)
b		1.96 br d OH (2.0)				
5	136.0		135.5		138.0	
6	132.0	6.07 br dd (6.2, 1.7)	132.5	6.08 br d (4.8)	131.3	6.00 dd (6.2, 1.7))
7a	31.2	2.25 ddd (18.4, 6.3, 4.6)	31.2	2.20 dt (17.9, 4.2)	32.6	2 12 m
b		1.67 ddd (18.4, 11, 1.7)		1.65 m		1.73 ddd(16,10.5,1.7)
8	32 5	2.05 qd (11.0, 4.6)	32.7	2.03 qd (11.0, 4.2)	33.2	2.14 m
9	42.6	1.24 dt (11.0, 5.0)	42.2	1.25 dt (11.0, 4.8)	43.7	1.35 m
10	46 7		46.8		48.0	
11a	21.1	1.84 m+	20.8	1.75 m	21.9	1.82 m
b		1.62 m+		1.55 m		1.68 m
12a	35.6	1.85 m+	36.9	1.76 m	35.7	2.48 m
b		1.20 m+		1.07 dt (13.5, 4.0)		1.43 m
13	43.7		43.7		47.5	
14	54.3	0.95 ddd (12.5,11.0, 6.3)	55.2	1.00 dt (11.0, 7.5)	57.2	1.41 m
15a	24.2	1.75 m+	24.6	1.70 m	32.0	2.41 m
b		1.38 m+		1.38 dt (11.0, 5.9)		2.16 m
16a	26.3	2.35 br dd (17.0, 9.4)	27.3	1.80 m	147.0	6.90 dd (3.4, 1.9)
b		2.26 m		1.60 m		
17	151 0		55.2	1.98 q (8.0)	156.2	
18	18.9	0 90 m	12.7	0.75 s	16 2	1 03 s
19	176.0		177.2		178.7	
20	110.6	5.06 tq (6.6, 2.5)	139.5	5.76 ddd (15,10.7,7.5)	200.0	
21a	13 8	1.56 d (6.6)	114.8	5.00 ddd (7.5, 2.1, 1.0)	27 1	2.25 s
b				4.97 ddd (15, 2.1, 1.0)		

+Resolved by COSY
*Recorded in $CDCl_3$
**Recorded in CD_3OD

This structure was confirmed by x-ray crystallography after **1** was crystallized from ethanol. Fig. 1 is an ORTEP drawing of the final structure.

The structure of the second pregnane, 2,3-dihydroxy-5,20-pregnadien-10,2-carbolactone (**2**) could be elucidated by comparing ^{1}H and ^{13}C NMR data of the two compounds. Substantial chemical shift differences for carbons and protons at C-16, 17, 18, 20 and 21 suggested that the C-17 olefin had shifted to C-20. Particularly revealing were the proton signals for the terminal (C-21) olefin: 5.00 ppm (ddd) and 4.97 ppm (ddd) (see Table I).

A third pregnane, 3,4-dihydroxy-5,16-pregnadien-20-one-10,2-carbolactone (**3**) was isolated, along with additional quantities of **1** and **2**, from the precipitates resulting from the aqueous ethanol - methylene chloride partition. It was purified by repeated reversed phase HPLC as a white powder. A new carbonyl band at 1790 cm^{-1} in the IR spectrum pointed to an α,β-unsaturated ketone. Examination of the NMR spectra and comparison with those of **1** and **2** located the carbonyl at C-20 and the double bond at C-16,17. Principal features were the H-16 resonance at 6.90 ppm, the H_3-21 singlet at 2.25 ppm and its HMBC correlations to C-20 (200.0 ppm) and C-17 (156.2 ppm) (partial structure C). Complete NMR data are tabulated in Table I.



C

These three sponge pregnanes exhibit a new structural feature among known marine invertebrate pregnanes, a 10,2-carbolactone. Conventional bioassays against human epidermoid carcinoma (KB) and human colorectal adenocarcinoma (LoVo) cell lines showed marginal cytotoxicity for **3** and no activity for **1** and **2**. Pregnane **3** had an MIC of 1 μg/mL in the KB assay and 5 μg/mL in the LoVo assay.

EXPERIMENTAL PART

General Procedures

Infrared spectra were recorded on a Perkin-Elmer Model 1420 spectrometer and a Nicolet Model 740 FTIR spectrometer. Ultraviolet spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrophotometer. Mass spectra were measured on a VG-70SE instrument,

NMR spectra on a General Electric GN OMEGA 500 instrument. Optical rotation was determined on a Jasco DIP 370 spectrophotometer. Solvents were freshly distilled before use.

Isolation

On November 19, 1989, 1 kg (wet) of a sponge, identified as a *Strongylophora* sp. (Haplosclerida), was collected by SCUBA at Puako, Hawai'i. A concentrated EtOH extract of the sponge was diluted with water and partitioned with CH_2Cl_2. Upon concentration of the CH_2Cl_2 extract, two layers formed. The top layer was chromatographed using HPLC on a silica stationary phase (Rainin Microsorb Si, 2.5% iPrOH in CH_2Cl_2) yielding a white powder (**1**, 4.3 mg, 4.3 x 10^{-4}% of wet sponge weight). Pregnane **1** co-crystallized from EtOH with a disordered water molecule in the asymmetric unit.

On April 9, 1991, 2 kg (wet) of the sponge was recollected at the same location. Upon re-isolation following the same protocol as described above, a new pregnane (**2**) co-eluted with **1** during silica HPLC. Pregnane **2** (12.1 mg, 1.21 x 10^{-3}% of wet sponge weight) was separated from **1** (8.6 mg, 4.3 x 10^{-4}% of wet sponge weight) using reverse phase HPLC (Phenomenex Ultracarb 5 ODS 30, MeOH/H_2O (50:3)).

Pregnane **3** was isolated from the combined CH_2Cl_2 bottom layers with 12.0 mg of **1** (24.9 mg, 8.3 x 10^{-4}% of total wet sponge weight) and 10.2 mg of **2** (22.1 mg, 7.4 x 10^{-4}% of total wet sponge weight) using C-18 reverse phase HPLC. Reverse phase HPLC (Phenomenex Ultracarb 5 ODS 30, MeCN/H_2O (10:1)) of the combined CH_2Cl_2 precipitates yielded an early fraction that contained **3** and two later fractions that contained pure **2** and **1**. Pregnane **3** (1.0 mg, 3.3 x 10^{-5}% of wet sponge weight) was isolated by repeated reverse phase HPLC (Phenomenex Ultracarb 5 ODS 30, MeCN/H_2O (1:1))

3,4-Dihydroxypregna-5,17-diene-10,2-carbolactone (**1**): $[\alpha]_D$ -82° (*c* 1.2, $CHCl_3$); IR neat (KBr): 3300 (m, br), 1775 (s), 1455 (m), 1375 (m), 1300 (m), 1215 (m), 1085 (s); mass spectrum HREI, *m/z* (relative intensity): 344.1996 (M^+,1.2) (calc for $C_{21}H_{28}O_4$: 344.1988), 326(42), 298(25), 282(65), 257(30), 213(100), 159(42), 128(40), 105(58), 91(68)

Crystal data for **1**: $C_{21}H_{28}O_4 \cdot H_2O$; *M*w=360.4; Hexagonal space group $P6_5$; a = 21.511(5) and c = 7.176(1) Å, $\alpha = \beta = 90.0°$ and $\gamma = 120.0°$; Z = 6; D_c = 1.249 g/cm^3. A colorless crystal with dimensions of 0.10 X 0.14 X 0.52 mm was selected. Data collection was carried out on a Siemens R3m/V diffractometer using Cu Kα radiation (λ = 1.54178) at room temperature. The ω scan mode was used at a variable rate from 2.00 to 29.30°/min. A total of 2,606 independent reflections were measured, of which 2,052 ($F > 4.0\sigma(F)$) were used in the refinement.

The structure was solved by direct methods with Siemens SHELXTL PLUS (VMS). The positional and thermal parameters of all non-hydrogen atoms were refined anisotropically, and the hydrogen atoms were introduced in fixed positions with a fixed isotropic thermal parameter. The final refinement included 233 variables. The converged model had unweighted and weighted R agreement factors of 8.12% and 10.76%, respectively, $w^{-1} = \sigma^2(F) + 0.0010F^2$. Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data

Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

3,4-Dihydroxypregna-5,20-diene-10,2-carbolactone (**2**): $[\alpha]_D$ -96° (*c* 5.0, $CHCl_3$); IR neat (KBr): 3390 (m, br), 1772 (s), 1450 (m), 1375 (m), 1285 (m), 1215 (m), 1083 (s); mass spectrum HREI, *m/z* (relative intensity): 344.1963 (M^+, 5) (calc for $C_{21}H_{28}O_4$: 344.1988), 326(40), 298(20), 282(62), 257(28), 213(100), 159(38), 129(40), 91(55).

3,4-Dihydroxypregna-5,15-dien-20-one-10,2-carbolactone (**3**): $[\alpha]_D$ -130° (*c* 1.5, $CHCl_3$); IR neat (KBr): 3450 (m, br), 1790 (s), 1760 (m), 1655 (m), 1260 (m), 980 (s); UV (MeOH) λ_{max} 260 nm (ε = 2,280), 204 nm (ε = 10,620).

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3 Identified by Dr. C. Battershill, New Zealand Department of Scientific and Industrial Research, Wellington, NZ, where a voucher specimen is deposited.

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