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Pectinoacetals A–C: Novel sterol hemiacetals from the gorgonian *Ctenocella pectinata*

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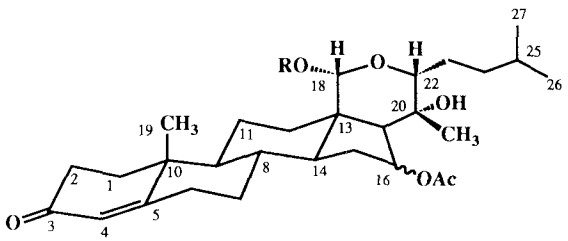
Abstract. Three polyhydroxylated sterol hemiacetals, pectinoacetals A–C (**1**–**3**) have been isolated as their acetyl derivatives (**4**–**6**) from the acetic anhydride treated organic extract of the Indo-Pacific gorgonian *Ctenocella pectinata*. These natural products were found to undergo very rapid epimerization at the C-18 chiral center and thus exist only as an equilibrium mixture of two diastereomers. The structure assignments are based on spectral studies and chemical modifications of the natural products.

Key words. Octocorallia; *Ctenocella pectinata*; sterol hemiacetals.

Soft corals[2], along with hydroids[3] and starfish[4], have been proven over the years to be some of the most prolific sources for a number of interesting steroidal structures. As a part of a program to isolate new biologically-active compounds from marine octocorals, we have turned our attention to organisms abundant in predator-rich habitats[5,6]. *Ctenocella pectinata* is a large sea whip very common along the coast of Western Australia. In this communication we report the structures of the three major chemical constituents isolated from the extract of this organism.

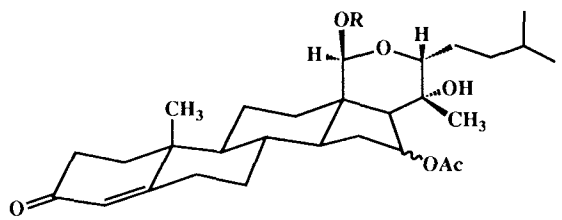
Materials and methods

Ctenocella pectinata was collected in Exmouth Bay in December, 1987, and immediately frozen. The organic extract ($CHCl_3/CH_3OH$) of the freeze-dried organism was examined by TLC analysis, which revealed the presence of some interesting compounds with UV activity. The extract was concentrated in vacuo and the residue was fractionated on silica gel (flash chromatography). Repetitive HPLC separations resulted in the isolation of the two major components of the extract. The ^{13}C NMR spectra of the isolated metabolites



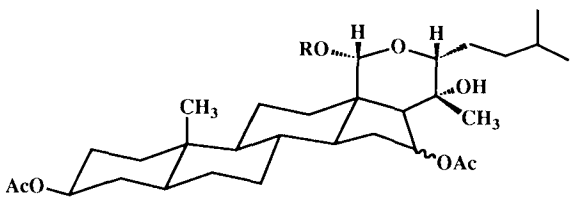
1: R = H

4: R = Ac



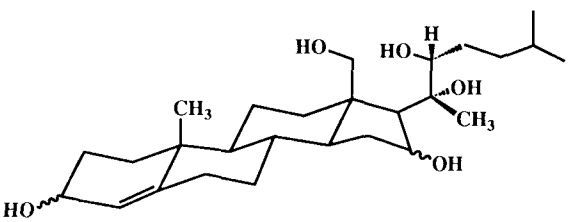
2: R = H

5: R = Ac



3: R = H

6: R = Ac



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demonstrated the presence of 54 and 58 carbon atoms in these molecules. Since metabolites with such high molecular weights have not been encountered before in similar organisms, the purity of the samples was questioned. Elaborate chromatographic analyses proved that these compounds where equilibrium mixtures of rapidly interconverting diastereomers. The ^{1}H NMR spectral features of the two stereoisomers were very similar, with the exception of a few low field signals that showed a considerable difference in their chemical shifts. The protons corresponding to these signals were suspected to be in close proximity to the chiral center undergoing epimerization. In an attempt to inhibit this interconversion, the mixture of the two diastereomers was acetylated to produce the acetyl derivatives (**4** and **5**) in a ratio of 95:5. The diastereomers were effectively separated by silica HPLC.

Structures of pectinoacetals A–C

A molecular formula of $C_{31}H_{46}O_7$ was established for acetyl-pectinoacetal A (**4**), by a combination of high resolution mass spectrometry[7] and ^{13}C NMR spectroscopy (table). Upon consideration of the degrees of unsaturation and the number of double bonds, a pentacyclic structure was deduced for this molecule. Spectral analysis of compounds **4** and **1** indicated that both compounds possessed a steroidal carbon skeleton and that compound **4** is the monoacetate derivative (at C-18) of pectinoacetal A (**1**). The enone system of ring A is responsible for the UV activity[7] of compound **4**. Comparison of the ^{13}C NMR chemical shifts of acetyl-pectinoacetal A (**4**) for rings A and B matched perfectly with those of 16-dehydroprogesterone[8]. The absence of a typical C-18 methyl group and the presence of a new low field methine carbon (94.5 ppm for ^{13}C and 5.97 ppm for ^{1}H NMR) suggested that C-18 was a hemiacetal carbon. Long range proton carbon correlation experiments (COLOC) exhibited correlations between H-18 and C-13 (46.1 ppm) and C-22 (83.1 ppm), indicating the presence of a six-membered ring. The multiplicity (singlet) and the chemical shift (72.1 ppm) of C-20 suggested that this chiral carbon possessed a

Carbon and selective proton NMR data for compounds **4**–**7**

	4		**5**		**6**		**7**	
1)	35.4		35.5		33.8		35.2	
2)	33.8		33.8		28.2		28.9	
3)	199.2	- - -	199.3	- - -	73.5	4.68 m	67.5	4.12 m
4)	124.1	5.69 s	124.0	5.72 s	36.5		123.5	5.24 s
5)	170.2	- - -	170.1	- - -	44.5	- - -	146.6	- - -
6)	32.8		32.4		27.3		32.9	
7)	32.3		31.6		31.9		31.9	
8)	34.9		34.4		34.7		35.4	
9)	51.8	1.20/1.45 m	52.7		52.2		53.4	1.02 m
10)	38.5		38.4		35.5		37.1	
11)	22.4		20.9		22.6		20.1	
12)	32.3		35.1		32.9		34.4	
13)	46.1	- - -	44.1		46.3	- - -	47.7	- - -
14)	53.6	1.10 m	53.6		54.0	1.10 m	54.4	0.85 m
15)	34.9	1.38/1.62 m	36.3		34.8		36.2	2.15 m
16)	73.3	5.21 m	74.3	5.27 m	73.4	5.24 m	72.3	4.45 m
17)	62.3	1.83 d (J = 8.6 Hz)	57.4	1.86 d (J = 9.1 Hz)	62.4	1.85 d (J = 8.2 Hz)	58.0	1.38 m
18)	94.5	5.97 s	92.6	6.33 s	94.5	5.97 s	59.9	3.95 d (J = 12 Hz) 3.77 d (J = 12 Hz)
19)	17.5	1.08 s	17.1	1.16 s	12.3	1.1 s	18.6	1.05 s
20)	72.1	- - -	70.8	- - -	72.1	- - -	78.9	- - -
21)	21.7	1.15 s	22.0	1.20 s	21.7	1.58 s	19.3	1.25 s
22)	83.1	3.92 t (J = 6.0 Hz)	74.6	4.09 dd (J_1 = 10.1 Hz, J_2 = 2.4 Hz)	83.1	3.97 t (J = 6.1 Hz)	75.2	3.95 m
23)	25.6		24.9		25.7		29.4	
24)	35.2		37.8		25.7		29.4	
25)	27.8	1.60 m	27.6	1.55 m	27.8	1.60 m	28.0	1.57 m
26)	22.2	0.85 d (J = 7.9 Hz)	22.8	0.90 d (J = 6.5 Hz)	22.3	0.89 d (J = 8.9 Hz)	22.2	0.90 d (J = 6.1 Hz)
27)	22.8	0.85 d (J = 7.9 Hz)	22.8	0.89 d (J = 6.5 Hz)	22.8	0.89 d (J = 8.9 Hz)	22.8	0.87 d (J = 6.1 Hz)
-OAc								
	169.6	- - -	169.8	- - -	170.1	- - -	-----	
	168.9	- - -	168.9	- - -	169.1	- - -	-----	
	-----		-----		67.9	- - -	-----	
	21.7	2.14 s	21.4	2.11 s	21.6	2.16 s	-----	
	21.4	2.05 s	21.3	2.09 s	21.5	2.08 s	-----	
	-----		-----		21.4	2.02 s	-----	

^{1}H NMR spectra were recorded at 360 MHz, ^{13}C NMR spectra were recorded at 50 MHz. Compounds **4**, **5** and **6** were dissolved in $CDCl_3$ while a mixture of $CDCl_3/CD_3OD$ was used as solvent for compound **7**. The carbon multiplicities were deduced by DEPT experiments and proton and carbon assignments were based on COSY, XHCORR and COLOC experiments.

tertiary hydroxyl group. The fact that the tertiary alcohol OH group was not acetylated under the reaction conditions that were employed (AcOAc/pyridine) was thus not surprising. The fourth oxygenated carbon was assigned by a combination of proton-proton and long range proton-carbon correlation experiments at C-16. The relative stereochemistry proposed for the new chiral centers is based on nOe difference experiments, which illustrated the spacial proximity of H-18 and H-22. The same set of data showed an intense enhancement of the H-22 and H-16 signals upon irradiation of H-21, and enhancement of H-21 resonance upon irradiation of H-16. The proposed overall stereochemistry is consistent with the nOe observed between H-19 and the acetate methyl at C-18. Although irradiation of the C-21 methyl group enhanced the proton at C-16, because of equal proximities, both epimers would be expected to be enhanced. Hence, the relative stereochemistry of the C-16 acetoxyl group cannot be assigned by nOe methods.

Acetyl-pectinoacetal B (**5**), isolated as 5% of the reaction mixture, exhibited spectral features similar to those of acetyl-pectinoacetal A (**4**). The two compounds (**4** and **5**) differed mainly in the chemical shifts of the H-18 and H-22 proton resonances. When the H-22 proton in **5** was irradiated in a set of nOe experiments it did not produce any enhancement of the H-18 signal, as was observed with **4**. When the H-21 methyl was irradiated it caused significant enhancement of H-16 and of the C-18 acetate methyl signal. Overall, these data support the assignment of **5** as the C-18 epimer of acetyl-pectinoacetal A.

Treatment of a mixture of pectinoacetal A and B with $LiAlH_4$ in ether afforded a single reduction product **7**[9]. It was clear from the spectral data that in this reduction product the lactol ring had opened (59.9 ppm for C-18 and an A-B system for H-18), the acetyl groups had been removed and the ketone had been reduced to the corresponding alcohol.

Acetylation of the second major natural metabolite pectinoacetal C (**3**), which was also found to exist as an equilibrium mixture of two diasteromers, produced exclusively acetyl-pectinoacetal C (**6**)[10]. The ^{13}C NMR data of compound **6** and especially the chemical shift of C-19 are only compatible with a cholestane skeleton[11]. The most significant difference between compound **6** and acetyl-pectinoacetal A (**4**) is the absence of the enone system and the presence of a third acetoxyl group. The new acetoxyl group was assigned, by spectroscopic methods, to C-3 in the equatorial position.

Pectinoacetals A, B and C represent a new class of polyhydroxylated sterol hemi-acetals, illustrating a novel lactol cyclization between C-18 and C-22. These rapidly interconverting secondary metabolites of *Ctenocella pectinata* had to be isolated as their acetate derivatives (**4**–**6**) before the structure elucidation could be accomplished.

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