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Ecdysteroids from the Antarctic Tunicate *Synoicum adareanum*

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Five new ecdysteroids, hyousterones A–D (**2–5**) and abeohyosterone (**6**), have been isolated from the Antarctic tunicate *Synoicum adareanum* along with the known ecdysteroid dialulusterol B (**1**). Hyousterones B (**3**) and D (**5**) are unusual ecdysteroids in bearing the 14 β -hydroxyl group, and abeohyosterone incorporates the 13(14 \rightarrow 8) abeo steroid skeleton, reflecting a rearrangement of the steroid C/D ring system. Abeohyosterone has moderate cytotoxicity toward several cancer cell lines. Hyousterones bearing the 14 α -hydroxy group (**2** and **4**) were weakly cytotoxic, while the 14 β -hydroxy hyousterones (**3** and **5**) were devoid of cytotoxicity. The 14 β -hydroxy function may be a thermodynamic pathway to the 13(14 \rightarrow 8) abeo steroid skeleton. Hyousterones, abeohyosterone, and dialulusterol B are the first ecdysteroids reported from tunicates.

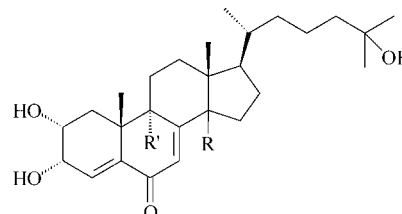
Ecdysteroids, molting hormones of arthropods, are found in a wide variety of animals and plants.^{1,2} In plants, ecdysteroids are thought to play the role of protecting agent against phytophagous insects since phytoecdysteroids are known to have insecticidal activities.^{3,4} Ecdysteroids also show pharmacological and physiological effects in vertebrates.⁵

Marine invertebrates are known as a rich source of bioactive metabolites.⁶ Although steroids are one of the major constituents of marine invertebrates,⁷ the number of ecdysteroids isolated from them is relatively small.⁸ The few reported marine ecdysteroids are, however, spread across a relatively broad taxonomic range including examples from sponges,^{9–12} corals,¹³ zoanthids,^{14–17} and a nudibranch.¹⁸ In the course of our continuing investigations of the chemical constituents of Antarctic marine invertebrates, we have examined extracts of the Antarctic tunicate *Synoicum adareanum*. Besides the recently reported palmerolides,¹⁹ this circumpolar tunicate was found to produce the known ecdysteroid dialulusterol B (**1**),¹⁸ as well as five new ecdysteroids, hyousterones²⁰ A–D (**2–5**) and abeohyosterone (**6**). Herein, we report the isolation and structure elucidation of the hyousterones and abeohyosterone.

Results and Discussion

Initial extraction of the tunicate with 1:1 dichloromethane/methanol yielded an organic extract, which was partitioned between EtOAc and H₂O. The EtOAc residue was subjected to silica gel column chromatography followed by reversed-phase HPLC to afford dialulusterol B (**1**) and hyousterone A (**2**). Inspection of the NMR spectral data for dialulusterol B, including ¹H, ¹³C, COSY, HMQC, and HMBC, identified it as an ecdysteroid. Comparison of the ¹H and ¹³C NMR spectra to those reported for dialulusterol B confirmed their identity.¹⁸ The relative stereochemistry was confirmed by analysis of the ROESY spectrum.

Hyousterone A (**2**) was found to have a molecular formula of C₂₇H₄₂O₅ on the basis of the HRESIMS analysis. The ¹H and ¹³C NMR data (Table 1) of **2** were similar to those of **1**, suggesting **2** was also an ecdysteroid. Analysis of the ¹H NMR and gHMBC spectra indicated the presence of five methyl, eight methylene, and seven methine groups as well as six quaternary sp³ carbons and



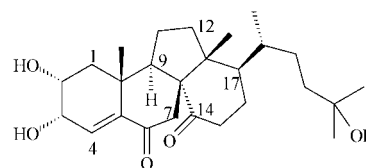
Dialulusterol B (**1**, R = R' = H)

Hyousterone A (**2**, R = α -OH, R' = H)

Hyousterone B (**3**, R = β -OH, R' = H)

Hyousterone C (**4**, R = α -OH, R' = OH)

Hyousterone D (**5**, R = β -OH, R' = OH)



Abeohyosterone (**6**)

one ketone carbonyl. Two methines and two quaternary carbons appeared to bear oxygen on the basis of their chemical shift (Table 1). With regard to the ecdysteroid ring system, the gCOSY spectrum established the connectivity of C-1 to C-4, C-9/C-11/C-12, and C-15 to C-17. C-17 was further connected by gCOSY data to the side chain, which displayed contiguous connectivity (Figure 1).

The gHMBC spectrum was useful in the assembly of the partial structures established by gCOSY and in assigning the quaternary carbons (Figure 1). Correlations of H-1 to C-5 and C-10, taken with H-4 correlation to C-5, C-6, and C-10, secured the planar structure of the A ring. The B ring could similarly be constructed on the basis of correlations of H-7 to C-5 and C-6, as well as H-9 to C-7, C-8, and C-10. Ring C was defined by correlations of H-11 to C-8 and C-13 and of H-12 to C-13 and C-14. Ring C connectivity to the B ring was established by the correlation of H-7 to C-14, while the D ring connection was based on correlation of H-15 to C-14. Positions of the angular methyl groups C-18 and C-19 were both secured by correlations of their respective protons to their α - and β -carbons. The C-26 and C-27 methyls were similarly assigned on the basis of correlations to their neighboring carbons. The chemical shift of C-14 (δ 83.0) and C-25 (δ 68.9) identified them as oxygen-bearing carbons.

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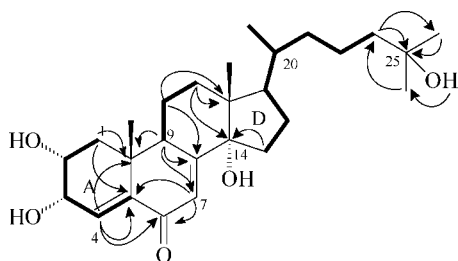
[§] University of Alabama at Birmingham.

[†] Henry Ford Health System.

Table 1. ^1H and ^{13}C NMR Spectral Data for Hyousterones A (**2**) and B (**3**)^a

position ^b		hyousterone A			hyousterone B		
		δ_{H}	δ_{C}	HMBC	δ_{H}	δ_{C}	HMBC
1	α	1.76 (m)	38.2	3, 19, OH-3	1.73 (m)	38.2	19
	β	1.56 (m)			1.60 (m)		
2		3.63 (m)	65.9	1 α,β , 3, 4, OH-3, OH-2	3.63 (m)	65.9	1 α,β , 4
3		3.99 (m)	64.6	1 α,β , 4, OH-2, OH-3	3.99 (m)	64.6	1 α,β , 4
4		6.27 (d, 5.3)	130.4	3, OH-3	6.29 (d, 5.2)	130.4	
5			144.1	1 β , 3, 4, 7, 19		143.6	1 β , 4, 7, 19
6			188.9	4		188.3	4
7		5.82 (s)	122.2	9	6.30 (s)	123.8	
8			167.1	9, 11 α , OH-14		170.0	7, 9, 11 α
9		2.82 (dd, 7.7, 11.4)	43.4	1 α,β , 7, 11 α , 11 β , 12 α , 19	2.56 (dd, 6.9, 10.0)	45.8	1 α , 7, 11 α , 19
10			39.5	1 α,β , 4, 9, 19		40.7	1 α,β , 4, 11 α,β , 9, 19
11	α	1.66 (m)	20.2	9, 12 α,β	1.66 (m)	20.4	1 α,β , 9
	β	1.55 (m)			1.51 (m)		
12	α	2.02 (m)	30.0	11 α,β , 18	1.90 (m)	40.0	18, OH-14
	β	1.63 (m)			1.33 (m)		
13			45.8	11 α , 12 α,β , 17, 18, OH-14		48.7	11 α,β , 15 β , 18, OH-14
14			83.0	7, 12 β , 15 α,β , 16 β , OH-14		83.3	7, 15 β , 16 α,β , 18, OH-14
15	α	1.79 (m)	30.4	16 α , 16 β , OH-14	1.50 (m)	40.5	16 α,β , OH-14
	β	1.58 (m)			1.44 (m)		
16	α	1.88 (m)	26.4	15 α,β , 17	1.68 (m)	26.3	
	β	1.28 (m)			1.68 (m)		
17		1.81 (m)	50.1	12 α , 15 β , 16 α , 18, 20, 21	1.50 (m)	55.4	12 β , 15 α,β , 18, 20, 21
18		0.63 (s)	15.3	12 α,β , 17	0.91 (s)	17.2	15 β , 17
19		1.03 (s)	20.5	1 α,β , 4, 9, 11 α	0.98 (s)	20.7	1 α,β , 4, 9
20		1.40 (m)	35.1	16 β , 17, 21, 22b	1.50 (m)	33.6	16 α,β , 17, 21
21		0.90 (d, 6.7)	18.9	17, 20, 22b	0.88 (d, 5.9)	20.6	17, 20, 22a
22	a	1.34 (m)	36.3	17, 20, 21, 23a,b, 24a	1.61 (m)	34.1	17, 20, 21
	b	1.02 (m)			0.92 (m)		
23	a	1.37 (m)	20.4	22a, 24a,b	1.36 (m)	21.5	20, 22a, 24a,b
	b	1.19 (m)			1.17 (m)		
24	a	1.34 (m)	44.2	23a,b, 26, 27, OH-25	1.34 (m)	44.3	22a, 23a,b, 26, 27, OH-25
	b	1.25 (m)			1.26 (m)		
25			68.1	24a,b, 26, 27, OH-25		68.7	23a, 24b, 26, 27, OH-25
26		1.07 (s)	29.5	24b, OH-25	1.06 (s)	29.3	23b, 24a,b, OH-25
27		1.07 (s)	29.5	24b, OH-25	1.06 (s)	29.3	23b, 24a,b, OH-25
2-OH		4.41			4.42		
3-OH		4.76			4.74		
14-OH		4.70			4.67		
25-OH		4.05			4.08		

^a 500 MHz for ^1H , 125 MHz for ^{13}C , DMSO- d_6 . ^b Position defined as α/β based on ROESY data (see text) or a/b when unknown.

**Figure 1.** Key COSY (bold) and HMBC (→) correlations for hyousterone A (**2**).

The coupling patterns found for H-2 and H-3 of hyousterone A (**2**) were in close agreement with those of dialusterol B (**1**) and much smaller than those from an analogous 2 β ,3 β -ecdysterone,¹⁸ indicating **1** and **2** had the same relative stereochemistry at C-2 and C-3. The relationship of the C-2/C-3 positions to the remainder of the ring system was determined on the basis of NOE data. ROESY correlations between H-2 and H₃-19 established H-2 as axial. H-9, which correlated in the ROESY spectrum to H-1 α , also showed proximity to OH-14, placing both of them on the opposite face from H-2 and H₃-19. Further support for these assignments derives from H-7, which displays ROESY correlations to H-9, H-15 α , and OH-14. H-17 correlates with OH-14. Irradiation of the methyl protons at δ 0.63 (H₃-18) produced 1D NOE enhancements of H-12 β , H-16 β , and H-20. Thus, the relative stereochemistry of hyousterone A was determined to reflect that of dialusterol B.

Subsequent extraction of additional *S. adareanum* biomass, following a similar fractionation scheme, resulted in additional hyousterones. That hyousterones B–D (**3**–**5**) belonged to the same family of ecdysteroids was evident from their low-field hydroxymethylene and olefinic signals. Hyousterone B (**3**) was found to have a molecular formula of C₂₇H₄₂O₅ and thus was isomeric with hyousterone A (**2**). In fact, analysis of 2D NMR spectra, particularly the HMBC experiment (Table 1), established the planar structure of **3** as identical to that of **2**. The ROESY spectrum confirmed the α -configuration of the 3,4-dihydroxy system as well as the β -orientation of the C-18 and C-19 methyl groups. H-9 displayed a ROESY correlation to H-1 α , confirming its configurational identity with hyousterone A. Differing from hyousterone A was a ROESY correlation between the 14-OH proton and H₃-18, while 14-OH did not show ROESY correlation to H-17 nor did H-7 to H-15 α or H-15 β . These ROESY data are consistent only with hyousterone B having the 14 β -hydroxyl configuration, a feature of ecdysteroids known only rarely in nature.²¹

Hyousterones C (**4**) and D (**5**) shared most spectral features (Table 2) with hyousterones A (**2**) and B (**3**). High-resolution ESIMS provided a molecular formula of C₂₇H₄₂O₆ for both hyousterones C and D, identifying them as isomeric and having one additional oxygen atom relative to **2** and **3**. The new oxygen atom could be placed at C-9 in both **4** and **5** on the basis of HMBC correlations of their respective H-1 α , H-7 and H₃-19 to new oxygen-bearing carbon at 73.5 and 72.7 ppm in **4** and **5**, respectively. Similarly, a new exchangeable proton appeared at δ 5.33 in **4** and at δ 4.83 in **5**, and in both cases, that exchangeable proton showed

Table 2. ^1H and ^{13}C NMR Spectral Data for Hyousterones C (**4**) and D (**5**)^a

position ^b		hyousterone C			hyousterone D		
		δ_{H}	δ_{C}	HMBC	δ_{H}	δ_{C}	HMBC
1	α	2.37 (t, 12.0)	31.5	19	2.29 (m)	31.5	3, 19
	β	1.28 (m)			1.27 (m)		
2		3.69 (m)	66.0	1 α , β , 4	3.68 (m)	66.0	1 α , β , 4
3		4.02 (br s)	64.4	1 α , β , 4			
4		6.46 (d, 5.2)	133.5	3, 7	6.50 (d, 5.1)	133.1	3, 7, OH-3
5			141.5	1 α , β , 3, 4, 7, 19		140.7	1 β , 3, 4, 7, 19
6			187.8	4		187.4	4
7		5.90 (s)	124.0		6.33 (s)	125.4	
8			162.2	7, 11 α , OH-9, OH-14		168.2	7, OH-9
9			73.5	1 α , 7, 11 α , 19, OH-9		72.7	1 α , 7, 19, OH-9
10			44.9	1 α , β , 4, 19, OH-9		45.7	1 α , β , 4, 19
11	α	1.61 (m)	27.6	18, OH-9	1.43 (m)	25.9	OH-9
	β	2.01 (m)			1.84 (m)		
12	α	1.24 (m)	29.3		1.75 (m)	36.9	17, 18, OH-14
	β	1.24 (m)			1.29 (m)		
13			46.0	11 α , β , 15 α , 17, 18, OH-14		47.4	11 α , 12 β , 17, 18, OH-14
14			84.9	7, 15 α , 16 β , 18, OH-14		82.8	7, 15 β , 18, OH-14
15	α	1.66 (m)	30.1	OH-14	2.23 (m)	41.2	OH-14
	β	1.77 (m)			1.36 (m)		
16	α	1.89 (m)	26.3	15 β , 17	0.89 (m)	33.7	17, 20
	β	1.28 (m)			1.63 (m)		
17		1.83 (m)	50.0	18, 21	1.49 (m)	55.9	15 β , 18
18		0.66 (s)	15.7	11 α , β , 17	0.91 (s)	16.5	
19		1.14 (s)	25.0	1 α , β , 4	1.11 (s)	24.5	1 α , β , 4
20		1.40 (m)	35.1	17, 21, 22a	1.48 (m)	33.6	17, 21, 22a,b, 23a,b
21		0.91 (d, 6.5)	18.9		0.88 (d, 5.7)	20.8	17, 20
22	a	1.33 (m)	36.4	21, 23b	1.63 (m)	25.7	20
	b	1.02 (m)			1.63 (m)		
23	a	1.89 (m)	20.5	22a, 24a,b	1.23 (m)	28.7	24a,b, 26, 27, OH-25
	b	1.35 (m)			1.23 (m)		
24	a	1.34 (m)	44.2	26, 27	1.34 (m)	44.0	26, 27, OH-25
	b	1.24 (m)			1.25 (m)		
25			69.0	24a,b, 26, 27, OH-25		68.7	24a,b, 26, 27, OH-25
26		1.06 (s)	29.5	24a,b, OH-25	1.06 (s)	29.2	24a,b, OH-25
27		1.06 (s)	29.5	24a,b, OH-25	1.06 (s)	29.2	24a,b, OH-25
2-OH		4.58			4.56		
3-OH		4.67			4.62		
9-OH		5.33			4.83		
14-OH		5.37			4.62		
25-OH		4.09			4.04		

^a 500 MHz for ^1H , 125 MHz for ^{13}C , DMSO- d_6 . ^b Position defined as α/β based on ROESY data (see text) or a/b when undetermined.

HMBC correlations to C-8, C-9, and C-11, securing the C-9 hydroxyl group as the difference between hyousterones A and B and hyousterones C and D. The stereochemical configuration of both **4** and **5** closely matched that of **2** and **3**. Hyousterone C had characteristic ROESY correlations between H-2 and H₃-19, H-1 β , and H-3, while H-3 had corresponding ROESY correlations to H₃-19 and H-1 β . Further, small couplings characteristic of *cis*-related vicinal cyclohexyl protons were observed between H-2 and H-3.

A second collection of *S. adareanum* contained, in addition to hyousterones A–D (**2–5**), an ecdysteroid with a different carbon skeleton. Analyzing for C₂₇H₄₂O₅ by HRESI MS, abeohyousterone (**6**) displayed low-field signals in the ^1H NMR spectrum characteristic of the other hyousterones, with the exception of the H-7 olefin, which is found between δ 5.8 and 6.3 in the other ecdysteroids, and 14-OH, which is found near δ 4.7–4.8 in all but hyousterone C, where H-bonding with 9-OH shifts it down to δ 5.37. In the ^{13}C NMR spectrum, a similar comparison of **6** to **2–5** reveals a new ketone at δ 214.3, as well as the absence of the C-7/C-8 olefin at δ 122–125/ δ 162–170, respectively. While H₃-18 resonates 0.3 ppm downfield in the 14 β -OH members (**3** and **5**), relative to the 14 α -OH, abeohyousterone was in the middle of that range. Abeohyousterone lacked a low-field oxygen-bearing carbon, characteristic (70–85 ppm) of C-9 and/or C-14 of the hyousterones.

Focusing on the areas where the hyousterones (**2–5**) differed from abeohyousterone (**6**), HMBC correlations (Table 3) indicated the C-6 carbonyl, while still conjugated to the C-4/C-5 olefin, was adjacent to a methylene, C-7. The protons of C-7 displayed HMBC

correlation to six different carbons, including the expected C-5, C-6, C-8, and C-9, as well as the new ketone at δ 214.3. Curiously, H₂-7 correlated to C-13. C-8 differed from hyousterone C-8's by virtue of being an aliphatic quaternary carbon, bearing no correlations in the HSQC spectrum, and displaying HMBC correlations from H₃-18 and H₂-12, correlations that are incompatible with the hyousterone skeleton. Securing the 13(14 \rightarrow 8) abeoecdysteroid skeleton was an HMBC correlation from H-15 β to C-8; the position of H-15 β was further confirmed by COSY and HMBC correlations of C-16 and C-17 and further into the steroid side chain, analogous to the hyousterones.

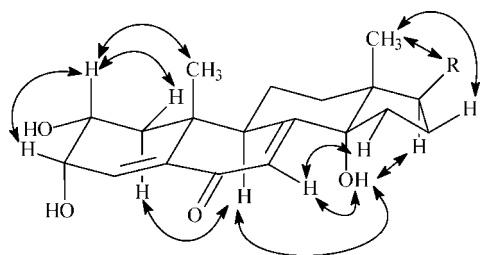
The ROESY spectrum of abeohyousterone (**6**) provided not only the stereochemical assignment but further confirmation of the planar structure (Figure 3). As observed in the other 2 α ,3 α -ecdysteroids from *S. adareanum*, abeohyousterone displayed ROESY correlations among H-1 α , H-2, H-3, and H₃-19 (Table 3), which, taken with the small $J_{2,3}$ coupling (recorded as a multiplet in Table 3 but simulation experiments suggest $J_{2,3} \approx 2$ Hz), confirmed the A ring stereochemistry as identical to hyousterones **2–5**. H-7 β correlates with H₃-19, securing its relative position on C-7, while both H-7 α and H-7 β display proximity to H₃-18, which requires H₂-7 and H₃-18 to be on the top face of the steroid nucleus.

Dialusterol B (**1**), hyousterones A–D (**2–5**), and abeohyousterone (**6**) were evaluated for solid tumor selective cytotoxicity using colon (colon 38, HCT-116), lung (H-125M), leukemia (L1210, CCRF-CEM), and normal (CFU-GM) cell lines in a soft-agar disk diffusion assay.²² The 14 β -OH-bearing ecdysteroids were largely

Table 3. ^1H and ^{13}C NMR Spectral Data for Abeohyosterone (**6**)^a

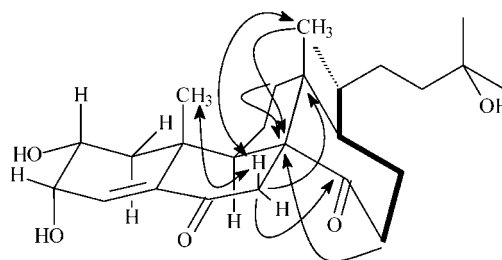
position ^b		δ_{H}	δ_{C}	HMBC	ROESY
1	α	1.75 (m)	42.0	3, 9, 19	1 β , 2, 9
	β	1.40 (m)			2, 3
2		3.69 (m)	66.1	1 α,β , 3, 4, 19	3, 19
3		3.97 (m)	65.9	1 α,β , 2, 4, OH-3	4, 2-OH
4		6.06 (d, 4.0 Hz)	131.1	3, 19, OH-3	
5			141.8	1 α,β , 3, 4, 7 α , 19	
6			200.1	4, 7 α,β	
7	α	2.28 (d, 16.5 Hz)			7 β , 15 β , 17, 18
	β	2.45 (d, 16.5 Hz)	40.6	9	12 α , 18, 19
8			62.0	7 α,β , 9, 11 α,β , 12 α,β , 15 β , 18	
9		2.94 (dd, 10.4, 6.1 Hz)	48.9	1 α,β , 7 α,β , 11 β , 12 α,β , 19	17
10			36.1	1 α,β , 2, 4, 9, 11 α,β , 19	
11	α	2.00 (m)	23.8	9, 12 α,β ,	17
	β	1.81 (m)			
12	α	1.53 (m)	37.2	9, 11 α,β , 18	18, 21
	β	1.67 (m)			
13			55.0	7 α,β , 11 α,β , 12 α,β , 16 α,β , 17, 18	
14			214.3	7 α,β , 9, 15 α,β , 16 α	
15	α	2.57 (m)	37.8	16 α,β , 17	15 β , 16 α , 17
	β	2.13 (m)			
16	α	1.78 (m)	22.5	17, 20	17, 21
	β	1.57 (m)			
17		1.56 (m)	46.6	12 α,β , 15 α,β , 16 β , 18, 20, 21	21
18		0.79 (s)	16.2	12 α,β , 17	
19		1.02 (s)	25.7	1 α,β , 9	
20		1.52 (m)	32.6	16 β , 17, 21, 22a,b	
21		0.97 (d, 6.8 Hz)	21.7	17, 20, 22a,b	
22	a	1.36 (m)	33.2	17, 20, 21, 23a,b, 24a,b	
	b	0.85 (m)			
23	a	1.34 (m)	23.2	23a,b, 24a,b	
	b	1.34 (m)			
24	a	1.34 (m)	44.0	22a, 26, 27, OH-25	
	b	1.21 (m)			
25			68.7	23a,b, 24a,b, 26, 27, OH-25	
26		1.05 (s)	29.3	24a,b, OH-25	
27		1.05 (s)	29.3	24a,b, OH-25	
2-OH	4.24				
3-OH	4.83				
25-OH	4.04				

^a 500 MHz for ^1H , 125 MHz for ^{13}C , DMSO- d_6 . ^b Position defined as α/β based on ROESY data (see text) or a/b when undetermined.

**Figure 2.** Key stereochemical relationships of hyousterone A (**2**) determined by NOE spectroscopy.

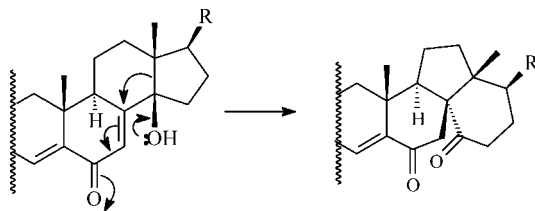
devoid of cytotoxicity, in agreement with prior studies.²³ Dialusterol B similarly lacked cytotoxicity, while hyosterones A, hyousterone C, and abeohyosterone displayed modest bioactivity. Abeohyosterone, for example, displayed an IC_{50} in the HCT-116 cell line of 3.0 μM , while hyousterone A was active at 10.7 μM and hyousterone C at 3.7 μM .

The co-occurrence of 14 β -hydroxy ecdysteroids and a 13(14 \rightarrow 8) abeo steroid is notable. Ecdysteroids bearing the 14 β -hydroxy functionality are rare in nature,²¹ and only one example, dankasterone,²⁴ exists of a natural product bearing the 13(14 \rightarrow 8) abeo steroid skeleton. Dankasterone was isolated from a fungi separated from a marine sponge, *Halichondria japonica*, and, similar to abeohyosterone, displayed modest cytotoxicity. Interestingly, the same fungal source of dankasterone, *Gymnascella dankaliensis*, produces gymnasterol, a related steroid that bears a β -epoxide between C-14 and C-15. This raises the question as to whether the 14 β -oxygenation pattern may be a thermodynamic pathway²⁵ into

**Figure 3.** Key COSY (bold), ROESY (\leftrightarrow), and HMBC (\rightarrow) correlations observed for abeohyosterone (**6**).

the 13(14 \rightarrow 8) abeo steroid skeleton (Scheme 1). Such a labile functional group could explain the paucity of 14 β -ecdysteroids despite the more common occurrence of the group among triterpenes. However, in our hands, hyousterone B (**3**) could not be converted thermally to abeohyosterone (**6**).

To our knowledge, this is the first report of ecdysteroids from a tunicate. The role of ecdysteroids in marine invertebrates has not been fully investigated. Phytoecdysteroids often have insecticidal properties thought to protect the plant from insect predation. The benthic flora²⁶ and invertebrate fauna (M. Amsler et al., unpublished) in the western Antarctic Peninsula communities where these collections were made appear to be heavily predated by crustaceans, particularly amphipods. Consequently, we believe that the ecdysteroids produced by *S. adareanum* may function as a chemical defense against crustacean predators.

Scheme 1. Hypothesized Origin of the 13(14→8) abeo Steroid Skeleton

Experimental Section

General Experimental Procedures. Optical rotations were measured on an Autopol IV automatic polarimeter using a Na lamp at 25 °C. ultraviolet-visible data were measured on a Hewlett-Packard 8452A diode array UV/vis spectrometer. Infrared spectra were obtained with a Nicolet Avatar 320FT-IR as KBr pellets. ^1H and ^{13}C NMR, HMQC, HMBC, and ^1H - ^1H COSY spectra were obtained on either a Varian Inova 500 instrument operating at 500 MHz for ^1H NMR and 125 MHz for ^{13}C or a Bruker Avance 250 instrument operating at 250 MHz for ^1H and 62.5 MHz for ^{13}C . Chemical shifts were referenced to solvent peaks: δ_{H} 2.50 and δ_{C} 39.51 for CD_3SOCD_3 . 2D NMR techniques were optimized as followed: HMQC, $J = 120$ Hz; HMBC, $J = 8$ Hz; COSY, $J = 7$ Hz. HPLC was carried out with a Shimadzu LC-8A multisolvent delivery system connected to a Shimadzu SPD-10A UV-vis tunable absorbance detector and/or an Alltech ELSD 2000 using a YMC-Pack ODS-AQ C-18 analytical column or a Waters prepLC (25 mm \times 30 cm) C-18 column for reversed-phase or Phenomenex Spherclone (250 \times 10 mm) for normal phase. EM Science silica gel 60 of 230–400 mesh was used in flash column chromatography. TLC was carried out on Whatman K6F silica gel 60 Å TLC plates with 0.25 mm thickness.

Animal Material. The specimens were collected by scuba between –20 and –40 m from the vicinity of Palmer Station (64°46' S, 64°03' W), Antarctica, in January and February 2003. The tunicate was identified as *Synoicum adareanum* (Herdman 1902; family Polyclinidae) by Dr. Linda Cole, Smithsonian Institution, Washington, DC. A voucher specimen is held at USF.

Extraction and Isolation. The freeze-dried tunicate (2 kg) was extracted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1, 1 L \times 3). The combined extract was concentrated, and the residue was partitioned between EtOAc and H_2O . Subsequently, the EtOAc layer was dried with MgSO_4 and concentrated *in vacuo*. The residue was subjected to Si gel column chromatography with an EtOAc/MeOH solvent system to give nine fractions. The fraction eluted with EtOAc/MeOH (95:5) was fractionated by RP HPLC (YMC-PAK ODS-AQ) with 50% aqueous CH_3CN to yield **1** (7.1 mg) and **2** (6.7 mg).

A second collection of freeze-dried tunicate (1.1 kg) was extracted, partitioned, and fractionated as described above. The column chromatography fraction eluting with EtOAc/MeOH (92:8) was further fractionated on ODS flash chromatography with aqueous MeOH, followed by RP HPLC (YMC-PAK ODS-AQ) following a linear gradient of aqueous MeOH to afford **2** (5.2 mg), **3** (2.2 mg), **4** (3.0 mg), and **5** (2.0 mg). The aqueous extract was concentrated and partitioned between BuOH and H_2O . The BuOH residue was concentrated and subjected to ODS flash column chromatography with aqueous MeOH to give 12 fractions. The fraction eluting with 70% MeOH was separated on RP HPLC with aqueous CH_3CN to yield abeohyosterone (**6**, 1.3 mg).

IC₅₀ Determination. Human tumor cells were plated at 5×10^4 cells in T25 tissue culture flasks (Falcon Plastics, NJ) with 5 mL of RPMI 1640 media (Cellgro, VA) supplemented with 15% BCS (Hyclone, UT), 5% Pen. Strep., and 5% glutamine (Cellgro). Three days later (cells in logarithmic growth phase; 5×10^5 cells/flask), test compound was added to the flasks to achieve concentrations ranging from 10^1 to 10^{-5} $\mu\text{g/mL}$. At day 3, the flasks were washed, trypsinized, and spun down, and the cells counted for both viable and dead cells using 0.08% trypan blue (Gibco, MD). Viable cell number as a function of concentration was plotted and the IC₅₀ value determined by interpolation.

Dialuolster B (1): yellow solid; $[\alpha]_{\text{D}}^{25} +46.4$ (c 0.05, MeOH); UV (MeOH) λ_{max} 213 nm (ϵ 1348), 262 nm (ϵ 715); IR ν_{max} 3330, 3263, 2945, 2832, 1659, 1578, 1317, 1154, 1022 cm^{-1} ; ^1H NMR (integration, multiplicity, assignment) ($\text{DMSO}-d_6$, 500 MHz) δ 0.59 (3H, s, H₃-18), 0.93 (1H, d, 6.6, H-21), 1.02 (3H, s, H₃-19), 1.02 (1H,

m, H-22b), 1.05 (6H, s, H₃-26, –27), 1.18 (1H, m, H-23b), 1.24 (1H, m, H-24b), 1.31 (2H, m, H-16b, –17), 1.32 (1H, m, H-22a), 1.34 (1H, m, H-24a), 1.36 (1H, m, H-23a), 1.37 (1H, m, H-20), 1.45 (2H, m, H-12b, –15b), 1.56 (1H, m, H-1b), 1.62 (1H, m, H-15a), 1.66 (1H, m, H-11b), 1.75 (1H, m, H-1a), 1.77 (1H, m, H-11a), 1.89 (1H, m, H-16a), 2.08 (1H, m, H-12a), 2.14 (1H, dd, 7.1, 11.9, H-14), 2.43 (1H, dd, 7.1, 12.0, H-9), 3.63 (1H, m, H-2), 3.98 (1H, m, H-3), 4.41 (1H, m, OH-2), 5.66 (1H, s, H-7), 6.26 (1H, d, 5.4, H-4); ^{13}C NMR (assignment) ($\text{DMSO}-d_6$, 125 MHz) δ 12.2 (C-18), 18.6 (C-21), 20.3 (C-23), 20.8 (C-19), 21.4 (C-11), 22.1 (C-15), 27.3 (C-16), 29.4 (C-26, –27), 35.4 (C-20), 36.0 (C-22), 37.8 (C-12), 38.0 (C-1), 39.7 (C-10), 43.9 (C-13), 44.1 (C-24), 46.7 (C-9), 55.0 (C-14), 55.4 (C-17), 64.6 (C-3), 65.9 (C-2), 68.9 (C-25), 122.7 (C-7), 130.2 (C-4), 143.9 (C-5), 166.7 (C-8), 187.8 (C-6); ESIMS (positive) m/z 453 [$\text{M} + \text{Na}$] $^+$; HRESIMS (positive) m/z 453.2992 [$\text{M} + \text{Na}$] $^+$ (453.2981 calcd for $\text{C}_{27}\text{H}_{42}\text{O}_4\text{Na}$).

Hyosterone A (2): yellow solid; $[\alpha]_{\text{D}}^{25} +24.2$ (c 0.1, MeOH); UV (MeOH) λ_{max} 215 nm (ϵ 1420), 261 nm (ϵ 729); IR ν_{max} 3566, 3130, 2960, 2859, 1723, 1123, 1071 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) m/z 469 [$\text{M} + \text{Na}$] $^+$; HRESIMS (positive) m/z 469.2924 [$\text{M} + \text{Na}$] $^+$ (469.2930 calcd for $\text{C}_{27}\text{H}_{42}\text{O}_5\text{Na}$).

Hyosterone B (3): yellow solid; $[\alpha]_{\text{D}}^{25} +24.2$ (c 0.1, MeOH); UV (MeOH) λ_{max} 213 nm (ϵ 1676), 272 nm (ϵ 586); IR ν_{max} 3389, 2927, 1655, 1618, 1457, 1031 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; ESIMS (negative) m/z 491 [$\text{M} + \text{HCOO}$] $^-$; HRESIMS (negative) m/z 491.3008 [$\text{M} + \text{HCOO}$] $^-$ (491.3009 calcd for $\text{C}_{28}\text{H}_{43}\text{O}_7$).

Hyosterone C (4): colorless solid; $[\alpha]_{\text{D}}^{25} +7.0$ (c 0.1, MeOH); UV (MeOH) λ_{max} 208 nm (ϵ 1859), 266 nm (ϵ 693); IR ν_{max} 3734, 3628, 3371, 2962, 1669, 1635, 1458, 881 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2; ESIMS (negative) m/z 461 [$\text{M} - \text{H}$] $^-$; HRESIMS (negative) m/z 461.2893 [$\text{M} - \text{H}$] $^-$ (461.2903 calcd for $\text{C}_{27}\text{H}_{41}\text{O}_6$).

Hyosterone D (5): colorless solid; $[\alpha]_{\text{D}}^{25} -34.2$ (c 0.1, MeOH); UV (MeOH) λ_{max} 206 nm (ϵ 1795), 272 nm (ϵ 616); IR ν_{max} 3587, 3385, 2924, 2854, 1733, 1684, 1457, 1375, 1073 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2; ESIMS (negative) m/z 461 [$\text{M} - \text{H}$] $^-$; HRESIMS (negative) m/z 461.2893 [$\text{M} - \text{H}$] $^-$ (461.2903 calcd for $\text{C}_{27}\text{H}_{41}\text{O}_6$).

Abeohyosterone (6): colorless solid; $[\alpha]_{\text{D}}^{25} +74.1$ (c 0.1, MeOH); UV (MeOH) λ_{max} 215 nm (ϵ 1058), 252 nm (ϵ 511); IR ν_{max} 3726, 3628, 1684, 1653, 1558, 1215 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 3; ESIMS (positive) m/z 469 [$\text{M} + \text{Na}$] $^+$; HRESIMS (positive) m/z 469.2943 (469.2930 calcd for $\text{C}_{27}\text{H}_{42}\text{O}_5\text{Na}$).

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Supporting Information Available: ^1H , ^{13}C , COSY, HM/SQC, HMBC, and ROESY NMR spectra for new compounds **2–6**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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