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4-DEHYDROECDYSTERONE, A NEW ECDYSTEROID FROM THE ZOANTHID *PARAZOANTHUS* SP.

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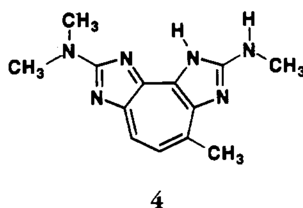
ABSTRACT.—A novel ecdysteroid, 4-dehydroecdysterone [**3**], has been isolated from the zoanthid *Parazoanthus* sp. collected from Port Phillip Bay, Australia. The known ecdysteroids ecdysterone [**1**] and ajugasterone C [**2**] were also isolated, together with the known alkaloid paragracine [**4**].

Relatively few chemical studies of zoanthids have been reported, despite evidence of their rich natural products chemistry (1). In our continuing study of antifungal natural products from marine invertebrates, we have examined a zoanthid, *Parazoanthus* sp. (Parazoanthidae), collected from Port Phillip Bay, Australia, and have isolated a novel ecdysteroid [**3**], together with ecdysterone [β-ecdysone, **1**] and ajugasterone C [**2**], previously reported from the Mediterranean zoanthid *Gerardia savaglia* (2,3), and paragracine [**4**], earlier isolated from *Parazoanthus gracilis* (4) and *Epizoanthus* sp. (5).

Parazoanthus sp. (90-13-120) was collected in May 1990, in Port Phillip Bay, Victoria, Australia. Freeze-dried animals were extracted with MeOH and the extract partitioned against organic solvents according to a modified Kupchan scheme (6). Tlc analysis of the $CHCl_3$ and *n*-BuOH fractions indicated several uv-active components that were visualized as green spots when treated with 1% vanillin/EtOH/H_2SO_4. Purification of a portion of the *n*-BuOH fraction by gel filtration, reversed-phase flash chromatography and hplc afforded compounds **1–3**.



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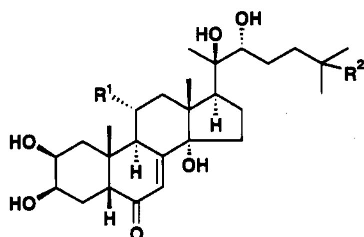
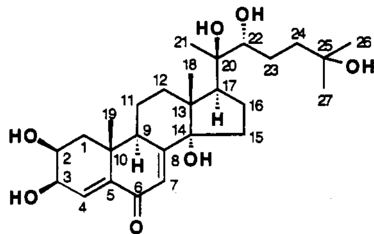


1 R^1=H, R^2=OH
2 R^1=OH, R^2=H



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Ecdysterone [**1**], obtained as a crystalline solid [mp 234–235° (dec) from CH_3CN], was the most abundant component of the extract (0.22% dry wt of animal). Identification was accomplished by ^{1}H-nmr, ^{13}C-nmr, and hrfabms techniques, and by comparison with reported data. In particular, the ^{13}C-nmr spectrum of **1** in C_5D_5N was identical ($\Delta\delta_C$<0.2) with reported values (7). Ajugasterone C [**2**] was obtained as an amorphous yellow solid (0.050%), and identified from its ^{1}H-nmr, ^{13}C-nmr, and hrfabms data. The ^{13}C-nmr spectrum of **2** in C_5D_5N was also in excellent agreement ($\Delta\delta_C$<0.1) with reported values (8).

Compound **3** (0.014%) was obtained as a colorless crystalline solid [mp 236–238° (dec) from CH_3CN]. The uv spec-

trum showed an absorbance at 258 nm (ε 9300) ascribed to an α,β-unsaturated enone. The high-resolution fabms indicated a molecular ion at *m/z* 479.3014 (MH^+, Δ 0.5 mmu), consistent with a molecular formula of $C_{27}H_{42}O_7$. Analysis of the ^{1}H- and ^{13}C-nmr data for **3** in CD_3OD, and of its COSY, HMQC, and HMBC nmr spectra (Table 1), established that **3** was the 4-dehydro analogue of ecdysterone [**1**]. The position of the double bond was located by the combined application of COSY and HMBC. The ^{1}H-nmr signal due to H-4 (δ 6.24, d, J=4.4 Hz) was coupled to H-3 (δ 4.18, dd, J=4.4 and 3.9 Hz), which in turn was coupled to H-2 (δ 3.90, dt, J=8.8 and 3.9 Hz). The HMBC experiment showed correlations from H-4 to the C-6 carbonyl group (198.2, s) and the ring junction C-10. The remainder of the structure is identical to ecdysterone [**1**].

The relative stereochemistry of **3** was established using a combination of coupling constant analysis and comparison of the nmr data with those of known compounds. The small coupling (J=3.9 Hz) between H-2 and H-3 suggests a cis arrangement of these protons (9). The relatively large (8.8 Hz) coupling be-

TABLE 1. Nmr Data for **3**.[a]

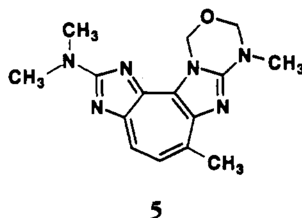
Position	^{13}C nmr δ (mult.)	^{1}H nmr δ (integral, mult., J Hz)	HMBC correlations
1	38.1 (t)	1.90 (2H, m)	C-2, C-3, C-5, C-9, C-10, C-19
2	68.2 (d)	3.90 (1H, dt, 8.8, 3.9)	
3	67.5 (d)	4.18 (1H, dd, 4.4, 3.9)	C-4, C-5
4	130.2 (d)	6.24 (1H, d, 4.4)	C-2, C-6, C-10
5	146.7 (s)		
6	192.8 (s)		
7	124.4 (d)	5.95 (1H, d, 2.4)	C-5, C-9, C-14
8	169.5 (s)		
9	44.6 (d)	2.94 (1H, ddd, 11.2, 7.3, 2.4)	
10	40.3 (s)		
11	21.8 (t)	1.72 (1H, m)	
		1.83 (1H, m)	
12	32.3 (t)	1.85 (1H, m)	
		2.13 (1H, td, 13.2, 4.9)	C-13, C-18
13	48.6 (s)		
14	85.2 (s)		
15	31.8 (t)	1.60 (1H, m)	C-13, C-14
		1.98 (1H, m)	
16	21.4 (t)	1.72 (1H, m)	
		1.98 (1H, m)	
17	50.6 (d)	2.37 (1H, t, 8.8)	C-13, C-16, C-21
18	18.0 (q)	0.91 (3H, s)	C-12, C-13, C-14, C-17
19	22.6 (q)	1.19 (3H, s)	C-1, C-5, C-9, C-10
20	78.0 (s)		
21	21.0 (q)	1.19 (3H, s)	C-20, C-22
22	78.4 (d)	3.31 (1H, dd, 10.0, 1.0)	C-20, C-21, C-24
23	27.3 (t)	1.28 (1H, m)	
		1.65 (1H, m)	
24	42.4 (t)	1.41 (1H, ddd, 13.2, 11.5, 4.4)	
		1.78 (1H, m)	C-23, C-25
25	71.3 (s)		
26	28.9 (q)	1.18 (3H, s)	C-24, C-25, C-27
27	29.7 (q)	1.20 (3H, s)	C-24, C-25, C-26

[a]Recorded in CD_3OD at 500 MHz for ^{1}H and 125 MHz for ^{13}C. HMBC experiment optimized for J_{CH}=8 Hz.

tween H-2 and one of the H-1 protons implies a pseudo-axial H-2. Analysis of molecular models shows that only the half-chair conformation with H-2 in a pseudo-axial position and 2β,3β-dihydroxyl substituents, would result in a relatively short distance (ca. 2.8 Å) between H-2 and H-9. Irradiation of the H-9 signal (δ 2.94, ddd, J=11.2, 7.3, and 2.4 Hz) produced a 6% nOe enhancement of the H-2 signal (δ 3.90, dt, J=8.8 and 3.9 Hz), thus establishing the relative configuration of the A and B rings. The relative configuration of the side-chain hydroxyl groups was determined by comparison of the ^{13}C-nmr data of **3** in C_5D_5N. Comparison of the C-20 and C-22 chemical shifts (δ_C 76.9, s; 77.6, d) with reported values for the four possible 20,22-dihydroxy sterol diastereomers (10), confirmed the 20β,22α-dihydroxyl stereochemistry of compound **3**. In addition, the ^{13}C-nmr values of C-11 to C-18 and C-20 to C-27 for **3** in CD_3OD are identical ($\Delta\delta_C$<0.2 ppm) to those for ecdysterone [**1**]. Thus, the relative stereochemistry of **3** is the same as **1**, with trans-fused C,D rings.

The crude MeOH extract of the zoanthid exhibited an intense blue fluorescence. A portion of the $CHCl_3$-soluble fraction was further purified by fractionation on Sephadex LH-20, followed by flash chromatography (silica). An amorphous yellow alkaloid (0.13%) was obtained which showed an intense yellow-blue fluorescence in dilute solution. Comparison of its ^{1}H- and ^{13}C-nmr data with literature values (4,5) suggested that the fluorescent alkaloid was paragracine [**4**]. This was confirmed by conversion of **4** to the known 1,3,5-oxadiazine [**5**] (4).

Ecdysteroids have previously been isolated from *Gerardia savaglia* (2,3,11). Compounds similar to **3**, containing a 3,4-double bond but different configurations at C-2,3 and C-22, have also been isolated from the red alga *Laurencia pinnata* (12,13).



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Ecdysterone and related ecdysteroids released from specialized cells in insect larvae and crustaceans act on epidermal cells and initiate molting (ecdysis) (14). We considered the suggestion that, like plants that express phytoecdysteroids and are immune from insect predation (8,10,15,16), marine invertebrates that contain ecdysteroids may gain protection from predation by marine crustaceans. We tested this hypothesis by feeding freeze-dried squid (ca. 3-mm cubes), treated with aliquots of solutions **1**–**3** or solvent alone, to blue-handed hermit crabs (*Pagurus samuelis*). All treatments, up to 1% ecdysteroid dry wt, were found to be palatable and readily eaten by the crabs. Compounds **1**–**4** were found to be inactive against the human fungal pathogen *Candida albicans*.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Optical rotations were measured on a Jasco DIP-370 spectropolarimeter. Nmr spectra were recorded at 300 MHz or 500 MHz for ^{1}H, and 75 MHz or 125 MHz for ^{13}C. ^{1}H- and ^{13}C-nmr spectra are referenced to residual CD_3OD signals at 3.30 ppm and 49.0 ppm, or C_5D_5N signals at 7.19 ppm and 123.5 ppm, respectively. Multiplicities of ^{13}C-nmr spectra were assigned by DEPT experiments. Standard pulse sequences (General Electric Instruments) were employed for DEPT, magnitude COSY, HMBC, and phase-sensitive HMQC experiments. Ir spectra were recorded on a Mattson Galaxy 3000 instrument. Uv spectra were recorded on a Hewlett-Packard 8450A spectrophotometer. Mass spectra were provided by the University of Minnesota Chemistry Department Mass Spectrometry Service Laboratory. Tlc was carried out on Merck Kieselgel $60F_{254}$ or Whatman $MKC_{18}F$ plates developed with 1% vanillin/EtOH/H_2SO_4. Solvents were distilled in glass before use.

ANIMAL MATERIAL.—The bright yellow commensal zoanthid *Parazoanthus* sp. (90-13-120)

was found at a depth of 20 m in Port Phillip Bay, Victoria, Australia, in May 1990, attached to an identified sponge. The animals were collected by hand using scuba, immediately frozen, and stored at −20° until required. This zoanthid is common throughout deeper waters off Victoria and Tasmania. The polyps are approximately 3–4 cm long when extended and have distinctive red lines radiating from each mouth, extending to the edge of the tentacles (17,18). Colonies numbering 4–50 polyps are connected by a common stolon which is directly attached to the sponge exoderm. The zoanthid colonies readily separated from the sponge in one piece with little force. Spirit-preserved samples display a characteristic blue fluorescence in sunlight. Voucher specimens of this animal were indistinguishable from yellow *Parazoanthus* sp. specimens archived in the invertebrate collection of the National Museum of Victoria. A voucher specimen is archived at the Department of Chemistry, University of California, Davis.

EXTRACTION AND ISOLATION.—Lyophilized animals (62.5 g) were extracted twice with MeOH The extracts were combined and concentrated to leave an orange oil (11.3 g). A portion (10.6 g) was dissolved in MeOH (500 ml) and successively extracted using a modified Kupchan partition (6) as follows. The H_2O content (% v/v) of the MeOH extract was adjusted prior to sequential partitioning against *n*-hexane (10% v/v H_2O), CCl_4 (20%), and $CHCl_3$ (40%). The aqueous phase was concentrated to remove MeOH then extracted with *n*-BuOH. Tlc indicated that the compounds of interest were contained in the $CHCl_3$ (0.755 g) and *n*-BuOH (1.67 g) extracts.

A portion (1.30 g) of the *n*-BuOH extract was fractionated on a column of Sephadex LH-20 (105 cm×2.5 cm) with MeOH (two batches) to afford an orange-brown gum (420 mg). Purification by flash chromatography (C_{18}-bonded silica, 75 mm×43 mm, MeOH-H_2O, 1:1) yielded ajugasterone C [**2**] (22.7 mg) and a mixture of **1** and **3**. Further purification of this mixture by hplc (Dynamax C_{18}, MeOH-H_2O, 6:4) gave ecdysterone [**1**] (99.4 mg) and 4-dehydroecdysterone [**3**] (6.2 mg).

A portion (740 mg) of the $CHCl_3$ extract was purified by elution through a column of Sephadex LH-20 (105 cm×2.5 cm) with MeOH, followed by flash chromatography (Si gel, 240 mm×25 mm, $CHCl_3$-MeOH, 9:1), to afford paragracine [**4**] (73.9 mg).

Ecdysterone [**1**].—Colorless plates (99.4 mg, 0.22%), mp 234–235° (dec) from CH_3CN. Identified by ^1H nmr, ^{13}C nmr, and fabms. Spectral data were identical to reported values (C_5D_5N) (7). ^{13}C nmr (CD_3OD) δ 18.1 (q, C-18), 21.1 (q, C-21), 21.5 (2×t, C-11, C-16), 24.4 (q, C-19), 27.3 (t, C-23), 29.0 (q, C-26), 29.7 (q, C-27), 31.8 (t, C-15), 32.4 (t, C-12), 32.8 (t, C-4), 35.0 (d, C-9), 37.3 (t, C-1), 39.2 (s, C-10), 42.3 (t, C-24), 48.6 (s, C-13), 50.4 (d, C-17), 51.7 (d, C-5), 68.4 (d, C-2), 68.6 (d, C-3), 71.3 (s, C-25), 77.9 (s, C-20), 78.3 (d, C-22), 85.2 (s, C-14), 122.1 (d, C-7), 167.9 (s, C-8), 206.4 (s, C-6).

Ajugasterone C [**2**].—Amorphous powder (22.7 mg, 0.050%). Identified by ^1H nmr, ^{13}C nmr, and fabms. Spectral data identical to reported values (8).

4-Dehydroecdysterone [**3**].—Colorless needles (6.2 mg, 0.014%); mp 236–238° (dec) from CH_3CN; $[\alpha]_D$ −33° (c=0.09, MeOH); uv (MeOH) λ max 258 nm (ε 9300); ir (film) ν max 3415 br, 2965, 2935, 1660, 1615, 1380, 1070 cm^{-1}; ^1H-(CD_3OD) and ^{13}C-nmr (CD_3OD) data, see Table 1; ^{13}C nmr (C_5D_5N) δ 17.9 (q, C-18), 21.3 (t, C-11*), 21.4 (t, C-16*), 21.7 (q, C-21), 22.5 (q, C-19), 27.5 (t, C-23), 30.0 (q, C-26), 30.2 (q, C-27), 31.8 (t, C-15), 31.9 (t, C-12), 39.1 (t, C-1), 39.2 (s, C-10), 42.7 (t, C-24), 44.6 (d, C-9), 48.1 (s, C-13), 50.2 (d, C-17), 67.4 (d, C-2), 67.7 (d, C-3), 69.6 (s, C-25), 76.9 (s, C-20), 77.6 (d, C-22), 84.2 (s, C-14), 124.2 (d, C-7), 130.4 (d, C-4), 145.2 (s, C-5), 167.1 (s, C-8), 190.0 (s, C-6). *Assignments interchangeable; fabms *m/z* 501 ($M+Na^+$, 7), 479 (MH^+, 8), 443 (MH^+-2H_2O, 5), 176 (90); hrfabms found *m/z* 479.3014 (MH^+), $C_{27}H_{43}O_7$ requires 479.3009.

Paragracine [**4**].—Yellow amorphous powder (73.9 mg, 0.13%); identified by ^1H and ^{13}C nmr, and by comparison with literature data (4,5). Treatment of **4** with formic acid and formaldehyde according to the procedure of Komoda *et al.* (4) gave the dioxazine **5** [30%, mp 238–240° (dec), MeOH; lit. (4) 246–249° (dec)]; ^1H nmr ($CDCl_3$) δ 2.82 (3H, br s, CH_3), 3.32 (3H, s, NCH_3), 3.35 (6H, s, $N(CH_3)_2$), 4.97 (2H, s, $N(CH_3)CH_2O$), 6.34 (2H, s, OCH_2N), 7.49 (1H, d, J=10.5 Hz), 7.83 (1H, d, J=10.5 Hz).

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