A New Cyclized 9,11-Secosterol Enol−Ether from the Australian Sponge *Euryspongia arenaria*

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The polar fraction of the crude extract from the sponge *Euryspongia arenaria* was separated by chromatography. Structure elucidation by spectrometric methods allowed the identification of a new steroid, stellattasterenol (**2**), containing an unprecedented seven-membered cyclic enol−ether in ring C. A related known compound, stellettasterol (**3**), a pentahydroxy-9,11-secosteroid, was also identified.

Our studies of sponges deterrent to settlement of the larvae of the ascidian *Clavellina mollucensis* have previously shown that the sponge *Crella incrustans* contains *lyso*-platelet activating factor (*lyso*-PAF, **1**) as its major chemical settling deterrent.[1,2] The sponge *Euryspongia arenaria*, also part of this study, was shown to contain three known compounds, thiofurodysin acetate, thiofurodysinin acetate, and dehydrodendrolasin, and two previously unreported epoxy lactone derivatives.[3] Subsequent work on a new collection of *E. arenaria* to obtain more material for bioassays led to the isolation of two 9,11-secosteroids, **2** and **3**. Despite the fact that sponges have been a prolific source of new steroids[4] with modified side chains, diverse levels of oxidation on the rings and cleavage of the carbon skeleton, isolation of 9,11-secosteroids has been limited to relatively few examples. Herbasterol (**4**) from *Dysidea herbacea*[5] is an early example and has been joined by others from *D. fragilis*,[6] *Spongia officinalis*,[7−9] *Aplysilla glacialis*,[10] and the genera *Pleraplysilla*,[11] *Stelletta*,[12] and *Euryspongia*.[13]
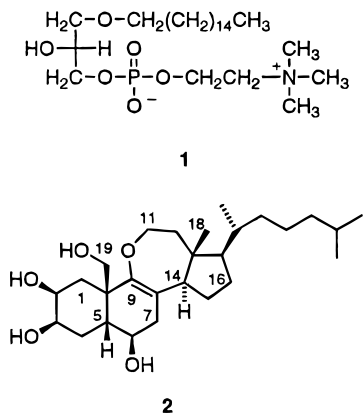

E. arenaria Bergquist, 1961, was collected by scuba diving from the jetty piers at Edithburgh, South Australia. A polar fraction of an extract of the sponge was submitted, in turn, to Sephadex LH-20, normal- and reversed-phase column, centrifugal, and reversed- and normal-phase HPLC chromatography to yield two steroids, stellettasterenol (**2**) and stellettasterol (**3**), in 0.01% and 0.05% yield, respectively.

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3 R₁ = H, R₂ = OH
4 R₁ = OH, R₂ = H
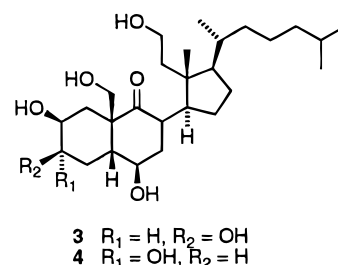
Stellettasterenol (**2**) was obtained as a white solid, [α]²⁵_D +70°, and was shown to have a molecular formula of $C_{27}H_{46}O_5$ by HRLSIMS. ¹³C NMR spectroscopy indicated the presence of only one double bond [¹³C 119.3 (s), 150.4 (s) ppm], suggesting that the remaining four double-bond equivalents are present as rings. IR spectroscopy only showed absorbances involving oxygens singly bonded to hydrogen (3351 cm⁻¹) and carbon (1062 cm⁻¹). The level of substitution of carbon atoms was determined a DEPT experiment and HMQC data was used to correlate carbons to their respective attached hydrogens (Table 1). One extensive hydrogen spin system containing three methinyloxy groups, inferred from their significant downfield chemical shifts (partial structure **2a**), and several shorter ones were established from COSY-45 data. Correlations (Table 2) allow the sequential connection of H-1 through H-7 and, further, H-14 through H-16. Although H-7 and H-14 are correlated, this must be due to significant long-range coupling, because the HMBC data (correlation of C-9 with H-7, see below) requires that an atom be interposed between them. In the shorter ones, hydrogens on C-11 and C-12, C-20 and C-21, and C-25 to C-24, C-26, and C-27 showed correlations indicating the presence of −OCH₂CH₂−, CH₃CH−, and *iso*-butyl groups, respectively.

The overall planar structure of stellettasterenol (**2**) was determined using an HMBC spectrum. It showed correlations between quaternary C-10 and H-5, H-4β, H-6, H₂-1, and H-2 in partial structure **2a**, placing it between C-1 and C-5, consequently forming the first ring. The sp² C-9 correlates with H₂-1 (showing that it is attached to C-10) as well as H₂-7, which indicates, with its necessary sp² partner, C-8, closure of the second ring involving partial structure **2a**. The fourth substituent on C-10 is the methylenoxy group (C-19), which has correlations to H₂-1 and H-5.

The second quaternary sp³ carbon, C-13, is correlated to H-14, the methyl group, H₃-18, and H₂-11 (δ 3.81 and 3.91). Because H₂-11 is also correlated to C-9 as well as

**Table 1.** NMR Spectral Data for Stellettasterenol (**2**)[a]

carbon no.	^{13}C δ (mult.)	^1H δ (mult., J in Hz)
1	30.7 (t)	1.83 (Hα, dd, 12.9, 3.4)
		1.32 (Hβ, m)
2	68.9 (d)	3.44 (m)
3	68.7 (d)	3.78 (br s)
4	32.5 (t)	1.64 (Hβ, m)
		1.27 (Hα, m)
5	40.7 (d)	1.93 (br d, 13)
6	68.3 (d)	3.62 (br s, $W_{1/2}$ 13)
7	34.6 (t)	2.28 (Hα, dd, 18.6, 5.9)
		2.06 (Hβ, d, 18.6)
8	119.3 (s)	
9	150.4 (s)	
10	45.1 (s)	
11	66.8 (t)	3.91 (Hβ, dd, 12.2, 12.2)
		3.81 (Hα, ddd, 12.2, 4.5, 2.5)
12	46.6 (t)	1.90 (Hβ, bd, 15)
		1.59 (Hα, m)
13	43.3 (s)	
14	50.7 (d)	2.78 (dd, 10.3, 10.3)
15	23.9 (t)	1.64 (Hβ, m)
		1.48 (Hα, m)
16	27.3 (t)	1.75 (Hβ, m)
		1.30 (Hα, m)
17	57.4 (d)	1.26 (m)
18	12.3 (q)	0.73 (s)
19	70.2 (t)	3.45 (d, 11.0)
		3.37 (d, 11.0)
20	35.6 (d)	1.33 (m)
21	19.9 (q)	0.85 (d, 6.4)
22	36.3 (t)	1.30 (m)
		0.95 (m)
23	24.5 (t)	1.28 (m)
		1.05 (m)
24	39.8 (t)	1.05 (m)
25	28.3 (d)	1.44 (nonet, 6.6)
26	22.7 (q)	0.78 (d, 6.6)
27	22.9 (q)	0.78 (d, 6.6)

[a] ^1H 500.1 MHz, ^{13}C 125.8 MHz, solvent: CDCl$_3$–CD$_3$OD, chemical shifts referenced to CDCl$_3$.
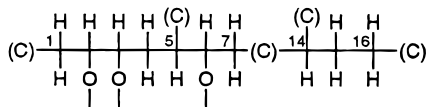
2a

being adjacent to C-12 in the $-OCH_2CH_2-$ group, C-11 must be connected to C-13 via C-12 in one direction and to C-9 via oxygen in the other. This results in the formation of the third ring, a seven-membered enol–ether. Among other things, this orientation of the C-11−C12 unit is supported by the ^{13}C NMR chemical shifts of C-9 and C-8 (150.4 and 119.3 ppm, respectively). The final ring could be formed by the insertion of the methine, C-17, between C-16 and C-13. It has correlations to H-16α and H$_3$-18. The remaining eight carbons, including three methyl groups [^1H δ 0.85 (d, J = 6.4 Hz), 0.78 (d, J = 6.6 Hz), 0.78 (d, J = 6.6 Hz)], one of which is correlated to C-17, could readily be assigned to a steroidal 'tail' as shown (**2**). The four remaining oxygen functionalities must be hydroxyl groups.

The relative stereochemistry of stellettasterenol (**2**) was determined largely by NOESY. NOESY showed a strong correlation between H-5 and H-19b, indicating that the A/B ring junction had the unusual cis stereochemistry. H-6 showed correlations to both H-5 and one of the hydrogens on C-4 (^1H δ 1.64), which, in light of the presence of the cis ring junction, can only occur if H-6 is on the concave or α face. The other hydrogen on C-4 (^1H δ 1.27) is correlated with a hydrogen on C-7 (^1H δ 2.28) and H-3 indicating, as H-4 (δ 1.27) must be axial due to its spatial proximity to
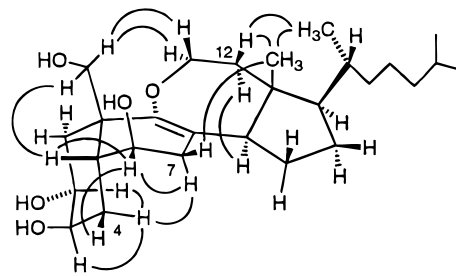
Figure 1. Significant NOE correlations found for stellettasterenol (**2**).

H-7 (δ 2.28), that all three also must lie on the α face. The disposition of H-4α also means that ring A is in the chair conformation. Another correlation from H-4α, this time to H-2, indicates that they must be 1,3-diaxial, again placing H-2 on the α face.

The hydrogen H-7β (δ 2.06) correlates to the methyl group, H$_3$-18, which, in turn, correlates to both H$_3$-21 and H-12 (δ 1.90), indicating that they are on the upper face of the molecule and that C-13 and C-17 have the normal steroid relative stereochemistry. H-12α(^1H δ 1.59), the geminal partner of H-12β, shows a correlation back to H-14, indicating that the C/D ring junction is trans, completing the assignment of relative stereochemistry in the rings. An energy minimized structure (Chem3D, CambridgeSoft Corp.) of stellettasterenol (**2**) gave a conformation in accord with the observed NOE and coupling constant data. It is represented in Figure 1 with the significant NOESY correlations indicated. The normal absolute stereochemistry for steroids is assumed.

Compound **3** was obtained as a white solid, $[\alpha]^{25}_D$ −31.4°, and was shown to have a molecular formula of $C_{27}H_{48}O_6$ by HRLSIMS. Although there were many similarities in the NMR data of **2** and **3**, compound **3** possessed a ketone (^{13}C 216.4 ppm, IR 1701 cm^{-1}) instead of an alkene and had one less ring incorporated into its structure. Analysis of the 2D spectral data led to the conclusion that **3** was the known compound stellettasterol, previously isolated by Li et al. from a sponge *Stelletta* sp.[12]

On the face of it, stellettasterenol (**2**) can be visualized as arising from the nucleophilic attack of the primary alcohol (C-11) on the carbonyl of stellettasterol (**3**) and subsequent elimination of water during the isolation process. Normally, the equilibrium is expected to vastly favor the alcohol/ketone rather than the enol−ether, and ether formation must be driven to completion by continuous removal of water. On the other hand, intramolecular cyclization may favor the reaction. Molecular modeling indicates that the approach vector of the primary alcohol to the ketone is extremely sterically hindered. The suggestion that the enol−ether might be an artifact of the isolation procedure was tested by stirring stellettasterol (**3**) in chloroform−methanol in the presence of Si gel for 22 h and for a further 26 h with added acetic acid without any apparent change, as judged by TLC.

Stellettasterol (**3**) differs from herbasterol (**4**) only by the stereochemistry at C-3. Herbasterol was isolated by Capon and Faulkner from the sponge *Dysidea herbacea*,[5] a member of the same family as *Euryspongia* spp. 9,11-Secosterols epimeric at C-3 have previously been found in *Euryspongia* sp.; however, they also carry a hydroxy at C-4 and their A/B ring junction is trans, not cis.[13] It is interesting that Andersen and Pika have reported the isolation of a 9,11-secosteroid, furodysin, and furodysinin from a sponge, *Pleraplysilla* sp.,[11] mirroring the compounds found in our studies of *E. arenaria*,[3] despite their being collected in very different locations. This is, however, in line with the

Table 2. 2D NMR Correlations for Stellettasterenol (**2**)[a−c]

C	H	COSY	HMBC	NOESY
1	Hα	H 1β, H 2	H 2	H 2
	Hβ	H 1α, H 2		
2	H	H 1α,β, H 3	H 1α,β	H 1α, H 3, Hα 4
3	H	H 2, H 4α,β	H 4β	H 2, H 4α
4	Hα	H 3, H 4β, H 5	H 5	H 2, H 3, H 7α
	Hβ	H 3, H 4α,		H 6
5	H	H 4α, H 6	H 1α, H 4β, H 12a,b, H 7β	H 6, H 19a,b
6	H	H 5, H 7α,β	H 5, H 7β	H 4β, H 5, H 7α
7	Hα	H 6, H 7β, H 14		H 4α, H 6, H 7β
	Hβ	H 6, H 7α, H 14		H 7α, H 15β, H 18
8			H 6, H 7α,β, H 14	
9			H 1α,β, H 7α,β, H 11α,β, H 14, H 19a,b	
10			H 1α,β, H 2, H 4β, H 5, H 6	
11	Hα	H 11β, H 12α,β		H 11β, H 12α,β, H 19a
	Hβ	H 11α, H 12α,β		H 11α, H 18, H 19a
12	Hα	H 11α,β, H 12β	H 18	H 11α, H 12β, H 14
	Hβ	H 11α,β, H 12α		H 11α, H 12α, H 18, H 21
13			H 11α,β, H 14, H 18	
14	H	H 7α,β, H 15α,β	H 12β, H 18	H 12α, H 15α
15	Hα	H 14, H 15β, H 16α,β	H 14	H 14, H 16α
	Hβ	H 14, H 15α, H 16β		H 7β
16	Hα	H 15α, H 16β	H 17	H 15α
	Hβ	H 15α,β, H 16α		
17	H		H 16α, H 18, H 21, H 22b	
18	H₃		H 14	H 7β, H 11β, H 12β, H 21
19	Ha	H 19b	H 1α,β, H 5	H 5, H 11α,β
	Hb	H 19a		H 5
20	H	H 21	H 17, H 21	
21	H₃	H 20		H 12β, H 18
22	Ha	H 22b	H 21	
	Hb	H 22a		
23	Ha		H 24, H 25	
	Hb			
24	H₂	H 25	H 22b, H 25, H 26, H 27	H 25
25	H	H 24, H 26, H 27	H 24, H 26, H 27	H 24
26	H₃	H 25	H 24, H 25	
27	H₃	H 25	H 24, H 25	

[a] ¹H 300.13 MHz, solvent: CDCl₃−CD₃OD, chemical shifts referenced to CDCl₃ (residual ¹H 7.24 ppm, ¹³C 77.0 ppm). [b] Ha and Hb denote the downfield and upfield signals, respectively, of a diastereotopic pair. [c] Some uninformative correlations have not been included.

recommendation that the genus *Pleraplysilla* be transferred to the same family as the *Euryspongia* (Dysideidae) based on their terpenoid chemistry.[14]

Experimental Section

General Experimental Procedures. IR spectra were recorded as films on NaCl disks with a Perkin−Elmer Paragon 1000 FT-IR spectrometer. Optical rotations were measured using a Perkin−Elmer 241 polarimeter. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance DRX-500 or a Bruker Avance DPX-300 spectrometer. NMR spectra were recorded as CDCl₃−CD₃OD solutions and the CDCl₃ solvent signals used as the internal standard for chemical shifts (¹³C 77.0 ppm, residual ¹H 7.24 ppm). Mass spectrometry was done on a Kratos Concept ISQ mass spectrometer at the Central Science Laboratory, University of Tasmania, Australia, using a liquid secondary ion MS ionization mode (primary beam 10 kV cesium ions), *m*-nitrobenzyl alcohol liquid matrix, and calibrated internally using peaks from the liquid matrix as reference. TLC was performed using aluminum-backed plates (Si gel 60 F₂₅₄, 0.2 mm, Merck), 'speedy' (short) column chromatography used Si gel H (Merck),[15,16] reversed-phase flash chromatography with LiChroprep RP-18 (40−60 μm, Merck), and centrifugal chromatography (Chromatotron, Harrison Research) was performed using plates coated with Si gel 60 PF₂₅₄ containing gypsum (Merck). Gel permeation chromatography was performed using Sephadex LH-20 (Pharmacia) with CHCl₃−CH₃OH (1:1) as eluent. HPLC was accomplished using a Waters 600 controller fitted with a photodiode array detector (Waters 996) and semipreparative columns [7.8 × 300 mm, μPorasil (Waters), 10 × 250 mm, Exsil 100 Å 7 μ (Activon)

or 10 × 250 mm, Exsil 100/10 ODS (Activon)]. All solvents used were of HPLC grade or distilled from glass. Light petroleum refers to a mixture of alkanes that distill at 60−80 °C.

Animal Material. The pale blue sponge *Euryspongia arenaria* was collected at around −4 m using scuba from the piers of the jetty at Edithburgh, Yorke Peninsula, South Australia, on February 9, 1993. The sponge was transported on ice and then kept at −20 °C until required. Dr. John Hooper of the Queensland Museum, Australia, identified the sponge. A voucher specimen of *E. arenaria* has been deposited with the Queensland Museum, Brisbane, Australia (reference no. G 304072).

Isolation of Stellettasterenol (2) and Stellettasterol (3). The frozen sponge (96 g dry extracted wt) was macerated (Waring blendor) in Me₂CO (4 × 500 mL), filtered through diatomaceous earth, and the Me₂CO removed under vacuum to leave an aqueous residue (400 mL). The residue was extracted with Et₂O (4 × 200 mL) and the ether removed under vacuum. The crude lipophilic extract was partitioned between 20% aqueous MeOH and light petroleum. The light petroleum layer was back extracted with aqueous MeOH and both MeOH layers combined, dried with anhydrous MgSO₄, and evaporated under vacuum to provide 1.6 g (1.7% yield) of sponge extract. The major fraction from gel permeation chromatography of the extract was submitted to 'speedy' column chromatography.[15,16] The polar fractions containing EtOAc eluent were combined and separated using, in turn, reversed-phase flash chromatography (H₂O, MeOH, CH₂Cl₂ gradient), chromatotron (5−10% MeOH, CHCl₃), reversed-phase HPLC (55−100% aqueous MeOH gradient) and normal-phase HPLC (8% MeOH−CHCl₃). This procedure resulted in the isolation of stellettasterenol (**2**)

and stellettasterol (**3**) in 0.01% and 0.05% yield, respectively, based on the dry extracted weight of the sponge.

Stellattasterenol (2): isolated as a white solid; $[\alpha]^{25}_D$ +70° (*c* 0.025, CHCl$_3$−MeOH, 1:1); IR (film from MeOH) ν_{max} 3351 (O−H), 2955, 1590, 1466, 1160 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; LSIMS *m/z* 473 [M + Na]$^+$ (20%), 451 [M + H]$^+$ (93), 433 (79), 421 (47), 415 (28), 403 (100), 391 (34), 383 (37), 291 (15), 273 (17), 237 (19), 219 (18), 189 (20); HRLSIMS *m/z* 451.3420 [M + H]$^+$ (calcd for C$_{27}$H$_{47}$O$_5$ 451.3424).

Stellettasterol (3): isolated as a white solid; $[\alpha]^{25}_D$ −31.4° (*c.* 0.92, CHCl$_3$−MeOH, 1:1) {lit: $[\alpha]^{23}_D$ −18.5° (*c.* 0.35, MeOH)[12]}; IR (film from MeOH) ν_{max} 3365 (O−H), 2955, 2871, 1701 (C=O), 1050, 1020 cm^{-1}; ^{1}H and ^{13}C NMR data corresponds with the literature;[12] LSIMS *m/z* 491 [M + Na]$^+$ (90%), 469 (57) [M + H]$^+$, 452 (55), 450 (43), 433 (85), 421 (100), 403 (99), 391 (14), 385 (17), 291 (16), 273 (20), 237 (40), 166 (35), 153 (32); HRLSIMS *m/z* 469.3561 [M + H]$^+$ (calcd for C$_{27}$H$_{49}$O$_6$ 469.3529).

Attempted Cyclization of Stellettasterol (3) to Stellettasterenol (2). Stellettasterol (**3**) (10 mg) was dissolved in CHCl$_3$−MeOH (1:1, 2 mL) and stirred with preparative TLC Si gel (18 mg) at room temperature for 22 h. TLC [15% MeOH−CHCl$_3$, stellettasterol (**3**) R_f 0.26, stellettasterenol (**2**) R_f 0.48] indicated no change to the starting material. HOAc (1%) was added and the mixture stirred for a further 26.5 h without any apparent change.

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