Gelliusterols A−D, New Acetylenic Sterols from a Sponge, *Gellius* Species

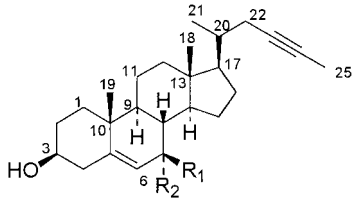
Winklet A. Gallimore,[†] Michelle Kelly,[‡] and Paul J. Scheuer*[,†]

Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, Hawaii 96822, and National Institute of Water and Atmospheric Research (NIWA), Taihoro Nukurangi, Private Bag 109-695 Newmarket, Auckland, New Zealand

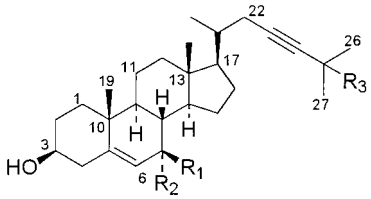
Received December 8, 2000

New acetylenic sterols, gelliusterol A (**1**, 26,27-bisnorcholest-5-en-23-yn-3β,7α-diol), its corresponding 7-ketone, gelliusterol B (**2**, 26,27-bisnorcholest-5-en-23-yn-3β-ol-7-one), and gelliusterols C (**4**, cholest-5-en-23-yn-3β,7-one) and D (**5**, cholest-5-en-23-yn-3β,25-diol-7-one), were isolated from an unidentified species of sponge, *Gellius* sp. The structures of the steroids were established from spectroscopic data.

Highly functionalized steroids featuring biogenetically unprecedented structures have been found in a vast array of marine organisms, particularly sponges.[1,2] It has been hypothesized that the sponges effect chemical modification of their dietary precursors in order to produce the diverse variation in steroidal content obtained from these sources. Many such steroids exhibit potent pharmacological properties, including cytotoxic[3] and ichthyotoxic[4] effects. Others effect enzymatic inhibition and display antifungal and antiviral bioactivity including anti-HIV effects.[2,5] The *Gellius* sp. represents a little-investigated genus from which indole alkaloids,[6] lipids, including phospholipids, and sterols have been isolated.[7−9] In the course of our chemical investigation of an unidentified *Gellius* sp., steroidal components possessing an uncommon acetylenic bond in the side chain were identified and are the subject of this paper.



(1) R_1 = H, R_2 = OH
(2) R_1, R_2 = O
(3) R_1 = R_2 = H



(4) R_1, R_2 = O, R_3 = H
(5) R_1, R_2 = O, R_3 = OH
(6) R_1 = R_2 = R_3 = H

Results and Discussion

The lyophilized sponge was exhaustively extracted with CH_2Cl_2/2-propanol (1:1) to yield a greenish-brown gum, which was subjected to vacuum liquid chromatography on Si gel, eluting with increasing concentrations of ethyl acetate in hexane. The fractions eluting in 20−80% ethyl acetate in hexane were subjected to normal and reversed-phase HPLC purification to afford gelliusterols A−D (**1**, **2**, **4**, **5**).

High-resolution mass spectral analysis of gelliusterol A (**1**) established a molecular formula of $C_{25}H_{38}O_2$ (*m*/*z* 370.2864), corresponding to seven degrees of unsaturation. ^{1}H NMR data revealed the presence of two hydroxyl methines (3.47 and 3.75 ppm), three methyl singlets (0.71, 1.00, 1.73 ppm), a methyl doublet (1.06 ppm), and a trisubstituted olefin (5.53 ppm). These resonances were accompanied by cyclic methylene signals (1.15−2.26 ppm), suggesting that the compound was steroidal in nature. The steroid nucleus was established through ^{1}H−^{1}H COSY and HMBC correlations (Figure 1). HMBC cross-peaks between H-4/C-3, C-5, C-6 and Me-19/C-1, C-5, C-9 established rings A and B. Rings C and D were connected through HMBC cross-couplings between Me-18/C-12, C-14, and C-17. ^{1}H−^{1}H COSY couplings between the olefinic proton (3.75 ppm, H-6) and the oxymethine at H-7 established their relative positions. The three-proton triplet centered at 1.73 ppm (H-25) correlated with a carbon shift of 3.3 ppm, suggesting a methyl group attached to a strongly shielding group such as an acetylenic group. This was borne out by an HMBC correlation to the quaternary carbon signal at 77.04 ppm, typical for an acetylene. Long-range COSY coupling with H-22 (2.14 ppm, J = 2.50 Hz) was observed through the alkyne bond (J = 2.44 Hz), thus establishing their relative positions. The mass spectral fragmentation pattern demonstrated fission of the C-20/22 bond (*m*/*z* 252, $M^+ - C_4H_5 - 2H_2O - H$), the side chain being of the formula C_4H_5. The 7-hydroxymethine was concluded to be of β-orientation on the basis of the established correlation between the orientation of the H-7 proton and the magnitude of the coupling constant (J = 5.0 Hz) and nature of the splitting pattern (doublet) for the adjacent olefinic methine.[10]

A molecular formula of $C_{25}H_{36}O_2$ (M^+ 368.2720) was deduced for gelliusterol B (**2**) from HREIMS, differing from **1** by 2H. Examination of the ^{1}H and ^{13}C NMR data led one to surmise that the C-7 alcohol was oxidized to the corresponding ketone, indicated by resonances at 202.1 ppm for the carbonyl group and a downfield shift of the C-5 olefin (165.0 ppm), typical of an α,β-unsaturated system. Of the eight double bond equivalents implied by the molecular formula, the steroid nucleus represented four double bond equivalents and the α,β-unsaturated system accounted for two double bond equivalents. The connectivities within the molecule were established through ^{1}H−^{1}H COSY and HMBC correlations (Figure 1). The remaining two double bond equivalents were attributed to the triple bond in the side chain, substantiated by the mass

* To whom correspondence should be addressed. Tel: (808) 956 5904. Fax: (808) 956 5908. E-mail: scheuer@gold.chem.hawaii.edu.
[†] University of Hawaii at Manoa.
[‡] NIWA.

10.1021/np000585a CCC: $20.00
Published on Web 05/24/2001

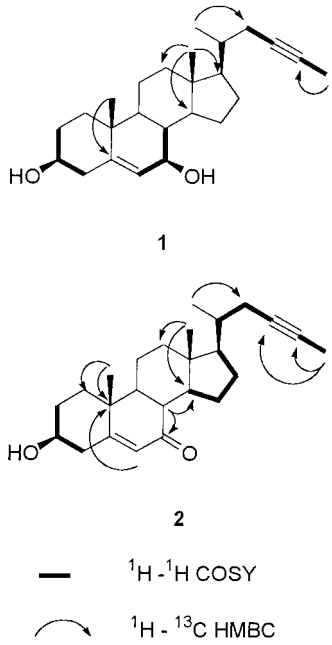


Figure 1. Key COSY and HMBC correlations in **1** and **2**.

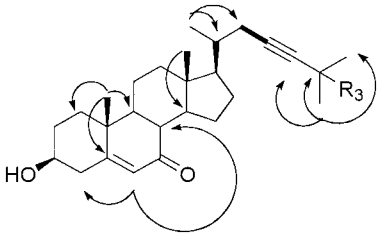


Figure 2. Key HMBC correlations in **4** and **5**.

spectral cleavage pattern, where a strong signal at *m/z* 315 ($M^+ - C_4H_5$) was observed, indicative of facile cleavage at C-20/22. HMBC correlations were observed between the methyl singlet of the side chain (H-25) and both acetylenic carbons, while a long-range COSY coupling to the adjacent methylene (H-22) could be identified.

Gelliusterol C (**4**), obtained as a colorless oil, gave a molecular formula of $C_{27}H_{40}O_2$, established from the HREIMS data (*m/z* 396.3032), thus denoting eight degrees of unsaturation. Many of the carbon and proton resonances were almost identical to **2**, implying a similarity in their gross structures. The ^{1}H NMR spectrum of **4** differed from that of **2** by the absence of a three-proton singlet at 1.75 ppm, being replaced with a six-proton doublet centered at 1.14 ppm ($J = 6.9$ Hz). The H-21 methyl doublet displayed HMBC coupling to C-17, 20, and 22. Major fragments at *m/z* 315 ($M^+ - C_6H_9$) and the base peak at *m/z* 81 (C_6H_9) represented C-20/22 cleavage. The base peak of molecular formula C_6H_9 consisted of two double bond equivalents, which could be accounted for by an alkyne bond, on the basis of the magnitude of the chemical shifts observed (87.2 and 77.8 ppm). HMBC coupling (Figure 2) of the six-proton doublet in this C-6 fragment, Me-26, 27 to C-24 (87.2 ppm) and C-25 (20.6 ppm), established their relative positions on the side chain, thus securing the final structure of **4**.

Gelliusterol D (**5**), a more polar analogue, lacked the molecular ion in the mass spectrum, displaying peaks at *m/z* 394.2853 ($M^+ - H_2O$) and 379.2654 ($M^+ - H_2O - CH_3$). The ^{13}C NMR spectrum displayed signals similar to those obtained in **4**, suggesting a similar steroidal backbone consisting of the 3β-alcohol, the 5,6-olefinic bond, and the C-7 ketone. Significant differences between **4** and **5** in the

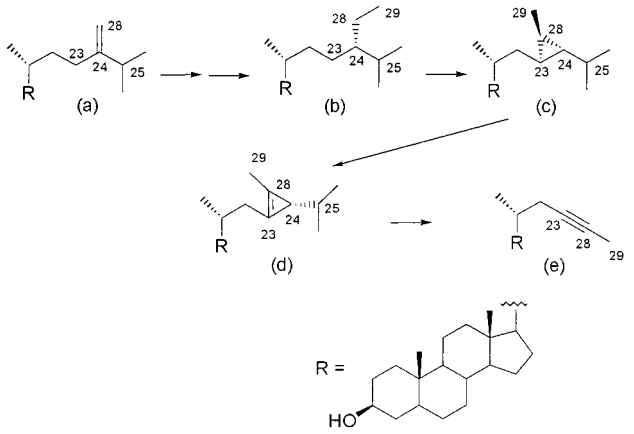


Figure 3. Proposed biogenetic pathway of 23-alkyne steroids from 24-methylenecholesterol (a).

^{1}H and ^{13}C NMR spectra were restricted to the side chain, where the six-proton doublet was replaced by a six-proton singlet which was shifted further downfield to 1.49 ppm. The acetylenic carbons were shifted downfield to 82.2 and 84.0 ppm and the methine signal at 20.6 ppm (C-25) previously observed in **4** was absent in **5**. Extensive HMBC couplings were observed between H-21/C-17, C-20, and C-22 (Figure 2), while HMBC cross-peaks from H-22 to the acetylenic carbons (C-23 and C-24) and C-17 served to confirm its similarity in structure to gelliusterol C (**4**). The outstanding quaternary resonance at 78.0 ppm was HMBC coupled to the six-proton methyl singlet which displayed cross-peaks to the acetylenic carbon, C-24. Due to the magnitude of the quaternary carbon, it was deduced to be attached to an OH functional group, thus defining a tertiary alcohol and hence the assigned structure **5**.

Steroids containing the atypical acetylenic unit as a component of the side chain have been obtained from the sponge *Calyx nacaaensis*, where 26,27-bisnorcholest-5-en-23-yn-3β-ol (**3**) and cholest-5-en-23-yn-3β-ol (**6**) were isolated as minor components.[11]

Biosynthetically, these alkynes have been found to arise from extensive modification of 24-methylenecholesterol, from which dihydrocalysterol was obtained and converted to 24H-isocalysterol (Figure 3). The acetylene could then subsequently be obtained by the resultant cyclopropene undergoing a formal retro-carbene reaction.[12]

Biological evaluation of gelliusterols A (**1**), B (**2**), and C (**4**) was conducted with cancer cell lines P-388, HT-29, A-549, DU-145, and MEL-28. Gelliusterols A (**1**) and B (**2**) exhibited moderate activity with IC_{50} values greater than 1 μg/mL. An activity of 0.5 μg/mL was observed with gelliusterol C (**4**) against HT-29, while the other cell lines gave IC_{50} values greater than 1 μg/mL. The quantity of gelliusterol D (**5**) was insufficient for biological testing.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C spectra were recorded on either a General Electric GN Omega 500 spectrometer or a Varian Unity INOVA 400 MHz instrument. Ultraviolet spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer. Mass spectral data were measured on a VG 70ZAB2SE mass spectrometer. Optical rotations were determined on a Jasco DIP-370 polarimeter. Infrared spectra were obtained on a Perkin-Elmer 1600 FTIR instrument.

Animal Material. The sponge was collected by hand while snorkeling in the shallow subtidal at a depth of about 1.1 m on Boca del Toros Isle, on the Caribbean side of Panama, in December 1999. In life, the sponge forms relatively thick-walled tubes and the texture is tough and brittle. The surface

Table 1. ^{1}H NMR and ^{13}C NMR Data of Gelliusterols A (**1**)[a,c,d] and B (**2**)[b−d]

	1		**2**	
carbon	^{13}C	^{1}H	^{13}C	^{1}H
1	38.1	1.13, m 1.84, m	36.3	1.15, m 1.95, m
2	32.1	1.47, m 1.78, m	31.2	1.60, m 1.93, m
3	72.1	3.47, m	70.5	3.68, m
4	42.9	2.25, m	41.8	2.40, m 2.49, ddd (1.9, 4.8, 14.1)
5	146.7		165.0	
6	125.0	5.53, d (5.0)	126.1	5.69, br s
7	65.9	3.75, br s	202.1	
8	39.0	1.44, m	45.4	2.22, m
9	43.4	1.30, m	49.9	1.52, m
10	38.5		38.3	
11	21.8	1.53, m	21.2	1.58, m
12	40.5	1.15, m 2.00, dt (2.9, 12.7)	38.5	1.15, m 2.02, dt (3.0, 12.9)
13	43.2		43.1	
14	50.7	1.45, m	49.9	1.35, m
15	25.1	1.10,m 1.78, m	26.3	1.24, m 2.41, m
16	29.1	1.26, m 1.85, m	28.4	1.25, m 1.87, m
17	56.3	1.27, m	53.8	1.15, m
18	12.2	0.71, s	12.1	0.69, s
19	18.6	1.00, s	17.3	1.20, s
20	37.0	1.50, m	35.8	1.51, m
21	19.6	1.06, d (6.5)	19.2	1.07, d (6.5)
22	26.7	1.95, m 2.14, dt (2.5, 16.5)	26.0	1.95, m 2.19, m
23	78.2		77.9	
24	77.0		76.4	
25	3.3	1.73, t (2.4)	3.5	1.79, br t (2.2)

[a] NMR spectra were recorded in CD_3OD. [b] NMR spectra were recorded in $CDCl_3$. [c] *J* values given in hertz are recorded in parentheses. [d] Multiplicities were determined by the HMQC and DEPT experiments.

Table 2. ^{1}H and ^{13}C NMR Data of Gelliusterols C (**4**) and D (**5**)[a−c]

	4		**5**	
carbon	^{13}C	^{1}H	^{13}C	^{1}H
1	36.4	1.20, m 1.96, m	36.3	1.20, m 1.94, m
2	31.2	1.63, m 1.93, m	31.1	1.60, m 1.94, m
3	70.5	3.68, m	70.5	3.68, m
4	41.8	2.43, m 2.50, m	41.8	2.40, m 2.51, ddd (1.9, 4.4,13.6)
5	165.0		165.1	
6	126.1	5.69, d (1.52)	126.0	5.70, d (1.63)
7	202.1		202.1	
8	45.4	2.24, m	45.4	2.24, dd (10.8,12.2)
9	49.9	1.49, m	49.8	1.36, m
10	38.3		38.2	
11	21.2	1.56, m	21.2	1.58, m
12	38.6	1.14, m 2.02, dt (3.0, 12.9)	38.5	1.14, m 2.02, m
13	43.1		43.1	
14	49.9	1.36, m	49.9	1.34, m
15	26.3	2.41, m	26.2	2.44, m
16	28.3	1.25, m 1.93, m	28.4	1.88, m
17	53.9	1.20, m	53.9	1.18, m
18	12.1	0.69, s	12.1	0.70, s
19	17.3	1.20, s	17.3	1.20, s
20	35.8	1.58, m	35.5	1.60, m
21	19.1	1.07, d (6.6)	19.2	1.08, d (6.6)
22	25.9	1.95, m 2.20, m	25.8	2.06, d (7.9) 2.30, dd (3.5, 16.6)
23	77.8		84.0	
24	87.2		82.2	
25	20.6	2.50, m	78.0	
26	23.5	1.14, d (6.9)	26.6	1.49, s
27	23.5	1.14, d (6.9)	26.6	1.49, s

[a] NMR spectra were recorded in $CDCl_3$. [b] *J* values given in hertz are recorded in parentheses. [c] Multiplicities were determined by the HMQC experiment.

is relatively smooth and granular to the touch. The color in life was unrecorded but is most likely to be either dark mauve-lilac or deep bluish-green. The interior is rust-colored with depositions of ferric oxide. The spicules are robust oxea packed in a dense irregular reticulation. The sponge is an undescribed species of *Gellius* (order Haplosclerida, family Adociidae). A voucher specimen has been deposited in the Natural History Museum, London (BMNH 1999.7.19.3).

Extraction and Isolation. The frozen sponge was lyophilized prior to exhaustive extraction with CH_2Cl_2/2-propanol (1:1) to yield a brownish-green gum (5.64 g), which was fractionated by vacuum liquid chromatography, eluting with increasing portions of EtOAc in hexane, with final elution with acetone and MeOH. Of the 11 fractions obtained, the fractions eluting with 20%, 40%, 60%, and 80% EtOAc/hexane, fractions 5 (577 mg), 6 (412 mg), 7 (134 mg), and 8 (165 mg), respectively, were subjected to normal-phase HPLC on Si-80-199-C5 with 2-propanol/hexane (1:9). Eight peaks were obtained from HPLC of fraction 5 (2.5 mL/min). Peak 5 was purified on Si-80-199-C5 (25% 2-propanol/hexane, 1.5 mL/min) to afford gelliusterol C (**4**) (3.4 mg), which was further purified on Si Sep Pak. Peak 7 underwent purification (25% 2-propanol/hexane, 2.0 mL/min) to yield gelliusterol D (**5**) (1.1 mg). Fractions 6, 7, and 8 from the initial chromatography were also subjected to normal-phase HPLC on Si-80-199-C5 with 2-propanol/hexane (1:9) (1.2 mL/min), where similar peaks were combined from successive runs. Further purification of peak 10 (16.1 mg) on reversed-phase HPLC (Luna, MeCN/H_2O (9:1), 2.0 mL/min) yielded gelliusterol B (**2**) (4.7 mg). Peaks 11−15 were successively purified and similar peaks combined (Luna, MeCN/H_2O (85:15), 2.0 mL/min). Reversed-phase HPLC (Ultracarb, MeCN/H_2O/MeOH (8:1:1), 2.0 mL/min; MeCN/H_2O (9:1), 1.5 mL/min) served to purify gelliusterol A (**1**) (0.7 mg).

Biological Evaluation. Assays for IC_{50} values (recorded in μg/mL) of selected cancer lines were determined against P-388 (mouse lymphoma, ATCC: CCL 46), HT-29 (human colon carcinoma, ATCC: HTB 38), A-549 (human lung carcinoma, ATCC: CCL 8), DU-145, and MEL-28.

Gelliusterol A (**1**): $[\alpha]_D$ −25.7° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (ϵ) 230 (176); IR (film) ν_{max} 3386 cm^{-1}; ^{1}H NMR and ^{13}C NMR (CD_3OD), see Table 1; LREIMS *m*/*z* (rel int) 370 (11), 352 (68) [$M^+ - H_2O$], 334 (6) [$M^+ - 2H_2O$] 252 (11), 204 (18), 181 (18), 165 (11), 145 (12), 131 (31), 105 (38), 69 (100); HREIMS *m*/*z* 370.2864 (calcd for $C_{25}H_{38}O_2$, Δ +0.7 mmu).

Gelliusterol B (**2**): $[\alpha]_D$ −16.6° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (ϵ) 237 (283); IR (film) ν_{max} 3500, 1660 cm^{-1}; H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; LREIMS *m*/*z* (rel int) 368 (59), 353 (20) [$M^+ - CH_3$], 335 (50) [$M^+ - H_2O - CH_3$], 315 (52) [$M^+ - C_4H_5$], 297 (23) [$M^+ - C_4H_5 - H_2O$], 285 (27), 269 (15), 258 (24), 227 (30), 173 (37), 107 (86), 91(100); HREIMS *m*/*z* 368.2720 (calcd for $C_{25}H_{36}O_2$, Δ −0.5 mmu).

Gelliusterol C (**4**): $[\alpha]_D$ −36.6°(*c* 0.28, MeOH); UV (MeOH) λ_{max} (ϵ) 238 (4767); IR (film) ν_{max} 3416, 1667, 1462 cm^{-1}; ^{1}H NMR and ^{13}C NMR ($CDCl_3$), see Table 2; LREIMS *m*/*z* (rel int) 396 (54), 378 (27), 363 (19), 315 (88), 297 (20), 285 (55), 267 (11), 259 (17), 227 (16), 205 (17), 174 (48), 161 (74), 135 (45), 109 (48), 105 (58), 91 (82), 81 (100), 77 (34), 69 (49); HREIMS *m*/*z* 396.3032 (calcd for $C_{27}H_{40}O_2$, Δ −0.4 mmu).

Gelliusterol D (**5**): $[\alpha]_D$ −6.7° (*c* 0.11, MeOH); UV (MeOH) λ_{max} (ϵ) 236 (23656); IR (film) ν_{max} 3422, 1654, 1458, 1376 cm^{-1}; ^{1}H NMR and ^{13}C NMR ($CDCl_3$), see Table 2; LREIMS *m*/*z* (rel

int) 394 (5), 379 (4), 353 (4), 315 (20), 285 (25), 267 (8), 187 (29), 161 (45), 135 (29), 121 (25), 119 (32), 107 (53), 95 (42), 91 (100), 81 (68), 77 (38), 69 (31); HREIMS *m/z* 394.2853 (calcd for $C_{27}H_{38}O_2$, mmu +1.9) [$M^+ - H_2O$], 379.2654 [$M^+ - H_2O - CH_3$].

Acknowledgment. We acknowledge financial assistance from the Sea Grant College Program, National Science Foundation, Instituto Biomar S.A., PharmaMar, S.A., which supported this research. We also thank Mr. Wesley Yoshida for conducting the NMR experiments and Mr. Mike Burger for mass spectral measurements. The sponge was collected by Dr. Jorge Jimenez.

References and Notes

(1) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839−1895, and references therein.
(2) Aiello, A.; Fattorusso, E.; Menna, M. *Steroids* **1999**, *64*, 687−714, and references therein.
(3) Jurek, J.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod.* **1994**, *57*, 1004−1007.
(4) Capon, R.; Faulkner, D. *J. Org. Chem.* **1985**, *50*, 4771−4773.
(5) Sun, H.; Cross, S.; Gunasekera, M.; Koehn, F. *Tetrahedron* **1991**, *47*, 1185−1190.
(6) Bifulco, G.; Bruno, I.; Minale, L.; Riccio, R.; Calignano, A.; Debitus, C. *J. Nat. Prod.* **1994**, *57*, 1294−1299.
(7) Dembitsky, V.; Gorina, I.; Fedorova, I.; Solovieva, M. *Comp. Biochem. Physiol.* **1989**, *92B*, 733−736.
(8) Xiao, D.; Deng, S.; Wu, H.; Wu, H. *Tianran Chanwu Yanjiu Yu Kaifa* **1999**, *11*, 6−9.
(9) McClintock, J. B. *Mar. Biol.* **1987**, *94*, 479−487.
(10) Notaro, G.; Piccialli, V.; Sica, D. *J. Nat. Prod.* **1992**, *55*, 1588−1594.
(11) Steiner, E.; Djerassi, C.; Fattorusso, D.; Magno, S., Mayol, L.; Santacroce, C.; Sica, D. *Helv. Chim. Acta* **1977**, *60*, 475−481.
(12) Djerassi, C.; Silva, C. J. *Acc. Chem. Res.* **1991**, *24*, 371−378.

NP000585A