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ASTROGORGIADIOL AND ASTROGORGIN, INHIBITORS OF CELL DIVISION IN FERTILIZED STARFISH EGGS, FROM A GORGONIAN ASTROGORGIA SP.[1]

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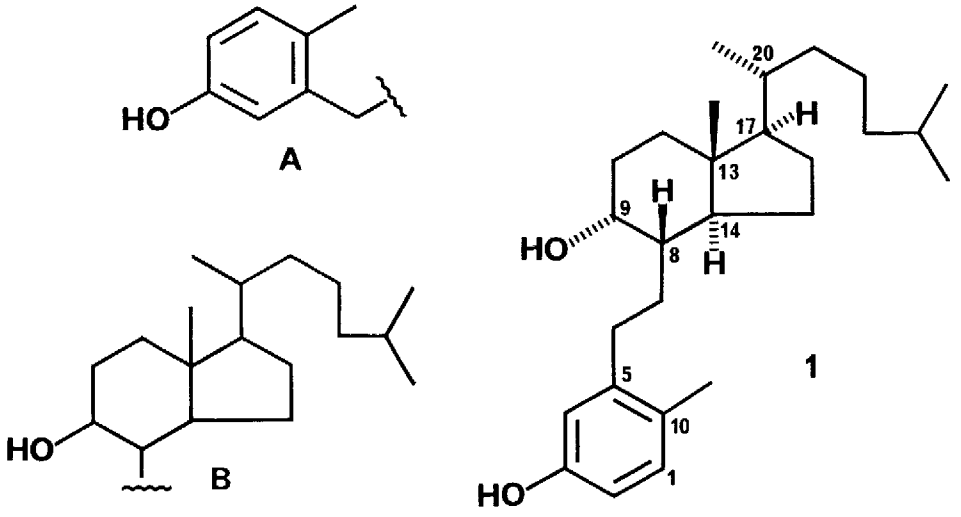
Abstract: A novel secosterol, astrogorgiadiol(**1**), a known diterpene ophirin(**2**) and a closely related diterpene, astrogorgin(**3**), have been isolated from a gorgonian Astrogorgia sp. as inhibitors of cell division in fertilized starfish eggs.

In our continuing search for bioactive metabolites from Japanese invertebrates, we encountered a gorgonian of the genus Astrogorgia during the cruise on the R/V Toyoshio-maru of Hiroshima University in Okino-shima Island off Shikoku (-10 to -20m) whose lipophilic extract showed significant activity in the starfish egg assay. The ethanol extract of this gorgonian afforded three active substances; a novel secosterol named astrogorgiadiol(**1**), a known diterpene ophirin(**2**) and its closely-related diterpene named astrogorgin(**3**). The present paper describes the structure elucidation of the two new metabolites.

The ether soluble portion of the ethanolic extract of frozen colonies (1kg) was fractionated by the Kupchan procedure. The CCl_4 fraction was subjected to flash chromatography on Kieselgel 60F(E.Merck) with CH_2Cl_2/MeOH. The active fractions eluted with CH_2Cl_2 were separated on Sephadex LH-20 with n-hexane/CH_2Cl_2/MeOH(2:1:1) to yield two active materials, of which the more polar material was further purified by HPLC on SI60-5(Yamamura Chem. Res. Co., Ltd.) with hexane/EtOAc(4:1) followed by on CAPCELL PAK C_{18}(Shiseido Co.,Ltd.) with aq MeCN to give astrogorgiadiol(**1**) as a colorless solid(6.7mg).

Astrogorgiadiol(**1**)[2] had a molecular formula of $C_{27}H_{44}O_2$ which was established by EIMS [m/z 400 (M^+)] and ^{13}C NMR data. The partial structure **A** was straightforward from UV[280nm(ε1560)], IR(3320cm^{-1}), ^1H NMR[6.93(1H, d, J=8.1Hz), 6.48(1H, dd, 8.1, 2.6), 6.63(1H, d, 2.6), 2.21(3H, s), 2.69(1H, ddd) and 2.36(1H, ddd)], and ^{13}C NMR spectra[142.9s, 154.9s, 131.3d, 112.9d, 116.1d, 127.4s, 18.5q and 31.3t] as well as from the HMBC[3] spectrum. This was also supported by methylation with CH_2N_2 which afforded a methyl ether(M^+,

m/z 414). In addition to this partial structure, ^{1}H and ^{13}C NMR spectra contained 4 methyls, 8 methylenes, 5 methines, one oxymethine and one quaternary carbon, which indicated the presence of two more carbocyclics. Partial structure **B** was implied by ^{1}H-^{1}H and ^{1}H-^{13}C COSY, HOHAHA[4] and HMBC spectra measured in C_6D_6 (Table 1) as well as by EIMS fragment ions (m/z 269, 247, 135 and 121). Moreover, the position of the remaining hydroxyl group was secured by COSY and HOHAHA spectra measured after addition of $Eu(fod)_3$-d_{27}(Table 1). The gross structure **1** was also confirmed by experiments mentioned above, in which the partial structure **A** could be linked to **B** through a methylene. It is likely that **1** is biosynthesized from cholestanol via dienol-phenol rearrangement and cleavage of the 9,10-bond whose precursors were reported from gorgonians closely related to Astrogorgia.[5] Therefore, the stereochemistry is presumed to be as shown in structure **1**, which was supported by NMR data reported for synthetic 9-hydroxy-9,10-seco-1,3,5(10)-cholestatrienes.[6] Incidentally, a 5,6-secosterol, hipposterol is known from the marine sponge Hippospongia communis.[7]



The nonpolar active material eluted from the LH-20 column was further purified by HPLC on SI60-5 with n-hexane/EtOAc(4:1) followed by CAPCELL PAK C_{18} with aq MeOH to yield ophirin(138mg) and astrogorgin(34.4mg).

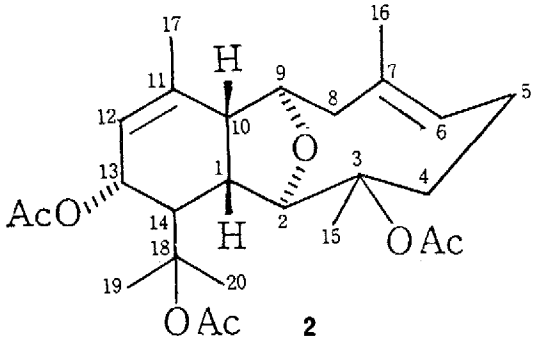
Identification of ophirin(**2**)[8] was readily performed by comparison with literature data.[9] The spectral data of **3**[10] were quite similar to those of ophirin except for the presence of an exomethylene(δ_H 5.25s, 5.11s; δ_C 114.6t, 143.9s) and an additional acetoxyl group[δ_H 2.09(3H,s), 2.00(3H,s) and 1.99(6H,s); δ_C 170.3s, 170.0s, 169.91s and 169.87s]. This was supported

Table 1. NMR Assignments(C_6D_6) and LIS Values($CDCl_3$) for Astrogorgiadiol(1)

C	^{13}C	^{1}H	Δ LIS*	C	^{13}C	^{1}H	Δ LIS*
1	131.3d	6.93d	1.2	15	24.7t	1.00	1.5
2	112.9d	6.48dd	0.6			1.50	1.5
3	154.9s			16	28.1t	1.18	1.2
4	116.1d	6.63d	0.5			1.75	1.1
5	142.9s			17	56.5d	1.10	1.6
6	31.3t	2.36ddd	1.5	18	11.2q	0.60s	1.8
		2.69ddd	3.6	19	18.5q	2.21s	0.5
7	30.9t	1.52m	3.2	20	36.2d	1.38	0.9
		1.64m	5.6	21	18.9q	0.98d	0.5
8	41.2d	1.35	3.1	22	36.6t	1.05	0.4
9	66.9d	3.79s	4.5			1.40	0.4
10	127.4s			23	24.3t	1.25	0
11	30.6t	1.40	4.0			1.40	0
		1.53	a	24	39.9t	1.21	0.2
12	34.5t	1.45	4.6	25	28.4d	1.55	0.1
		1.64	3.6	26	22.7q	0.93	0
13	43.1s			27	23.0q	0.93	0
14	47.8d	1.47	6.0				

* Molar ratio [1/Eu(fod)$_3$-d_{27}] ≒ 1 to 0.5
a Undetactable

by a FABMS spectrum which showed a pseudomolecular ion at m/z 626(M+H+ diethanolamine)$^+$, establishing a molecular formula of $C_{28}H_{40}O_9$. The gross structure **3** was deduced by ^{1}H-^{1}H and ^{1}H-^{13}C COSY and HMBC experiments as well as by comparison of spectral data with those of ophirin. The positions of the exomethylene and the acetoxyl group were verified by HMBC; exomethylene protons were correlated with C-7(δ_C 143.9), C-6(76.3) and C-8(40.4) carbons. The relative stereochemistry was established as **3a** by phase sensitive NOESY.[11]



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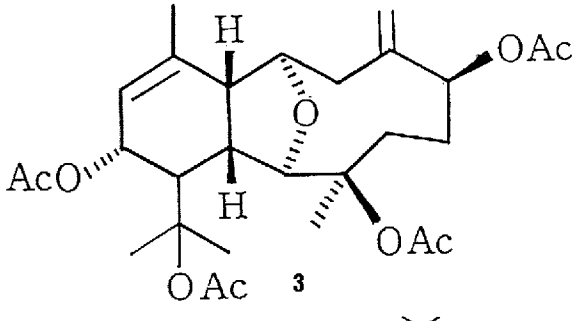
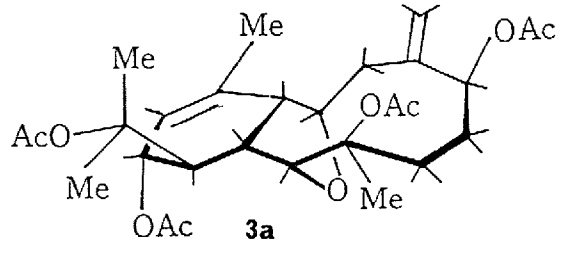


3



3a

Astrogorgidiol, ophirin and astrogorgin inhibited cell division of the fertilized starfish(Asterina pectinifera) eggs at concentrations of 50, 10 and 10 μg/mL, respectively.

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References and **Notes**

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2. **1**, $C_{27}H_{44}O_2$; $[\alpha]_D$ -16.40° (c 0.058, $CHCl_3$); λ_{max}(MeOH) 280nm(ε1560); IR(film), 3320(br), 2950, 2860, 1610, 1580, 1500, 1460, 1380, 1290, 1260, 1150, 1075, 960, 915, 860 and 810cm^{-1}; EIMS, m/z 400(M^+), 382(M^+-H_2O), 367(M^+-H_2O-CH_3), 269, 247, 147, 135, 134(base peak), 122 and 121; ^1H and ^{13}C NMR data in Table 1.
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8. **2**, $C_{26}H_{38}O_7$, $[\alpha]_D$ -120.20° (c 0.186, $CDCl_3$); IR(film), 2940, 1730, 1440, 1370, 1255, 1085, 1020, 950, 930, 880, 850, 810 and 740 cm^{-1}; FABMS(diethanolamine), m/z 568(M+H+diethanolamine)$^+$, 550 and 508; NMR ($CDCl_3$), $^{13}C\delta$ and $^1H\delta$(mult.,J in Hz): [C-1] 35.9d, 2.67(t, 9.2); [C-2] 87.2d, 4.55(d, 10.0); [C-3] 90.1s; [C-4] 30.8t, 2.42[b], 2.06[c]; [C-5] 22.0t, 2.42(m), 2.15(m); [C-6] 129.5d, 5.45(t, 8.7); [C-7] 125.9s[f]; [C-8] 45.1t, 1.98[b], 2.51(dd, 6.1, 13.5); [C-9] 80.3d, 4.38(d, 6.0); [C-10] 48.4d, 2.42[b]; [C-11] 139.6s[f]; [C-12] 120.6d, 5.38(d, 5.6); [C-13] 66.3d, 5.66(d, 5.6); [C-14] 43.2d, 3.11(s); [C-15] 21.3q, 1.80(s); [C-16] 18.3q, 1.83(s); [C-17] 21.8q, 1.79(s); [C-18] 83.7s; [C-19] 25.4q, 1.55(s); [C-20] 25.1q, 1.39(s); [OAc] 170.4s, 169.9s, 169.9s: 22.7q, 2.01(s): 22.5q, 1.99(s)[c]: 21.3q, 1.95(s). (b-c; mutually overlapping. f; the values may be interchanged.)
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10. **3**, $C_{28}H_{40}O_9$; $[\alpha]_D$ -118.70° (c 0.064, $CHCl_3$); IR(film), 3450, 3000, 2950, 1740, 1640, 1440, 1370, 1250, 1130, 1080, 1020, 980, 960, 820 and 765 cm^{-1}; FABMS(diethanolamine), m/z 626(M+H+diethanolamine)$^+$, 594 and 566; NMR ($CDCl_3$), $^{13}C\delta$ and $^1H\delta$(mult.,J in Hz) values: [C-1] 36.3d, 2.94[d]; [C-2] 86.7d, 4.39[e]; [C-3] 85.4s; [C-4] 24.3t[i], 2.42(m)[g], 1.85(m)[h]; [C-5] 24.9t[i], 2.38(m)[g], 1.91(m)[h]; [C-6] 76.3d, 5.33(brs); [C-7] 143.9s; [C-8] 40.4t, 2.46(dd, 4.3, 14.4), 2.28(dd, 2.9, 14.4); [C-9] 81.2d, 4.39[e]; [C-10] 46.1d, 2.66(d, 7.9); [C-11] 139.7s; [C-12] 121.4d, 5.70(d, 5.6); [C-13] 66.6d, 5.41(d, 5.7); [C-14] 44.9d, 2.94[d]; [C-15] 22.8q, 1.70(s); [C-16] 114.6t, 5.11(s), 5.25(s); [C-17] 21.9q, 1.81(s); [C-18] 83.6s; [C-19] 25.5q, 1.58(s); [C-20] 25.4q, 1.38(s); [OAc] 170.3s; 170.0s; 169.9s; 169.9s; 22.5q, 1.99(s); 22.5q, 1.99(s); 21.3q, 2.00(s); 21.1q, 2.09(s). (d-e; mutually overlapping. g-i; the values may be interchanged.)
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