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NEW SECOSTEROIDS FROM AN UNDESCRIBED GORGONIAN OF THE GENUS *MURICELLA*

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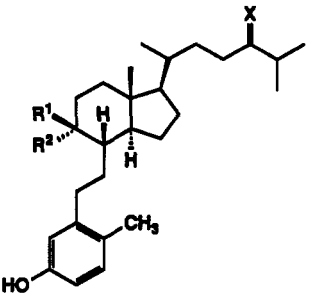
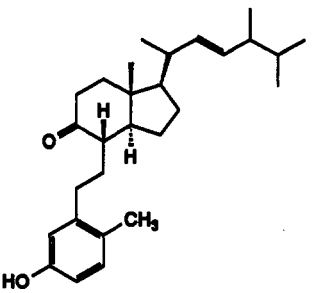
ABSTRACT.—Three new 9,10-secosteroids, calicoferols C-E [**2–4**] have been isolated from an undescribed gorgonian of the genus *Muricella*, and their structures determined by a combination of spectroscopic methods. Calicoferol D [**3**] exhibited potent antiviral activity and brine-shrimp lethality.

Marine octocorals (phylum Cnidaria) of the order Gorgonacea produce a wide variety of both biologically active and structurally unique secondary metabolites (1); terpenoids and steroids are the major groups of metabolites from these animals. Several bioactive gorgonian steroids possess unusual carbon skeletons and functionalities. Thus, astrogorgiadiol, a 9,10-secosteroid isolated from the Japanese gorgonian *Astrogorgia* sp. (2), was reported to inhibit cell division of fertilized starfish eggs. Recently, the structurally related secosteroids calicoferols A and B were isolated from the gorgonian *Calicogorgia* sp. and were found to possess potent brine-shrimp lethality (3).

In our search for bioactive compounds from organisms native to Korean waters, we collected an undescribed gorgonian of the genus *Muricella* offshore of Jaeju Island. Si gel vacuum flash chromatography followed by silica and C_{18} reversed-phase hplc of the CH_2Cl_2 extract yielded several 9,10-secosteroids. Herein we report the isolation and structure elucidation of astrogorgiadiol [**1**] and three new secosteroids, calicoferols C-E [**2–4**].



1 R^1=H, R^2=OH, X=H_2
2 R^1=H, R^2=OH, X=CH_2
4 R^1, R^2=O, X=H_2



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Compound **1** was isolated as a colorless oil that analyzed for $C_{27}H_{44}O_2$ by hreims and ^{13}C-nmr spectral methods. The presence of 27 signals in the ^{13}C-nmr spectrum, combined with the presence of five methyl proton signals in the ^{1}H-nmr spectrum, revealed that compound **1** was a steroid. Six downfield carbon signals in the region of δ 110–160 and the corresponding proton signals indicated the existence of an aromatic ring (Table 1), which was shown by proton decoupling and a ^{1}H-^{1}H COSY experiment to be part of a 3-hydroxy-6-methyl-benzyl moiety. With this information, a literature survey revealed that compound **1** was

TABLE 1. ^{13}C-Nmr Assignments for Astrogorgiadiol [**1**] and Calicoferols C–E [**2–4**].[a]

Position	Compound			
	1	**2**	**3**	**4**
1	130.99 (d)	131.01 (d)	130.99 (d)	130.99 (d)
2	112.44 (d)	112.40 (d)	112.45 (d)	112.47 (d)
3	153.72 (s)	153.61 (s)	153.56 (s)	153.62 (s)
4	115.47 (d)	115.47 (d)	115.65 (d)	115.66 (d)
5	142.61 (s)	141.70 (s)	142.53 (s)	142.53 (s)
6	30.80 (t)	30.85 (t)	31.00 (t)	31.02 (t)
7	30.22 (t)	30.26 (t)	27.62 (t)	27.65 (t)
8	40.87 (d)	40.89 (d)	50.43 (d)	50.43 (d)
9	67.31 (d)	67.17 (d)	213.00 (s)	213.20 (s)
10	127.74 (s)	127.91 (s)	128.07 (s)	128.04 (s)
11	30.02 (t)	30.13 (t)	38.28 (t)	38.29 (t)
12	34.09 (t)	34.12 (t)	38.37 (t)	38.51 (t)
13	42.83 (s)	42.91 (s)	42.69 (s)	42.83 (s)
14	47.74 (d)	47.73 (d)	55.28 (d)	55.23 (d)
15	24.41 (t)	24.43 (t)	25.17 (t)	25.13 (t)
16	27.71 (t)	27.71 (t)	29.64 (t)	29.05 (t)
17	56.13 (d)	55.99 (d)	54.86 (d)	55.07 (d)
18	10.99 (q)	11.01 (q)	11.72 (q)	11.53 (q)
19	18.35 (q)	18.35 (q)	18.37 (q)	18.37 (q)
20	35.74 (d)	35.71 (d)	40.15 (d)	35.64 (d)
21	18.65 (q)	18.66 (q)	20.94 (q)	18.56 (q)
22	36.10 (t)	34.58 (t)	135.22 (d)	35.88 (t)
23	23.75 (t)	30.85 (t)	132.50 (d)	23.79 (t)
24	39.47 (t)	156.81 (s)	43.05 (d)	39.45 (t)
25	27.99 (d)	33.79 (d)	33.18 (d)	28.01 (d)
26	22.80 (q)	21.99 (q)	20.16 (q)	22.81 (q)
27	22.55 (q)	21.85 (q)	19.66 (q)	22.55 (q)
28		105.94 (t)	18.00 (q)	

[a]Measured in $CDCl_3$ at 125 MHz with TMS as internal standard. Signal multiplicity was observed by DEPT experiments. Assignments for **1** and **3** were based on HMQC and HMBC experiments. Assignments for **2** and **4** were aided by comparison with **1** and **3**.

astrogorgiadiol, a 9,10-secosteroid previously isolated from the gorgonian *Astrogorgia* sp. (2). Comparison of the spectral data of **1** showed very good correlation with published data for this compound.

Calicoferol C [**2**] was isolated as an oil whose molecular formula was determined as $C_{28}H_{44}O_2$ by a combination of hreims and ^{13}C-nmr spectrometry. The spectral data for **2** were very similar to those obtained from **1**. The only difference in the ^{13}C-nmr data was the appearance of a new exo-methylene group [δ 156.81 (C) and 105.94 (CH_2)]. In the ^{1}H-nmr spectrum, corresponding proton signals were found at δ 4.71 (1H, br s) and 4.65 (1H, d, J=1.5 Hz). A combination of ^{1}H-^{1}H COSY and HMQC experiments assigned this exomethylene to C-24. Thus, calicoferol C [**2**] was determined as a secosteroid of the astrogorgiadiol class.

Calicoferol D [**3**] was isolated as a white solid. A molecular formula of $C_{28}H_{42}O_2$ was deduced by a combination of hreims and ^{13}C-nmr spectrometry. Spectral data for **3** were similar to those obtained for **1**. Differences in the ^{13}C-nmr spectrum were a replacement of the C-9 hydroxyl group of **1** by a carbonyl group and the appearance of a new double bond and a methyl group. Corresponding differences were found in the ^{1}H-nmr spectrum, in which signals of an addi-

tional double bond and a methyl group appeared at δ 5.22 (1H, dd, J=15.1 and 8.0 Hz), 5.15 (1H, dd, J=15.1 and 8.3 Hz), and 0.92 (3H, d, J=6.8 Hz), respectively. In addition, a strong absorption band at 1700 cm^{-1} in the ir spectrum revealed that the carbonyl group was an isolated keto group. A ^1H-^1H COSY nmr experiment assigned the positions of the ketone, double bond, and new methyl group to the C-9, C-22, and C-24 carbons, respectively. Therefore, **3** must be the C-24 methyl derivative of calicoferol A, a substance recently isolated from the gorgonian *Calicogorgia* sp. (3). This interpretation was supported by a combination of COSY, TOCSY, HMQC, and HMBC nmr expeirments (Experimental). Thus, calicoferol D [**3**] was determined to be a 9,10-secosteroid of the astrogorgiadiol class.

Calicoferol D [**3**] possessed several asymmetric carbon centers. NOeds nmr experiments revealed that the configurations of these centers were identical to those of astrogorgiadiol. However, the stereochemistry of the additional asymmetric center at C-24 was not determined. Comparison of the ^1H- and ^{13}C-nmr data for **3** with spectra of compounds possessing the C-24*R* and C-24*S* configurations failed to give sufficient information on the stereochemistry of this center. It is well known that the configuration of the C-24 asymmetric center can be determined from the chemical shift of the C-21 methyl protons (4,5). However, this method is reliable only when compounds of both configurations are isolated.

Finally, calicoferol E [**4**] was isolated as a white solid which analyzed for $C_{27}H_{42}O_2$ by a combination of hreims and ^{13}C-nmr spectral methods. Spectral data for **4** were very similar to those of **3**. The main difference in their nmr spectra was due to the absence of the C-22 double bond in **4** which was confirmed by combined nmr experiments. Therefore, **4** was assigned as the 9-oxo derivative of astrogorgiadiol [**1**].

Astrogorgiadiol [**1**] and calicoferols A and B have been reported to possess potent bioactivity. In particular, calicoferols A and B have exhibited potent lethality to brine-shrimp larvae (LD_{50} 1.8 and 2.3 ppm, respectively) (3). In our studies, calicoferol D [**3**] exhibited significant activity against *Herpes simplex* viruses I and II (EC_{50} 1.2 μg/ml for both strains) and polio virus (EC_{50} 0.4 μg/ml). In addition, calicoferol D [**3**] was moderately toxic (LD_{50} 37.0 ppm) against brine-shrimp larvae. In contrast to the results with structurally similar compounds, however, **1** and **2–4** were not toxic (LD_{50}>100 ppm).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded in $CDCl_3$ solution on a Varian Unity-500 spectrometer. Proton and carbon nmr spectra were measured at 500 and 125 MHz, respectively. All chemical shifts were recorded with respect to internal Me_4Si. Ir spectra were recorded on a Mattson Galaxy spectrophotometer. Uv spectra were obtained in MeOH using a Milton-Roy spectrophotometer. Mass measurements were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. The optical rotations were measured on a Jasco digital polarimeter with a 5-cm microcell. Mps were measured on a Fisher-Jones apparatus and are reported uncorrected. All solvents used were spectral grade or were distilled from glass prior to use.

ANIMAL MATERIAL.—The animals were collected by hand using scuba at 20–25 meters depth in July 1992, along the shore of Jaeju Island, Korea. The collected samples were dried briefly in the shade and kept in a freezer. This gorgonian, *Muricella* sp. (family Paramuriceidae), is closely related to *M. perramosa* and *M. nitida* in general morphological features: horny axis with central chord, fan-shaped colonies, coenenchyme having two layers, truncated calyces with sclerites forming eight marginal points, anthocodial armature in chevron spicules resting on transverse collarets, deep reddish purple in color except for the yellowish tentacular operculum. However, these specimens differ in their characters as follows: colonies richly ramified at angles 70–90° at 5–25 mm intervals, twigs bent upwards, mostly the lateral arrangements of calyces on stem and branches, somewhat flattened, 4×3–7×5 mm, especially the spindles with complex tubercles, up to 0.44×0.19-mm long in calyces and coenenchymes.

The voucher specimens under the code name 92J-18 are on deposit in the octocorallian collection, Natural History Museum, Ewha Womans University, Seoul, Korea under the curatorship of J.-I.S.

EXTRACTION AND ISOLATION.—The animals (2.1 kg) were defrosted and repeatedly extracted with CH_2Cl_2. The crude extracts (7.7 g) were separated by silica vacuum flash chromatography using sequential mixtures of *n*-hexane and EtOAc. *Fractions eluted with non-polar solvents* (10–20% EtOAc in hexane) were combined and separated by semi-prep. silica hplc (7% EtOAc in hexane) followed by C_{18} reversed-phase hplc (100% CH_3CN) to yield compounds **1**–**4** in the order of **1**, **2**, **4**, and **3**: 0.14, 0.04, 0.11, and 0.12% of crude extract, respectively.

Astrogorgiadiol [**1**].—Colorless oil (10.9 mg, 0.14% of crude extract); $[\alpha]^{25}D$ −6.8° (*c*=1.0, $CHCl_3$); uv (MeOH) λ max (log ε) 218 (3.85), 281 (3.36) nm; ir (KBr) ν max 3400 (OH), 2950–2860 (CH, aliphatic), 1610, 1590, 1500, 1370, 1230, 1160, 980, 810 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 6.98 (1H, d, *J*=7.8 Hz, H-1), 6.65 (1H, d, *J*=2.9 Hz, H-4), 6.57 (1H, dd, *J*=7.8 and 2.9 Hz, H-2), 4.05 (1H, m, H-9), 2.69 (1H, ddd, *J*=13.7, 11.2, and 5.4 Hz, H-6), 2.42 (1H, ddd, *J*=13.7, 10.7, and 5.4 Hz, H-6), 2.22 (3H, s, Me-19), 1.81 (1H, m, H-16), 1.77 (1H, m, H-12), 1.75 (2H, m, H-11), 1.60 (1H, m, H-15), 1.55 (2H, m, H-7), 1.53 (1H, m, H-8), 1.51 (2H, m, H-14, H-25), 1.48 (1H, m, H-12), 1.36 (1H, m, H-20), 1.35 (1H, m, H-23), 1.34 (1H, m, H-22), 1.30 (2H, m, H-24), 1.23 (1H, m, H-16), 1.19 (1H, m, H-17), 1.15 (1H, m, H-23), 1.12 (1H, m, H-15), 0.99 (1H, m, H-22), 0.92 (3H, d, *J*=6.8 Hz, Me-21), 0.87 (3H, d, *J*=6.8 Hz, Me-26), 0.86 (3H, d, *J*=6.8 Hz, Me-27), 0.69 (3H, s, Me-18); hreims (50 eV) *m/z* $[M]^+$ 400.3345 (14) ($C_{27}H_{44}O_2$ requires 400.3341), 382 (6), 269 (3), 247 (5), 147 (5), 134 (100), 121 (24), 84 (11).

Calicoferol C [**2**].—Colorless oil (3.4 mg, 0.04% of crude extract); $[\alpha]^{25}D$ −9.5° (*c*=0.3, $CHCl_3$); uv (MeOH) λ max (log ε) 219 (3.89), 281 (3.40) nm; ir (KBr) ν max 3400, 2950, 2870, 1610, 1590, 1500, 1460, 1380, 1290, 1250, 1160, 890, 810 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 6.98 (1H, d, *J*=7.8 Hz, H-1), 6.65 (1H, d, *J*=2.4 Hz, H-4), 6.57 (1H, dd, *J*=7.8 and 2.4 Hz, H-2), 4.71 (1H, br s, H-28), 4.65 (1H, d, *J*=1.5 Hz, H-28), 4.04 (1H, m, H-9), 2.71 (1H, ddd, *J*=13.8, 11.2, and 5.4 Hz, H-6), 2.42 (1H, ddd, *J*=13.8, 13.8, and 5.1 Hz, H-6), 2.22 (1H, m, H-25), 2.22 (3H, s, Me-19), 2.10 (1H, ddd, *J*=15.9, 11.5, and 4.6 Hz, H-23), 1.88 (1H, m, H-23), 1.85 (1H, m, H-16), 1.75 (2H, m, H-11), 1.75 (1H, m, H-12), 1.60–1.50 (7H, m, H-7, H-7, H-8, H-12, H-14, H-15, H-22), 1.43 (1H, m, H-20), 1.30 (1H, m, H-17), 1.26 (1H, m, H-16), 1.16 (1H, m, H-22), 1.11 (1H, m, H-15), 1.03 (3H, d, *J*=6.8 Hz, Me-26), 1.02 (3H, d, *J*=6.8 Hz, Me-27), 0.96 (3H, d, *J*=6.8 Hz, Me-21), 0.70 (3H, s, Me-18); hreims (50 eV) *m/z* 412.3362 (21) ($C_{28}H_{44}O_2$ requires 412.3341), 394 (17), 310 (18), 274 (5), 174 (13), 155 (15), 134 (100), 122 (20), 88 (14).

Calicoferol D [**3**].—White crystals (hexane/Me_2CO, 7.9 mg, 0.11% of crude extract): mp 76–79°; $[\alpha]^{25}D$ +13.5° (*c*=0.4, $CHCl_3$); uv (MeOH) λ max (log ε) 219 (3.84), 281 (3.34) nm; ir (KBr) ν max 3400 (OH), 2960–2870 (CH, aliphatic), 1700 (C=O, ketone), 1610, 1590, 1500, 1370, 1300, 1230, 1160, 980, 810 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 6.98 (1H, d, *J*=7.8 Hz, H-1), 6.66 (1H, d, *J*=2.9 Hz, H-4), 6.57 (1H, dd, *J*=7.8 and 2.9 Hz, H-2), 5.22 (1H, dd, *J*=15.1 and 8.0 Hz, H-23), 5.15 (1H, dd, *J*=15.1 and 8.3 Hz, H-22), 2.65 (1H, ddd, *J*=13.0, 12.2, and 4.9 Hz, H-6), 2.50 (1H, ddd, *J*=14.2, 14.2, and 6.8 Hz, H-11), 2.42 (1H, ddd, *J*=13.0, 11.5, and 5.4 Hz, H-6), 2.37 (1H, m, H-8), 2.32 (1H, ddd, *J*=14.2, 5.1, and 2.0 Hz, H-11), 2.25 (3H, s, Me-19), 2.14 (1H, ddd, *J*=13.2, 6.8, and 2.0 Hz, H-12), 2.08 (1H, m, H-20), 1.84 (2H, m, H-16, H-24), 1.75 (1H, m, H-7), 1.69 (2H, m, H-14, H-15), 1.59 (2H, m, H-7, H-12), 1.47 (1H, m, H-25), 1.42 (1H, m, H-16), 1.28 (1H, m, H-15), 1.26 (1H, m, H-17), 1.02 (3H, d, *J*=6.8 Hz, Me-21), 1.00 (3H, s, Me-18), 0.92 (3H, d, *J*=6.8 Hz, Me-28), 0.84 (3H, d, *J*=6.6 Hz, Me-26), 0.82 (3H, d, *J*=6.6 Hz, Me-27); HMBC correlations: H-1–C-3, C-5, C-19; H-2–C-3, C-4, C-6; H-4–C-2, C-3, C-6, C-10; H-6–C-4, C-5, C-7, C-10; H-8–C-9, C-14; H-11–C-8, C-9, C-12, C-13; H-12–C-9, C-11, C-13, C-14; H-18–C-12, C-13, C-17; H-19–C-1, C-5, C-10; H-20–C-17; H-21–C-17, C-20, C-22; H-23–C-20, C-22, C-24, C-28; H-25–C-23, C-24, C-26, C-27, C-28; H-26–C-24, C-25, C-27; H-27–C-24, C-25, C-26; H-28–C-23, C-24, C-25; hreims (50 eV) *m/z* 410.3183 (30) ($C_{28}H_{42}O_2$ requires 410.3185), 276 (65), 261 (13), 205 (10), 151 (51), 134 (100), 121 (40), 109 (16), 93 (30), 83 (19).

Calicoferol E [**4**].—White crystals (hexane/Me_2CO, 9.4 mg, 0.12% of crude extract): mp 94–95°; $[\alpha]^{25}D$ +21.4° (*c*=0.6, $CHCl_3$); uv (MeOH) λ max (log ε) 218 (3.87), 282 (3.40) nm; ir (KBr) ν max 3400 (OH), 2950–2870 (CH, aliphatic), 1700 (C=O, ketone), 1610, 1590, 1500, 1460, 1230, 1160, 820 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 6.97 (1H, d, *J*=8.3 Hz, H-1), 6.66 (1H, d, *J*=2.9 Hz, H-4), 6.57 (1H, dd, *J*=8.3 and 2.9 Hz, H-2), 2.66 (1H, ddd, *J*=13.2, 11.7, and 4.9 Hz, H-6), 2.50 (1H, ddd, *J*=14.7, 13.7, and 6.8 Hz, H-11), 2.41 (1H, ddd, *J*=13.2, 11.7, and 5.4 Hz, H-6), 2.36 (1H, m, H-8), 2.31 (1H, ddd, *J*=14.2, 5.4, and 2.0 Hz, H-11), 2.25 (3H, s, Me-19), 2.17 (1H, ddd, *J*=12.9, 6.8, and 2.3 Hz, H-12), 1.98 (1H, m, H-16), 1.75 (1H, m, H-7), 1.69 (2H, m, H-14, H-15), 1.59 (2H, m, H-7, H-12),

1.54 (1H, m, H-25), 1.43 (2H, m, H-16, H-20), 1.34 (2H, m, H-22, H-23), 1.29 (1H, m, H-15), 1.21 (1H, m, H-17), 1.16 (1H, m, H-23), 1.13 (2H, m, H-24, H-24), 1.01 (1H, m, H-22), 0.98 (3H, s, Me-18), 0.93 (3H, d, J=6.8 Hz, Me-21), 0.88 (3H, d, J=6.8 Hz, Me-26), 0.87 (3H, d, J=6.8 Hz, Me-27); hreims (50 eV) *m/z* 398.3188 (40) ($C_{27}H_{42}O_2$ requires 398.3185), 264 (37), 249 (14), 193 (29), 180 (12), 151 (100), 134 (94), 121 (50), 109 (27).

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