# New Secosteroids from a Gorgonian of the Genus *Muricella*

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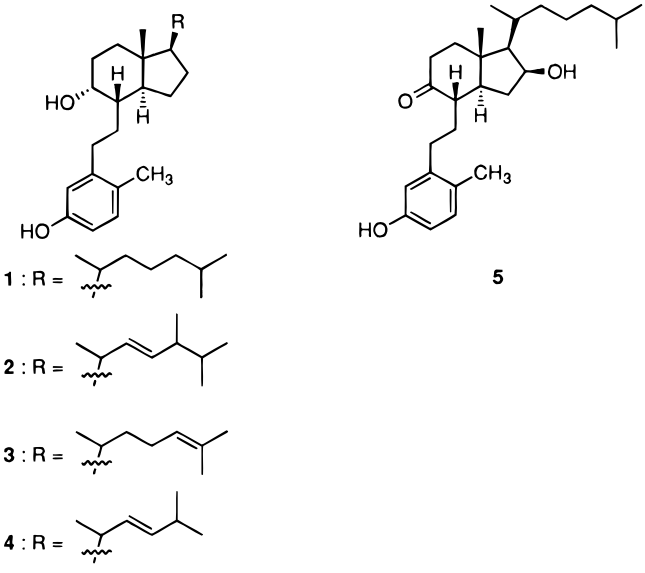
Calicoferols F−I (**2**−**5**), four new 9,10-secosteroids, have been isolated from a gorgonian of the genus *Muricella* collected from Jaeju Island, Korea. The structures of these compounds have been determined by combined spectroscopic methods. Calicoferols exhibited significant cytotoxicity and inhibitory activity against $PLA_2$.

Recently we reported the structures of calicoferols C−E, three new 9,10-secosteroids from an unidentified gorgonian of the genus *Muricella* (family Paramuriceidae, order Gorgonacea) collected from Jaeju Island, South Sea, Korea.[1] These compounds exhibited moderate antiviral activity and potent toxicity against brine-shrimp larvae. In our continuing search for bioactive substances from organisms of Korean waters, we encountered another specimen of the same animal whose crude extract exhibited significant inhibitory activity against $PLA_2$. Silica vacuum flash chromatography of the crude extract followed by silica and reversed-phase HPLC of bioactive fractions yielded several steroids along with the previously described secosteroids. Herein we report the structures and bioactivity of calicoferols F−I (**2**−**5**), four novel 9,10-secosteroids, structurally related to astrogorgiadiol (**1**), the major metabolite from the specimens.[1−3] These metabolites exhibited significant cytotoxicity against a human leukemia cell-line and moderate inhibitory activity against $PLA_2$.







**5**

Calicoferol F (**2**) was isolated as a colorless oil that analyzed for $C_{28}H_{44}O_2$ by combined HRCIMS and $^{13}C$ NMR analysis. The spectral data for this compound were very similar to those obtained for astrogorgiadiol. The only significant differences in the $^{13}C$ NMR data were the replacement of two upfield carbon signals of **1** by those of olefinic methines and the appearance of a new methyl carbon signal; $^{13}C$ $\delta$ 135.8(d), 131.9 (d), and 18.0 (q) (Table 1). The corresponding changes were also observed in the $^1H$ NMR spectrum in which signals of a new double bond and a methyl group appeared at $\delta$ 5.19 (1H, dd, $J$ = 15.6, 6.4 Hz), 5.14 (1H, dd, $J$ = 15.6, 6.6 Hz), and 0.91 (3H, d, $J$ = 6.8 Hz), respectively. Careful examination of the NMR data revealed that the structural difference occurred at the side chain of the molecule. A combination of $^1H$ COSY, TOCSY, HMQC, and HMBC experiments placed the double bond and methyl group at C-22 and C-24, respectively. Thus, the structure of calicoferol F was defined as the 22,23-didehydro-24-methyl derivative of astrogorgiadiol.

Compound **2** possessed an additional asymmetric carbon center at C-24. Comparison of the NMR data for **2** with those of compounds possessing the 24*R* and 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 of ergosterol-type steroids can be assigned on the basis of the $^1H$ NMR chemical shifts of the C-21 methyl protons.[4−6] Unless it is synthesized from a known precursor, however, this method is reliable only when compounds of both configurations are obtained.

The molecular formula of calicoferol G (**3**) was deduced as $C_{27}H_{42}O_2$ by HREIMS and $^{13}C$ NMR methods. The spectral data for this compound were highly compatible with those observed for **1** and **2**. The most noticeable change in the NMR data was the appearance of a trisubstituted double bond; $^{13}C$ $\delta$ 131.0 (s), 125.1 (d); $^1H$ $\delta$ 5.09 (1H, br t, $J$ = 6.8 Hz). In addition, the $^1H$ NMR spectrum showed that the signals of the C-26 and C-27 methyl groups were shifted downfield to $\delta$ 1.68 (3H, br s) and 1.60 (3H, br s), respectively. These changes could be accommodated by dehydrogenation of C-24 and C-25 of **1**, and this was confirmed by a combination of $^1H$ COSY, TOCSY, HMQC, and HMBC experiments. Thus, calicoferol G was defined as the 24,25-didehydro derivative of astrogorgiadiol.

Another related compound, calicoferol H (**4**), was isolated as a colorless oil. A molecular formula of $C_{26}H_{40}O_2$ was deduced by HRMS data. The spectral data of **4** were very similar to those derived from other secosteroids; however, only 26 carbon signals were observed in the $^{13}C$ NMR spectrum. A combination of $^1H$ COSY and HMQC experiments readily assigned the position of a double bond to C-22. The signal of the C-23 olefinic proton at $\delta$ 5.27 (1 H, dd, $J$ = 15.2, 6.6 Hz) was coupled with that of a proton at $\delta$ 2.19 (1 H, m), which, in turn, was coupled with a signal corresponding to two methyls at $\delta$ 0.94 (6 H, d, $J$ = 6.8

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**Table 1.** Carbon NMR Assignments for Compounds **2**−**5**[a]

| carbon no. | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 1 | 131.0 (d) | 131.0 (d) | 131.0 (d) | 131.0 (d) |
| 2 | 112.4 (d) | 112.4 (d) | 112.4 (d) | 112.5 (d) |
| 3 | 153.6 (s) | 153.5 (s) | 153.5 (s) | 153.5 (s) |
| 4 | 115.5 (d) | 115.4 (d) | 115.4 (d) | 115.6 (d) |
| 5 | 142.7 (s) | 142.7 (s) | 142.7 (s) | 142.4 (s) |
| 6 | 30.9 (t) | 30.9 (t) | 30.9 (t) | 31.1 (t) |
| 7 | 30.2 (t) | 30.3 (t) | 30.3 (t) | 27.7 (t) |
| 8 | 40.9 (d) | 40.9 (d) | 40.9 (d) | 50.0 (d) |
| 9 | 67.3 (d) | 67.2 (d) | 67.2 (d) | 212.4 (s) |
| 10 | 127.9 (s) | 127.9 (s) | 127.9 (s) | 128.1 (s) |
| 11 | 29.7 (t) | 30.2 (t) | 30.2 (t) | 38.0 (t) |
| 12 | 34.1 (t) | 34.1 (t) | 34.1 (t) | 38.8 (t) |
| 13 | 42.8 (s) | 42.9 (s) | 42.8 (s) | 42.7 (s) |
| 14 | 47.0 (d) | 47.8 (d) | 47.8 (d) | 52.7 (d) |
| 15 | 24.5 (t) | 24.5 (t) | 24.4 (t) | 37.0 (t) |
| 16 | 28.3 (t) | 27.7 (t) | 27.9 (t) | 72.8 (d) |
| 17 | 56.0 (d) | 56.1 (d) | 56.0 (d) | 60.5 (d) |
| 18 | 11.3 (q) | 11.1 (q) | 11.3 (q) | 12.7 (q) |
| 19 | 18.4 (q) | 18.4 (q) | 18.4 (q) | 18.0 (q) |
| 20 | 40.3 (d) | 35.6 (d) | 39.9 (d) | 29.6 (d) |
| 21 | 21.0 (q) | 18.6 (q) | 20.8 (q) | 18.4 (q) |
| 22 | 135.8 (d) | 36.1 (t) | 133.4 (d) | 36.1 (t) |
| 23 | 131.9 (d) | 24.7 (t) | 134.9 (d) | 24.2 (t) |
| 24 | 43.0 (d) | 125.1 (d) | 31.0 (d) | 39.5 (t) |
| 25 | 33.2 (d) | 131.0 (s) | 22.8 (q) | 28.1 (d) |
| 26 | 20.2 (q) | 25.8 (q) | 22.8 (q) | 22.8 (q) |
| 27 | 19.7 (q) | 17.7 (q) | | 22.6 (q) |
| 28 | 18.0 (q) | | | |

[a] Measured in $CDCl_3$ at 125 MHz. Signal multiplicity was observed in DEPT experiments. Assignments for **2** and **5** were based on HMQC and HMBC experiments. Assignments for **3** and **4** were aided by comparison with **2** and **5**.

Hz). This spin-correlation was also confirmed by a TOCSY experiment in which a correlation containing the signals of the olefinic and methyl protons was clearly observed. Therefore, compound **4** was defined as a 9,10-secosteroid of the 24-norcholestane class. Steroids possessing this type of truncated side chain have been isolated from a few sponges, the gorgonian *Acabaria undulata*, and the scallop *Placopecten megellanicus* as minor constituents, but the biogenesis remains to be investigated.[7−9]

The molecular formula for calicoferol I (**5**) was established as $C_{27}H_{42}O_3$ by HRMS and $^{13}C$ NMR spectrometry. In contrast to other secosteroids, the $^{13}C$ NMR data for this compound showed the carbonyl carbon signal at $\delta$ 212.4 (s) and an absorption band at 1700 $cm^{-1}$ in the IR spectrum, indicative of a ketone. A combination of $^1H$ COSY, TOCSY, HMQC, and HMBC experiments determined the location of this functional group at C-9, while the hydroxyl-bearing carbon at $\delta$ 72.8 (d) in the $^{13}C$ NMR spectrum was assigned to C-16. Thus, the structure of **5** was defined as the 9-dehydroxy-9-oxo-15-hydroxy derivative of **1** that made this compound structurally comparable to calicoferol B, a 9,10-secosteroid from the Japanese gorgonian *Calicogorgia* sp.[3]

The relative stereochemistry of **5** was determined by combined proton-coupling and NOESY experiments. A large coupling ($J \sim 13.6$ Hz) between the H-8 and H-14 protons assigned a trans diaxial orientation for these protons. This interpretation was supported by NOESY correlations of H-18 with H-8 and H-15$\beta$, and that of H-14 with H-15$\alpha$. Similarly, the $\beta$-orientation of the C-16 hydroxyl group was determined by NOESY correlations of H-16 with H-15$\alpha$ and H-17.

Gorgonian-derived 9,10-secosteroids exhibit diverse bioactivities, including antiviral activity, brine-shrimp lethality, and inhibition of cell division of fertilized starfish eggs.[1−3] In our measurement, compounds **1**−**5** exhibited significant cytotoxicity against the human leukemia cell-line K-562 with $LC_{50}$ values of 12.1, 3.2, 2.1, 10.7, and 9.6 $\mu$g/mL, respectively. In addition, compounds **1**, **3**, and **4** exhibited moderate inhibitory activity against $PLA_2$ showing 47, 55, and 67% inhibition at 50 $\mu$g/mL, respectively.

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded in $CDCl_3$ solutions on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the 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. MS were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. The optical rotations were measured on a JASCO digital polarimeter using a 5-cm cell. All solvents used were spectral grade or were distilled from glass prior to use.

**Collection, Extraction, and Isolation.** Samples of the gorgonian *Muricella* sp. (sample no. 96J-41) were collected by hand at a depth of 25−30 m in January 1996, off the coast of Jaeju Island, Korea. Morphological characters of the specimens were identical with those described previously.[1] The collected specimens were immediately frozen by dry ice and stored at −25 °C. The defrosted specimens (3.3 kg) were briefly dried under shade and repeatedly extracted with $CH_2Cl_2$ (6 L × 3). The crude extracts (11.1 g) were subjected to silica vacuum flash chromatography by using sequential mixtures of *n*-hexane and EtOAc as eluents. Fractions eluted with moderately polar solvents (20−25% EtOAc−hexane) were combined and separated by semipreparative silica HPLC (YMC silica column, 15% EtOAc in hexane) to yield compounds **1**−**5** in the order of **1**, **2**, **5**, **3**, and **4**. Final purifications were made by $C_{18}$ reversed-phase HPLC (100% $CH_3CN$) to afford pure compounds: 47.8, 5.8, 2.1, 2.6, and 4.9 mg for **1**−**5**, respectively.

**Calicoferol F** (**2**): colorless oil; $[\alpha]^{25}_D$ +13.7° (*c* 0.1, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 219 (3.81), 279 (3.38) nm; IR (KBr) $\nu_{max}$ 3400, 2960, 2920, 2860, 1610, 1500, 1460, 1370, 1270, 1020 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.98 (1H, d, $J$ = 8.1 Hz, H-1), 6.65 (1H, d, $J$ = 2.8 Hz, H-4), 6.57 (1H, dd, $J$ = 8.1, 2.8 Hz, H-2), 5.19 (1H, dd, $J$ = 15.6, 6.4 Hz, H-23), 5.14 (1H, dd, $J$ = 15.6, 6.6 Hz, H-22), 4.04 (1H, br s, H-9), 2.70 (1H, ddd, $J$ = 13.2, 11.2, 5.4 Hz, H-6), 2.42 (1H, ddd, $J$ = 12.7, 11.2, 5.4 Hz, H-6), 2.22 (3H, s, H-19), 2.01 (1H, m, H-20), 1.82 (1H, m, H-24), 1.75 (3H, m, H-11(×2), H-12), 1.68 (1H, m, H-16), 1.60 (1H, m, H-15), 1.51 (3H, m, H-7(×2), H-14), 1.50 (1H, m, H-12), 1.48 (1H, m, H-8), 1.46 (1H, m, H-25), 1.25 (1H, m, H-17), 1.22 (1H, m, H-16), 1.08 (1H, m, H-15), 1.01 (3H, d, $J$ = 6.8 Hz, H-21), 0.91 (3H, d, $J$ = 6.8 Hz, H-28), 0.83 (3H, d, $J$ = 8.0 Hz, H-26), 0.82 (3H, d, $J$ = 8.0 Hz, H-27), 0.70 (3H, s, H-18); HMBC correlations H-1/C-3, C-5, C-19; H-2/C-3, C-4, C-10; H-4/C-2, C-3, C-6, C-10; H-6/C-4, C-5, C-7, C-10; H-18/C-12, C-13, C-17; H-19/C-1, C-5, C-10; H-21/C-17, C-20, C-22; H-22/C-21; H-23/C-20, C-22, C-24, C-28; H-26(27)/C-24, C-25; H-28/C-23, C-24, C-25; LRCIMS *m*/*z* (rel int) 413 (28), 397 (6), 281 (5), 269 (7), 163 (9), 147 (22), 134 (100), 97 (13), 55 (43); HRCIMS $[M + H]^+$ *m*/*z* 413.3423 (calcd for $C_{28}H_{45}O_2$ 413.3419).

**Calicoferol G** (**3**): colorless oil; $[\alpha]^{25}_D$ −7.5° (*c* 0.1, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 218 (3.85), 281 (3.38) nm; IR (KBr) $\nu_{max}$ 3400, 2920, 2860, 1610, 1460, 1360, 1270, 1160, 895 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.98 (1H, d, $J$ = 7.8 Hz, H-1), 6.65 (1H, d, $J$ = 2.5 Hz, H-4), 6.57 (1H, dd, $J$ = 7.8, 2.5 Hz, H-2), 5.09 (1H, br t, $J$ = 6.8 Hz, H-24), 4.04 (1H, br s, H-9), 2.71 (1H, ddd, $J$ = 13.2, 11.2, 5.4 Hz, H-6), 2.42 (1H, ddd, $J$ = 13.2, 11.5, 5.1 Hz, H-6), 2.22 (3H, s, H-19), 2.01 (1H, m, H-23), 1.83 (2H, m, H-16, H-23), 1.77 (1H, m, H-12), 1.75 (2H, m, H-11), 1.68 (3H, br s, H-26), 1.60 (3H, br s, H-27), 1.58 (1H, m, H-15), 1.52 [3H, m, H-7 (×2), H-14), 1.50 (1H, m, H-8), 1.49 (1H, m, H-12), 1.40 (2H, m, H-20, H-22), 1.23 (1H, m, H-16), 1.21 (1H, m, H-17), 1.10 (1H, m, H-15), 1.06 (1H, m, H-22), 0.94 (3H, d, $J$ = 6.8 Hz, H-21), 0.69 (3H, s, H-18); LREIMS *m*/*z* (rel int) 398 (14),

365 (5), 269 (8), 246 (6), 147 (24), 134 (100), 121 (52), 93 (13); HREIMS $[M]^+$ *m/z* 398.3199 (calcd for $C_{27}H_{42}O_2$ 398.3185).

**Calicoferol H** (**4**): colorless oil; $[\alpha]^{25}_D$ −6.6° (*c* 0.1, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 219 (3.84), 281 (3.32) nm; IR (KBr) $\nu_{max}$ 3360, 2955, 2880, 1610, 1590, 1500, 1370, 1260, 1160, 970 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.98 (1H, d, $J$ = 8.3 Hz, H-1), 6.65 (1H, d, $J$ = 2.5 Hz, H-4), 6.57 (1H, dd, $J$ = 8.3, 2.5 Hz, H-2), 5.27 (1H, dd, $J$ = 15.6, 6.6 Hz, H-23), 5.16 (1H, dd, $J$ = 15.6, 8.3 Hz, H-22), 4.04 (1H, br s, H-9), 2.71 (1H, ddd, $J$ = 13.2, 11.5, 5.1 Hz, H-6), 2.42 (1H, ddd, $J$ = 13.2, 10.7, 5.4 Hz, H-6), 2.22 (3H, s, H-19), 2.19 (1H, m, H-24), 2.00 (1H, m, H-20), 1.77 (1H, m, H-12), 1.75 (2H, m, H-11), 1.65 (1H, m, H-16), 1.56 (1H, m, H-15), 1.53 (1H, m, H-8), 1.52 (3H, m, H-7 (×2), H-14), 1.50 (1H, m, H-12), 1.23 (1H, m, H-16), 1.20 (1H, m, H-17), 1.09 (1H, m, H-15), 1.00 (3H, d, $J$ = 6.8 Hz, H-21), 0.94 (6H, d, $J$ = 6.8 Hz, H-25, H-26), 0.70 (3H, s, H-18); LREIMS *m/z* (rel int) 384 (40), 366 (5), 349 (4), 269 (7), 229 (4), 147 (14), 134 (100), 121 (59), 97 (26); HREIMS $[M]^+$ *m/z* 384.3023 (calcd for $C_{26}H_{40}O_2$ 384.3028).

**Calicoferol I** (**5**): colorless oil; $[\alpha]^{25}_D$ +18.4° (*c* 0.1, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 218 (3.82), 283 (3.32) nm; IR (KBr) $\nu_{max}$ 3400, 2950, 2920, 2860, 1700, 1610, 1470, 1380, 1260, 1035 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.99 (1H, d, $J$ = 8.1 Hz, H-1), 6.66 (1H, d, $J$ = 2.8 Hz, H-4), 6.58 (1H, dd, $J$ = 8.1, 2.8 Hz, H-2), 4.45 (1H, ddd, $J$ = 7.8, 6.8, 4.2 Hz, H-16), 2.66 (1H, ddd, $J$ = 13.2, 13.2, 4.9 Hz, H-6), 2.51 (1H, ddd, $J$ = 13.7, 13.7, 6.8 Hz, H-11ax), 2.45 (1H, m, H-8), 2.43 (1H, ddd, $J$ = 13.2, 13.2, 5.7 Hz, H-6), 2.35 (1H, br dd, $J$ = 13.2, 7.8 Hz, H-15eq), 2.32 (1H, ddd, $J$ = 13.7, 5.6, 2.1 Hz, H-11eq), 2.25 (3H, s, H-19), 2.17 (1H, ddd, $J$ = 13.0, 6.8, 2.1 Hz, H-12), 1.93 (1H, m, H-20), 1.77 (1H, m, H-7), 1.56 (1H, m, H-7), 1.54 (1H, m, H-25), 1.53 (1H, ddd, $J$ = 13.7, 13.0, 5.6 Hz, H-12), 1.51 (1H, m, H-14), 1.49 (1H, m, H-22), 1.44 (1H, m, H-23), 1.42 (1H, ddd, $J$ = 13.2, 13.2, 4.2 Hz, H-15ax), 1.25 (1H, m, H-23), 1.18 (3H, s, H-18), 1.15 (2H, m, H-24), 1.11 (1H, m, H-22), 1.10 (1H, dd, $J$ = 10.8, 6.8 Hz, H-17), 1.00 (3H, d, $J$ = 6.8 Hz, H-21), 0.88 (3H, d, $J$ = 6.8 Hz, H-26), 0.87 (3H, d, $J$ = 6.8 Hz, H-27); HMBC correlations H-1/C-3, C-5, C-19; H-2/C-3, C-4, C-10; H-4/C-2, C-3, C-6, C-10; H-6/C-4, C-5, C-7, C-10; H-8/C-9; H-11/C-8, C-9, C-12, C-13; H-12/C-9, C-11, C-13; H-15/C-14, C-16; H-16/C-15, C-17; H-18/C-12, C-13, C-17; H-19/C-1, C-5, C-10; H-21/C-17, C-20; H-26(27)/C-24, C-25; NOESY correlations H-1/H-19, H-6/H-8, H-8/H-18, H-11$\beta$/H-18, H-12$\beta$/H-21, H-15$\alpha$/H-16, H-15$\beta$/H-18, H-16/H-17, H-18/H-20; LREIMS *m/z* (rel int) 414 (21), 280 (23), 265 (45), 221 (30), 208 (9), 134 (76), 109 (10), 69 (25), 43 (100); HREIMS $[M]^+$ *m/z* 414.3135 (calcd for $C_{27}H_{42}O_3$ 414.3134).

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