# Synthesis of Uprolide D−G Analogues. Revision of Structure of the Marine Cembranolides Uprolide F Diacetate and Uprolide G Acetate

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Among the cembranolides found in marine organisms, in particular gorgonian species of the genus *Eunicea*,[1,2] uprolide D (**1**), uprolide D acetate (**2**), uprolide E acetate (**3**), uprolide F diacetate (**4**), and uprolide G acetate (**5**) are structurally uniquely characterized by the presence of the rare 4,7-oxa-bridged functionality (Figure 1).[3] These cytotoxic natural products have been isolated at the ±0.001% level from the $CHCl_3$ extract of the gorgonian octocoral *Eunicea mammosa*, and it is quite likely that they are actually produced from eupalmerin acetate (**6**).[4]

Although details of the biological effects of uprolides D−G are not available, these compounds appear to have promising antitumor activity.[5] We became interested in a synthetic approach toward uprolides D−G (**1**−**5**) because the synthesis of these metabolites as well as analogues for SAR studies represents the only realistic supply of natural product necessary for further biological evaluation. We recently completed an efficient diastereoselective synthesis of uprolide D−G analogues **15** and **16** from eupalmerin acetate (**6**), and we were surprised to find that, while the $^1H$ and $^{13}C$ NMR data of furanether **15** were similar with those of uprolides **1**−**3**, the NMR data of pyranether **16** were in almost perfect congruity with those of uprolides **4** and **5**. We suspected than an error in the constitutional assignment of the latter natural products was the cause for this contradiction. We report here revised structures for uprolide F diacetate and uprolide G acetate (structures **17** and **18**, respectively), which were unequivocally established by the synthesis of analogues **15** and **16**. The complete structural assignment of these synthetic analogues was accomplished on the basis of comprehensive 2D NMR experiments (see Tables 1 and 2, Supporting Information). Furthermore, the molecular structure of cembranolide analogue **16** was confirmed by single-crystal X-ray crystallography. Since our starting material **6** is optically pure without ambiguity in the absolute configuration, we assumed that the absolute configuration of the analogues obtained is as that depicted.[6,7]

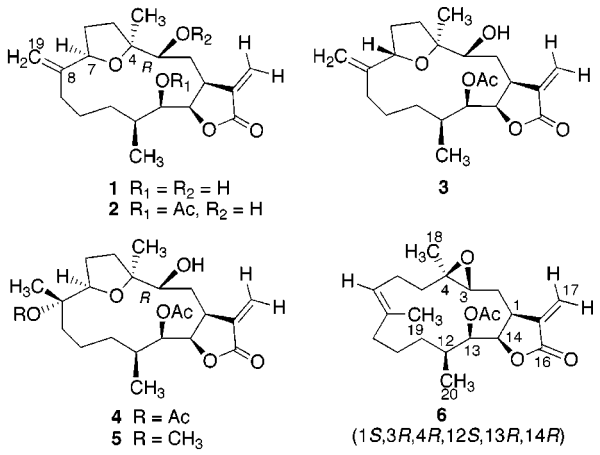


**Figure 1.** Originally reported structures for uprolide D (**1**), uprolide D acetate (**2**), uprolide E acetate (**3**), uprolide F diacetate (**4**), and uprolide G acetate (**5**); the structure of eupalmerin acetate (**6**) showing its absolute stereochemical assignments.

## Results and Discussion

A key feature of our approach was the use of cembranediol **7** as a precursor to the oxolane ring in uprolides **1**−**5**. At the outset, we envisioned that exposure to a protic acid in aqueous media would transform eupalmerin acetate (**6**) into the desired precursor. Indeed, exposure of **6** to PTSA·$H_2O$ in THF containing water at reflux temperature for 5 h gave a 13:2:1:3:2:1 mixture of products **7**−**12**, respectively, in 93% overall yield (Scheme 1). In this way, the desired 3,4-diol **7** was obtained in an isolated yield of 53% based on **6**. Thus, acidolytic cleavage of the epoxide favors the formation of kinetic **7**, which upon prolonged reaction times is slowly transformed to allylic alcohols **8** and **9**.[8] Evidently, diol **7** is the precursor of pyranether **10** upon regioselective protonation of the olefin function at C-7 followed by intramolecular attack of the C-4 hydroxyl, from the $\beta$ face, on a carbocation at C-8. With a view toward obtaining interesting analogues for future biological evaluation, we welcome the unexpected isolation of cembranolide analogues **8**−**10**. From a mechanistic point of view, the finding of products **11** and **12** was a most intriguing result.[9] The stereochemistry of crystalline THF adduct **11** was confirmed by X-ray crystallographic analysis.[10]

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(1) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55 and previous reports in this series.

(2) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618 and references therein.

(3) Rodríguez, A. D.; Soto, J. J.; Piña, I. C. *J. Nat. Prod.* **1995**, *58*, 1209−1216.

(4) (a) Ealick, S. E.; Van der Helm, D.; Weinheimer, A. J. *Acta Crystallogr.* **1975**, *B31*, 1618−1626. (b) Van der Helm, D.; Ealick, S. E.; Weinheimer, A. J. *Cryst. Struct. Commun.* **1974**, *3*, 167−171. (c) Fontán, L. A.; Yoshida, W. Y.; Rodríguez, A. D. *J. Org. Chem.* **1990**, *55*, 4956−4960.

(5) Rodríguez, A. D.; Piña, I. C.; Barnes, C. L. *J. Org. Chem.* **1995**, *60*, 8096−8100.

(6) For details pertaining to the extraction and isolation of eupalmerin acetate (**6**) from *E. mammosa*, see: Rodríguez, A. D.; Piña, I. C.; Soto, J. J.; Rojas, D. R.; Barnes, C. L. *Can. J. Chem.* **1995**, *73*, 643−654.

(7) In ref 4c the absolute configuration at C-3 of eupalmerin acetate (**6**) is erroneously described as 3*S*.

(8) Cembranediol **7** can be obtained in significantly higher yield (75−80%) upon using shortened reaction times (2−3 h) and THF that has been freshly distilled from sodium wire and sodium benzophenone ketyl.

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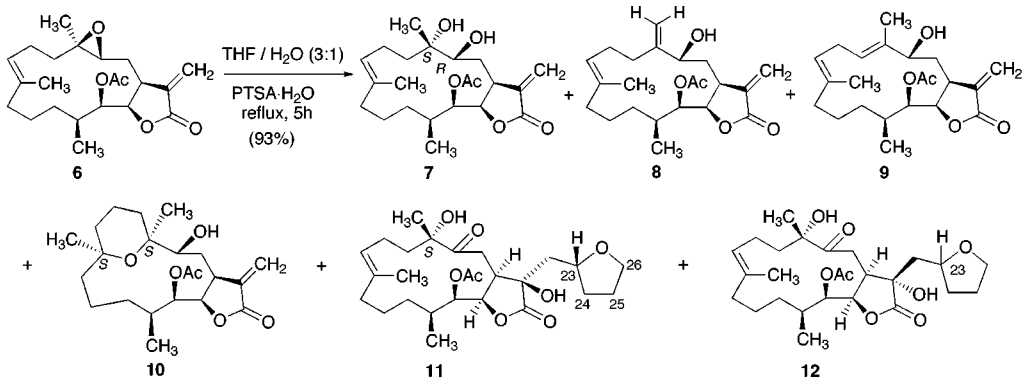
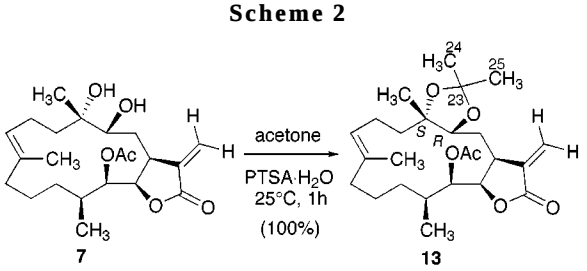
**Scheme 1**




**Scheme 2**




Cembranediol diol **7** was successfully converted to the lactone acetonide **13** in quantitative yield thus allowing structure **7** to be assigned as having the C-3(*R*),4(*S*) configuration (Scheme 2).[11] To our considerable surprise, the peracid oxidation of **7** in benzene using *m*-CPBA at 25 °C for 2 h gave only one isomer of epoxide **14** in 49% isolated yield along with a 1.3:1 mixture of cyclized ethers **15** and **16** in a combined 28% yield.[12] Subsequent treatment of **14** with PTSA·$H_2O$ in benzene at 25 °C for 1.5 h opened the epoxide ring in a regio- and stereoselective manner to give the desired ethers **15** and **16** in 50% and 38% yield, respectively (Scheme 3). The X-ray analysis of analogue **16** reveals that the product has the C-7(*R*),8(*S*) configuration, which means that the stereochemistry of the epoxide C-7 atom was inverted in the reaction. Except for the chemical shifts of C-4, C-5, and C-18, the $^{13}C$ NMR spectral data of synthetic *cis* 2,6-disubstituted pyranether **16** were remarkably similar to those of natural products uprolide F diacetate (**4**) and uprolide G acetate (**5**) (see Table 3, Supporting Information). Comparison of the overall spectral data of **16** with those reported for **4** and **5** indicated that, while these compounds have identical molecular constitution, the natural products must have the opposite C-4(*R*) configuration in order to explain the only significant variations in the NMR data of **16**.[13] On the basis of our synthetic

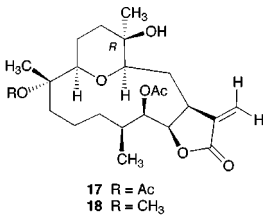
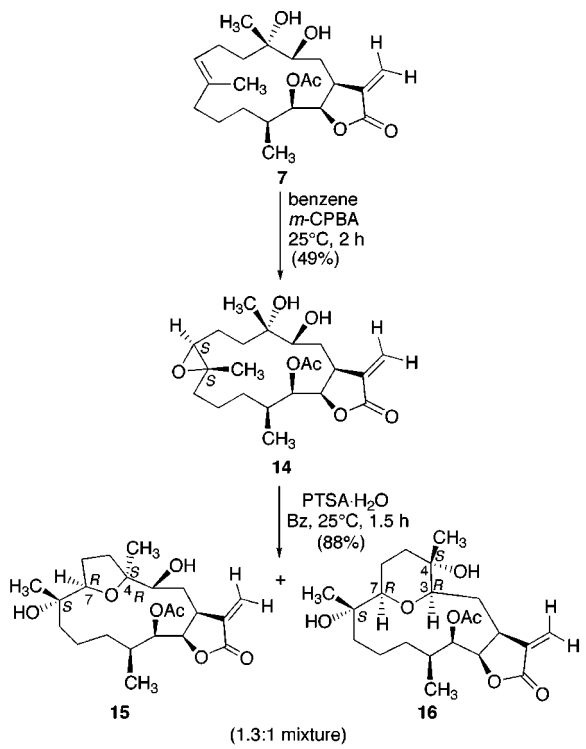



**Figure 2.** Revised structures for uprolide F diacetate (**17**) and uprolide G acetate (**18**).

**Scheme 3**




interconversions and reinterpretation of spectral and chemical data, we conclude that the structures reported for uprolide F diacetate (**4**) and uprolide G acetate (**5**) should be corrected to those of pyranethers **17** and **18**, respectively (Figure 2). Furthermore, the spectral data of synthetic furanether **15** correlated remarkably well with those reported for naturally occurring 4,7-

(9) A combination of factors could be responsible for the formation of THF adducts **11** and **12** from diol **7**. The use of undistilled THF could account for the presence of THF-hydroperoxide in the reaction media, which after thermal degradation could lead to epoxidation of the C-15,17 double bond from each face of the molecule. Ring opening of the epoxides upon attack at C-17 by a THF free-radical species leaves a hydroxyl group at C-15 in the α and *β* configuration. Chemoselective oxidation of a secondary alcohol by a free-radical mechanism could account for the genesis of the ketone functionality at C-3 furnishing lactones **11** and **12**.

(10) We could not confidently ascertain the configuration at C-23 of compound **12** from the spectroscopic data available.

(11) Marshall, J. A.; Andrews, R. C.; Lebioda, L. *J. Org. Chem.* **1987**, *52*, 2378−2388.

(12) The configuration at C-7 and C-8 of compound **14** is given as 7*S*,8*S* on the basis of the X-ray structure of compound **16** and the ring opening mechanistic speculation.

(13) The NOESY spectrum of uprolide G acetate showed that the C-3 proton signal was within NOE distance of the Me-18 protons. Moreover, the absence of NOE between H-3 and Me-18 in **16** was consistent with an absolute stereochemistry at C-4 opposite to that of **17** and **18**.

oxabridged cembranolides **1**−**2**, thus validating their proposed molecular structures.[14]

In summary, we were able to extend our original approach[5] toward the synthesis of 4,7-oxa-bridged cembranolides with some tactical modifications toward the synthesis of a structurally unique 3,7-oxa-bridged cembranolide analogue, **16**, in overall yields of 20−25% based on eupalmerin acetate (**6**). A careful comparison of $^1$H and $^{13}$C NMR resonances for both synthetic uprolide analogues with the data available for the natural products revealed that the pyranether isomer **16** provided an excellent spectroscopic match only to metabolites **4** and **5**. Accordingly, we conclude that the structures of uprolide F diacetate and uprolide G acetate have to be revised as shown for structures **17** and **18**, respectively. Despite the small differences in the $^{13}$C NMR spectra between synthetic analogue **15** and natural products **1** and **2**, the NMR analysis provides overwhelming support for their proposed structural assignments.[15]

## Experimental Section

**General Experimental Procedures.** Infrared spectra were determined as thin films and were referenced to polystyrene. $^1$H NMR, $^{13}$C NMR, DEPT, HMQC, HMBC, $^1$H−$^1$H COSY, $^1$H−$^{13}$C COSY (CSCMBB), RCT COSY, and 2D−NOESY spectra were recorded at 300 MHz for $^1$H and 75 MHz for $^{13}$C. Column chromatography was carried out with silica gel (35−75 mesh). Reactions were monitored by TLC on silica gel plates (0.25 mm) and visualized using UV light and $I_2$ vapors. THF, benzene, acetone, *m*-CPBA, and PTSA·$H_2O$ were obtained from commercial suppliers and were used as provided. Yields refer to chromatographically and spectroscopically pure materials.

**Reaction of Eupalmerin Acetate (6) with *p*-Toluenesulfonic Acid Hydrate.** A solution of **6** (1.02 g, 2.71 mmol) in 50 mL of a mixture of undistilled THF/$H_2O$ (3:1) was treated with 276 mg (1.45 mmol) of PTSA·$H_2O$, and the mixture was refluxed for 5 h. The solution was cooled, concentrated, quenched with saturated $NaHCO_3$ (50 mL), and extracted with chloroform (3 × 40 mL). The combined organic extracts were washed with saturated NaCl, dried, and concentrated to afford a colorless oil. The oil was flash chromatographed on a 50 cm × 2.5 cm silica gel column (40 g) eluted with 20% ethyl acetate in hexane to furnish 563 mg (53%) of **7**, 106 mg (10%) of **8**, 60 mg (6%) of **9**, 131 mg (12%) of **10**, 111 mg (9%) of THF adduct **11**, and 248 mg of a complex mixture. The latter was subjected to successive column chromatography [$SiO_2$ (10 g) with 15% acetone in hexane followed by $SiO_2$ (5 g) using 4% 2-propanol in chloroform] to give 43 mg (3%) of C-15 epimer **12**.

**Data for 7:** colorless oil; $[\alpha]^{25}_D$ +13.8° (*c* 4.4, $CHCl_3$); IR (neat) 3483, 1770, 1749 cm$^{-1}$; UV ($CH_3OH$) $\lambda_{max}$ 210 nm; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.42 (m, 1H, H-1), 3.44 (br d, 1H, $J$ = 10.5 Hz, H-3), 5.74 (br t, 1H, H-7), 5.04 (d, 1H, $J$ = 10.2 Hz, H-13), 4.49 (dd, 1H, $J$ = 5.7, 10.2 Hz, H-14), 5.68 (br s, 1H, H-17), 6.24 (br s, 1H, H-17′), 1.04 (s, 3H, Me-18), 1.53 (s, 3H, Me-19), 0.87 (d, 3H, $J$ = 6.6 Hz, Me-20), 2.07 (s, 3H, OCOC$H_3$), 3.09 (br s, exchangeable, −O$H$); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 38.9 (d, C-1), 28.2 (t, C-2), 74.0 (d, C-3), 74.9 (s, C-4), 39.5 (t, C-5), 22.0 (t, C-6), 129.4 (d, C-7), 134.4 (s, C-8), 36.3 (t, C-9), 20.7 (t, C-10), 31.0 (t, C-11), 28.9 (d, C-12), 75.5 (d, C-13), 79.9 (d, C-14), 138.7 (s, C-15), 171.2 (s, C-16), 123.8 (t, C-17), 28.9 (q, C-18), 14.6 (q, C-19), 11.5 (q, C-20), 170.4 (s, C-21), 21.5 (q, C-22); HRFAB-MS *m*/*z* [M + Na]$^+$ calcd for $C_{22}H_{34}O_6Na$ 417.2253, found 417.2243.

**Synthesis of Lactone Acetonide 13.** A mixture of diol **7** (81 mg, 0.20 mmol) and *p*-toluenesulfonic acid hydrate (two crystals) in acetone (25 mL) was stirred at 25 °C for 1 h and concentrated to leave a residue that was flash chromatographed on silica gel (15% 2-propanol in hexane), giving 89 mg (100%) of lactone acetonide **13**.

**Epoxidation of Diol 7 with *m*-Chloroperbenzoic Acid.** A mixture of diol **7** (403 mg, 1.02 mmol) and *m*-CPBA (230 mg, 1.33 mmol) was stirred in benzene (50 mL) at room temperature for 2 h, diluted with saturated $NaHCO_3$, and extracted with benzene (3 × 30 mL). The combined organic layers were dried and concentrated, leaving a residue that on chromatography ($SiO_2$, 40% ethyl acetate in hexane) gave 207 mg (49%) of epoxide **14** as a colorless oil and 134 mg of an oily mixture of cyclized products **15** and **16**. The latter oil was chromatographed on silica gel (3 g, 5% 2-propanol in chloroform) to provide 66 mg (16%) of furanether **15** and 49 mg (12%) of pyranether **16**.

**Data for 14:** colorless oil; $[\alpha]^{25}_D$ +9.7° (*c* 1.96, $CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.45 (m, 1H, H-1), 3.54 (br d, 1H, $J$ = 9.6 Hz, H-3), 3.33 (t, 1H, $J$ = 6.9 Hz, H-7), 5.28 (dd, 1H, $J$ = 3.3, 7.8 Hz, H-13), 4.68 (br t, 1H, $J$ = 6.6 Hz, H-14), 5.60 (d, 1H, $J$ = 1.5 Hz, H-17), 6.25 (d, 1H, $J$ = 1.8 Hz, H-17′), 1.15 (s, 3H, Me-18), 1.26 (s, 3H, Me-19), 0.90 (d, 3H, $J$ = 6.9 Hz, Me-20), 2.06 (s, 3H, OCOC$H_3$), 3.23 (br s, 2H, exchangeable, −O$H$); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 38.9 (d, C-1), 28.3 (t, C-2), 74.7 (d, C-3), 74.0 (s, C-4), 36.1 (t, C-5), 22.8 (t, C-6), 63.9 (d, C-7), 61.8 (s, C-8), 34.1 (t, C-9), 20.5 (t, C-10), 31.7 (t, C-11), 31.2 (d, C-12), 71.5 (d, C-13), 79.3 (d, C-14), 138.7 (s, C-15), 170.7 (s, C-16), 121.4 (t, C-17), 28.0 (q, C-18), 16.6 (q, C-19), 13.9 (q, C-20), 169.6 (s, C-21), 20.9 (q, C-22); HREI-MS *m*/*z* [M − $H_2O$]$^+$ calcd for $C_{22}H_{32}O_6$ 392.2199, found 392.2236.

**Data for 15:** colorless oil; $[\alpha]^{25}_D$ +119.6° (*c* 2.3, $CHCl_3$); IR (neat) 3482, 1768, 1744, 1665 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ 212 nm ($\epsilon$ 8200); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.43 (m, 1H, H-1), 3.34 (dd, 1H, $J$ = 3.1, 11.2 Hz, H-3), 3.94 (br t, 1H, $J$ = 7.1 Hz, H-7), 5.37 (d, 1H, $J$ = 10.5 Hz, H-13), 4.55 (dd, 1H, $J$ = 5.7, 10.5 Hz, H-14), 5.77 (br s, 1H, H-17), 6.28 (br s, 1H, H-17′), 1.38 (s, 3H, Me-18), 1.18 (s, 3H, Me-19), 0.96 (d, 3H, $J$ = 6.8 Hz, Me-20), 2.11 (s, 3H, OCOC$H_3$); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 37.6 (d, C-1), 28.0 (t, C-2), 72.2 (d, C-3), 84.5 (s, C-4), 35.2 (t, C-5), 29.0 (t, C-6), 87.1 (d, C-7), 74.0 (s, C-8), 36.5 (t, C-9), 21.7 (t, C-10), 33.4 (t, C-11), 29.3 (d, C-12), 70.1 (d, C-13), 78.9 (d, C-14), 138.1 (s, C-15), 169.6 (s, C-16), 123.5 (t, C-17), 25.7 (q, C-18), 23.4 (q, C-19), 15.5 (q, C-20), 170.9 (s, C-21), 20.9 (q, C-22); HREI-MS *m*/*z* [M − $CH_3CO_2H$]$^+$ calcd for $C_{20}H_{30}O_5$ 350.2093, found 350.2089, 350 (2), 332 (3), 314 (2), 164 (10), 128 (40), 85 (100).

**Data for 16:** white crystalline solid; $[\alpha]^{25}_D$ +145.5° (*c* 2.0, $CHCl_3$); IR (neat) 3429, 1769, 1747, 1668, 1464 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ 210 nm ($\epsilon$ 6900); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.16 (m, 1H, H-1), 3.28 (m, 1H, H-3), 3.28 (m, 1H, H-7), 5.38 (br d, 1H, $J$ = 10.8 Hz, H-13), 5.48 (br d, 1H, $J$ = 8.1 Hz, H-14), 5.34 (d, 1H, $J$ = 3.3 Hz, H-17), 6.10 (d, 1H, $J$ = 3.9 Hz, H-17′), 1.37 (s, 3H, Me-18), 1.15 (s, 3H, Me-19), 0.78 (d, 3H, $J$ = 6.9 Hz, Me-20), 1.87 (s, 3H, OCOC$H_3$); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 42.2 (d, C-1), 25.6 (t, C-2), 85.7 (d, C-3), 70.0 (s, C-4), 40.5 (t, C-5), 25.5 (t, C-6), 88.0 (d, C-7), 75.6 (s, C-8), 37.0 (t, C-9), 18.3 (t, C-10), 28.8 (t, C-11), 33.2 (d, C-12), 72.8 (d, C-13), 79.5 (d, C-14), 139.3 (s, C-15), 170.9 (s, C-16), 116.5 (t, C-17), 21.1 (q, C-18), 29.4 (q, C-19), 15.0 (q, C-20), 170.2 (s, C-21), 20.9 (q, C-22); HREI-MS *m*/*z* [M]$^+$ calcd for $C_{22}H_{34}O_7$ 410.2304, found 410.2309, 410 (5), 392 (4), 350 (12), 332 (11), 314 (4), 164 (36), 111 (100). The structural assignment to **16** was corroborated by X-ray crystallographic analysis.

**Reaction of Epoxycembranediol 14 with *p*-Toluenesulfonic Acid Hydrate.** After a solution of **14** (207 mg, 0.50 mmol) in 20 mL of benzene was treated with 5 mg of PTSA·$H_2O$ and stirred at room temperature for 1.5 h, the mixture was concentrated to afford a semisolid mass. The residue was layered on top of a 25 cm × 2.5 cm silica gel (15 g) column eluted with 40% ethyl acetate in hexane to furnish 104 mg (50%) of furanether **15** and 79 mg (38%) of pyranether **16**.

**Supporting Information Available:** Copies of $^1$H and $^{13}$C NMR spectra for analogues **7**−**11**, **15**, and **16**; X-ray characterization data for cembranolide analogues **11** and **16**, including tables of experimental details, selected bond lengths and bond angles, spectral data for compounds **8**−**13**, complete 1D and 2D NMR correlation data for compounds **15** (Table 1) and **16** (Table 2), and comparison of the overall $^{13}$C NMR spectral data of compounds **1**−**5** and **15** and **16** (Table 3). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) We conclude, therefore, that the uprolides could arise biosynthetically from **6** upon hydroxylation of the $\Delta^7$ olefin followed by intramolecular attack of a C-7 hydroxyl on the epoxide at C-3 and C-4.

(15) The small variations in the NMR spectra of **15** are explained by the absence at C-8 of a terminal methylene.