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UPROLIDES D–G, 2. A RARE FAMILY OF 4,7-OXA-BRIDGED CEMBRANOLIDES FROM THE CARIBBEAN GORGONIAN *EUNICEA MAMMOSA*

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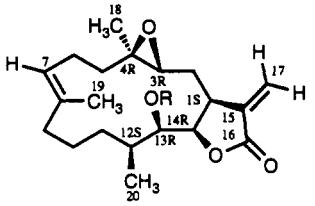
ABSTRACT.—Five new cytotoxic cembranolides possessing a rare 4,7-oxa-bridged functionality were isolated from the gorgonian *Eunicea mammosa*. The structures of **5**, **7–9**, and **10** were deduced from spectroscopic data and by chemical correlation experiments.

Gorgonians of the common Caribbean genus *Eunicea* were among the first marine invertebrates to be investigated chemically, with more than 15 species documented (1). Throughout the last three decades these animals have been shown to be a rich source of several different skeletal classes of terpenes (2). Cembrane diterpenoids, as a single class, represent the largest percentage of natural products isolated from *Eunicea* gorgonians (3,4). *Eunicea mammosa* (Lamouroux) (phylum Cnidaria), one particularly chemically productive and taxonomically complex species, has long been the subject of intensive investigations (5–11). As part of a long-term study of the secondary metabolite content of *E. mammosa*, we have examined exhaustively a number of specimens collected at different locations around Puerto Rico. From these specimens we have obtained new substances that were shown to be highly functionalized diterpenoids of the cembranolide class (12–15). In all cases, these substances accounted for the antibiotic and cytotoxic activity of the gorgonian extracts. Recently, we have described the isolation of 13 new lactones isolated in trace amounts from the hexane and $CHCl_3$ extracts of the gorgonian that could be regarded as closely related structural congeners of eupalmerin acetate [**1**], the most abundant cembranolide isolated from *E. mammosa* (16). These minor metabolites, which have been named collectively as the uprolides (e.g., **3** and **4**),[1] could in fact originate from **1** by oxidation of the Δ^7 unsaturation followed by successive hydrolysis of the epoxide and dehydration of the ensuing cembrane diol. We have now examined in more detail the diterpene cembranolide mixture occurring in the $CHCl_3$ extract of this gorgonian at the $\pm 0.001\%$ level, and found it to consist primarily of five new components possessing moderate cytotoxic activity. We found that the new compounds, named uprolides D-G, are characterized by the presence of an unusual 4,7-oxa-bridged functionality. This structural feature suggests a close structural relationship between the new compounds presented here and several of the previously described cembranolides belonging to the uprolide series (e.g., **3** and **4**) (16). We now report the results of this investigation.

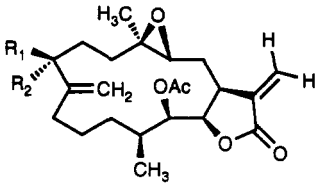
RESULTS AND DISCUSSION

Fresh specimens of *E. mammosa* from Puerto Rico were extracted as described previously (16). The main fractions obtained from the $CHCl_3$ extract (2.4% dry wt) consisted of several well known γ-cembranolides such as eupalmerin acetate [**1**] (13) and eupalmerin [**2**] (14), and several γ-lactonic cembranolide representatives of the already described uprolide C series (e.g., uprolide C acetate [**3**] and 7-*epi*-uprolide C acetate [**4**]) (16). A minor polar fraction (0.22% dry wt) contained the five new γ-lactonic cembranolides, **5**, **7–9**, and **10** which were isolated and purified by successive normal-

[1]Uprolide is an acronym of University of Puerto Rico.



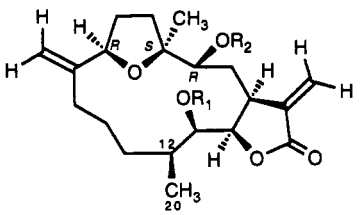
1 R=Ac
2 R=H



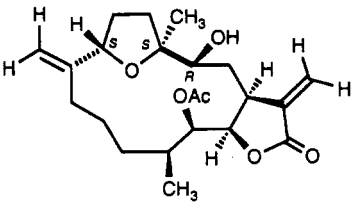
3 R_1=H, R_2=OH
4 R_1=OH, R_2=H

phase hplc. Each of the new metabolites was shown to possess the usual γ-lactone moiety and also a novel 4,7-oxa-bridged functionality.

Uprolide D acetate [**5**], a diterpene of the molecular formula $C_{22}H_{32}O_6$, contained two terminal methylene groups [δ 147.5 (s), 138.8 (s), 122.4 (t), and 115.7 (t)] and two ester carbonyl functions [δ 170.6 (s) and 169.7 (s)] apparent from ^{13}C-nmr and ir data; **5** is thus a tricyclic molecule. The ir spectrum of **5** contained absorptions for hydroxyl (3485 cm^{-1}), γ-lactone (1770 cm^{-1}), ester (1743 cm^{-1}), and olefin (1660 cm^{-1}) functionalities, some of the same structural features found in **1** and **2**. The α-methylene-γ-lactone functionality was supported by a uv absorption at λ max (MeOH) 208 nm (ε 18,700) and two broad singlets in the nmr spectrum at δ 6.24 (1H) and 5.70 (1H). The Me-12 and OAc-13 functionalities in the 14-membered ring of **5** were assumed to be intact on the basis of similar ir, uv, and nmr data [compare signals of related protons and carbons in Tables 1 and 2 with those reported for eupalmerin acetate [**1**] (13)]. Uprolide D acetate [**5**], however, had two terminal methylenes (vs. one in **1**), as indicated by ^{1}H-nmr signals at δ 5.03 (1H, br s) and 4.95 (1H, br s) and ^{13}C-nmr signals at δ 147.5 (s) and 115.7 (t). Also, the absence of resonances near δ 60.0 (s) and 58.0 (d) indicated the lack in this compound of an epoxy moiety, a feature found in **1**. A ^{1}H-^{1}H COSY experiment confirmed the spin-spin coupling system in **5** between H-3, resonating at δ 3.43 (1H, br m), and the protons around the α-methylene-γ-lactone ring including H-1, H-2α, -β, H-13, H-14, and the exomethylene protons H-17α and -β. The



5 R_1=Ac, R_2=H
6 R_1=R_2=Ac
7 R_1=R_2=H



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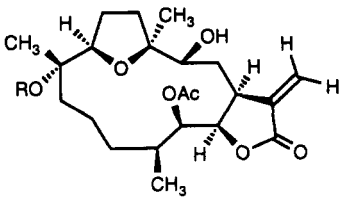

9 R=Ac
10 R=CH_3

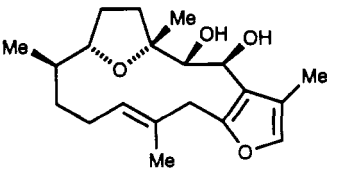

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TABLE 1. ^{1}H-Nmr Spectral Data of Compounds **5** and **7–10**.[a]

Proton	Compound				
	5	**7**	**8**	**9**	**10**
	δ, int. mult., *J* (Hz)	δ, int. mult., *J* (Hz)	δ, int. mult., *J* (Hz)	δ, int. mult., *J* (Hz)	δ, int. mult., *J* (Hz)
H-1	3.43, 1H, br m	3.45, 1H, m	3.41, 1H, m	3.16, 1H, m	3.14, 1H, m
H-2α	1.95, 1H, m	1.93, 1H, m	2.20, 1H, m	2.10, 1H, m	2.12, 1H, m
H-2β	1.48, 1H, m	1.32, 1H, m	1.78, 1H, m	1.91, 1H, m	1.80, 1H, m
H-3	3.43, 1H, br m	3.36, 1H, m	3.88, 1H, dd, 3.6, 8.4	3.54, 1H, dd, 3.1, 10.6	3.26, 1H, m
H-5α	1.82, 1H, m	1.76, 1H, m	1.97, 1H, m	1.72, 1H, m	1.49, 1H, m
H-5β	1.73, 1H, m	1.76, 1H, m	1.80, 1H, m	2.77, 1H, dt	2.16, 1H, m
H-6α	1.92, 1H, m	1.95, 1H, m	1.98, 1H, m	1.77, 1H, m	1.82, 1H, m
H-6β	1.92, 1H, m	1.78, 1H, m	1.77, 1H, m	1.47, 1H, m	1.49, 1H, m
H-7	4.49, 1H, dd, 6.4, 8.5	4.48, 1H, m	4.39, 1H, br d, 10.2	3.35, 1H, br d, 12.0	3.28, 1H, m
H-9α	2.49 1H, br t, 11.1	2.53, 1H, m	2.01, 1H, m	1.88, 1H, m	1.95, 1H, m
H-9β	1.71, 1H, m	1.63, 1H, m	1.78, 1H, m	1.33, 1H, m	1.47, 1H, m
H-10α	1.62, 1H, m	1.62, 1H, m	1.48, 1H, m	1.81, 1H, m	1.70, 1H, m
H-10β	1.62, 1H, m	1.62, 1H, m	1.48, 1H, m	1.75, 1H, m	1.48, 1H, m
H-11α	1.44, 1H, m	1.80, 1H, m	2.22, 1H, m	2.40, 1H, m	2.40, 1H, m
H-11β	1.18, 1H, m	1.18, 1H, m	1.81, 1H, m	1.32, 1H, m	1.44, 1H, m
H-12	1.87, 1H, m	1.82, 1H, m	2.19, 1H, m	2.10, 1H, m	2.14, 1H, m
H-13	5.41, 1H, br d, 9.6	3.98, 1H, d, 9.6	5.48, 1H, dd, 4.8, 7.2	5.36, 1H, d, 10.8	5.39, 1H, d, 10.8
H-14	4.60, 1H, dd, 6.0, 9.3	4.48, 1H, m	4.74, 1H, t, 6.9	5.54, 1H, d, 8.1	5.47, 1H, d, 8.1
H-17α	6.24, 1H, br s	6.26, 1H, br s	6.18, 1H, d, 1.8	6.11, 1H, d, 3.6	6.10, 1H, d, 3.6
H-17β	5.70, 1H, br s	5.74, 1H, br s	5.58, 1H, d, 1.8	5.32, 1H, d, 3.6	5.35, 1H, d, 3.0
Me-18	1.35, 3H, s	1.38, 3H, s	1.17, 3H, s	1.14, 3H, s	1.15, 3H, s
H-19α	5.03, 1H, br s	5.03, 1H, br s	4.92, 1H, br s	1.64, 3H, s	1.32, 3H, s
H-19β	4.95, 1H, br s	4.97, 1H, br s	4.87, 1H, br s	—	—
Me-20	0.96, 3H, d, 6.9	0.96, 3H, d, 6.0	0.93, 3H, d, 6.9	0.78, 3H, d, 6.9	0.79, 3H, d, 6.9
Me-22	2.08, 3H, s	—	2.04, 3H, s	1.87, 3H, s	1.87, 3H, s
Me-23	—	—	—	—	3.24, 3H, s
Me-24	—	—	—	1.99, 3H, s	—

[a]Spectra were recorded at room temperature in $CDCl_3$ using a ^{1}H observation frequency of 300.11 MHz. Assignments were aided by ^{1}H-^{1}H COSY, CSCM, NOESY, spin-splitting patterns, and comparison with *J* values. Chemical shifts are in ppm and are referenced to the residual $CHCl_3$ signal (7.26 ppm).

TABLE 2. ^{13}C-Nmr Spectral Data of Compounds **5** and **7–10**.[a]

Carbon	Compound				
	5	**7**	**8**	**9**	**10**
	δ (mult.)	δ (mult.)	δ (mult.)	δ (mult.)	δ (mult.)
1	37.8 (d)	37.5 (d)	39.1 (d)	41.9 (d)	42.2 (d)
2	28.8 (t)	28.7 (t)	31.1 (t)	25.7 (t)	25.8 (t)
3	72.4 (d)	72.5 (d)	73.5 (d)	83.4 (d)	84.9 (d)
4	84.8 (s)	84.8 (s)	82.7 (s)	75.6 (s)	75.6 (s)
5	35.5 (t)	35.3 (t)	37.3 (t)	35.4 (t)	35.1 (t)
6	33.9 (t)	34.3 (t)	33.2 (t)	36.6 (t)	36.9 (t)
7	83.3 (d)	83.2 (d)	78.3 (d)	88.2 (d)	88.0 (d)
8	147.5 (s)	147.9 (s)	147.6 (s)	80.4 (s)	73.7 (s)
9	29.7 (t)	29.8 (t)	30.0 (t)	18.3 (t)	18.4 (t)
10	27.8 (t)	28.7 (t)	21.8 (t)	24.9 (t)	24.8 (t)
11	32.9 (t)	33.1 (t)	32.7 (t)	28.6 (t)	28.8 (t)
12	29.7 (d)	29.8 (d)	30.6 (d)	33.2 (d)	33.2 (d)
13	70.4 (d)	69.2 (d)	71.3 (d)	72.8 (d)	72.8 (d)
14	79.0 (d)	82.2 (d)	80.8 (d)	79.4 (d)	79.5 (d)
15	138.8 (s)	139.0 (s)	140.5 (s)	139.2 (s)	139.3 (s)
16	169.7 (s)[b]	169.8 (s)	169.9 (s)[b]	169.9 (s)[b]	170.2 (s)[b]
17	122.4 (t)	123.0 (t)	120.0 (t)	116.4 (t)	116.6 (t)
18	25.1 (q)	25.6 (q)	21.8 (q)	29.7 (q)	29.5 (q)
19	115.7 (t)	115.5 (t)	110.9 (t)	18.2 (q)	16.6 (q)
20	15.4 (q)	14.7 (q)	15.1 (q)	14.9 (q)	15.0 (q)
21	170.6 (s)[b]	—	170.5 (s)[b]	170.3 (s)[b]	171.0 (s)[b]
22	20.9 (q)	—	20.9 (q)	20.9 (q)	20.9 (q)
23	—	—	—	170.9 (s)[b]	48.7 (q)
24	—	—	—	22.3 (q)	—

[a]Multiplicities were obtained by an Attached Proton Test (APT) experiment. Assignments were made on the basis of heteronuclear chemical shift-correlation methods, carbon atom multiplicities and chemical shift values. The δ values are in ppm and are referenced to the residual $CDCl_3$ signal (77.0 ppm).

[b]Values with identical superscripts in each column may be interchanged.

chemical shifts of the carbons associated with these protons were assigned by a HETCOR experiment. Acetylation of **5** with a mixture of Ac_2O and pyridine at 25° yielded the diterpene diacetate **6**, causing the signal ascribed to H-3 to shift to δ 5.00 (1H, dd, J=5.1 and 9.9 Hz). This observation was consistent with the secondary nature and location of the alcohol function as indicated in the proposed structure for **5**.

The ^{1}H-nmr spectrum also indicated the presence of a methyl group [δ 1.35 (3H, s, Me-18)] on a quaternary carbon atom bearing oxygen [δ 84.8 (s, C-4)] and a ^{1}H-^{13}C HETCOR experiment correlated the proton resonance at δ 4.49 (1H, dd, J=6.4 and 8.5 Hz), ascribed to H-7, with its corresponding carbon resonance at δ 83.3 (C-7). These combined ^{1}H- and ^{13}C-nmr data suggest that C-4 and C-7 participate in the formation of an ethereal linkage. The nature of these low-field signals was consistent with the presence of an oxolane ring system. These features unique to **5** were eventually confirmed through a selective INEPT (or INAPT) nmr experiment wherein the couplings between the C-19 exomethylene protons and C-7 and the Me-18 protons and C-4 were clearly demonstrated. All of the key two- and three-bond ^{1}H-^{13}C couplings (except for a correlation between H-7 and C-4 which are separated by an ether bridge) were observed with this technique. A NOESY experiment showed the quaternary methyl at C-4 and the proton at C-7 to be within nOe proximity; therefore, their relative spatial arrangement must be cis. The multiple nOes observed for **5**, particularly those between H-1 and

both H-14 and Me-18, and that between H-3 and H-12, clearly correlated with a Dreiding model representing the relative stereochemistry shown in the structure of **5**. Moreover, the absence of an nOe between H-7 and both H-3 and H-13 is consistent with the proposed stereochemistry of the sites involving these protons. Because the ^{1}H- and ^{13}C-nmr signals of **5** assigned to atoms at positions 1, 12, 13, and 14 were virtually identical to those observed for eupalmerin acetate [**1**], and because similar nOes were observed in these compounds, it was assumed that the relative stereochemistry of **5** at these centers was the same as that of **1**. The absolute configuration of **1** has been established (8) and because both **1** and **5** have identical relative configurations at all common chiral centers, one can safely assume that they also have the same absolute configuration. The C-3, C-4, C-7 constellation has been correlated with the C-12, C-13 array through their nOes, therefore the absolute configuration of chiral centers C-3, C-4, and C-7 must be as shown in structure **5**. Uprolide C acetate [**3**], a compound previously described from *E. mammosa* (16), could be envisioned as a precursor of **5** via epoxide ring opening by the C-7 hydroxyl group as an internal nucleophile.

A molecular formula of $C_{20}H_{30}O_5$ was confirmed for uprolide D [**7**] by hreims. Comparison of the nmr spectra of **7** with those of **5** revealed that these two molecules are structurally similar. From these data (Tables 1 and 2), **7** was recognized as a 13-deacetyl derivative of **5**. The ^{1}H-nmr spectrum of **7** was almost identical with that of **5**, with the exception that it did not contain an acetate signal and that the signal for H-13 had shifted from δ 5.41 (1H, br d, J=9.6 Hz) in **5** to δ 3.98 (1H, d, J=9.6 Hz) in **7**. The structure of **7** was correlated chemically with that of **5** upon acetylation of **7** with a mixture of Ac_2O/pyridine for 12 h at 25°. After workup, we obtained the same diacetate derivative **6** produced before by the acetylation of **5**. The rigorous assignment of the nmr spectra of **7** (see Tables 1 and 2) was established by application of the same 2D nmr techniques described earlier.

The structure of uprolide E acetate [**8**] showed it to be an isomer of **5**. A molecular formula of $C_{22}H_{32}O_6$ was established for **8** from hreims and ^{13}C-nmr data. The ir spectrum of **8** contained absorptions for hydroxyl (3498 cm^{-1}), γ-lactone (1750 cm^{-1}), ester (1737 cm^{-1}), and olefin (1670 and 1644 cm^{-1}) functionalities, the same structural features found in **5**. Like **5**, compound **8** showed a uv absorption at λ max (MeOH) 208 nm (ε=9,900) that indicated the presence of an α,β-unsaturated lactone. The ^{1}H-nmr spectrum of **8** was virtually superimposable on that of **5** except that this compound contained separate signals for H-3 [δ 3.88 (1H, dd, J=3.6 and 8.4 Hz)] and H-1 [δ 3.41 (1H, m)], and that the signal for H-7 had shifted from δ 4.49 (1H, dd, J=6.4 and 8.5 Hz) in compound **5** to δ 4.39 (1H, br d, J=10.2 Hz) in **8**. Moreover, key ^{1}H-^{13}C correlations were obtained by INAPT nmr by selective irradiation of the signals at δ 4.92 (H-19α) and 1.17 (Me-18) which caused enhancement of the ^{13}C-nmr signals at δ 78.3 (C-7) and 82.7 (C-4), respectively. These heteronuclear couplings effectively positioned the ethereal linkage in **8** between C-4 and C-7. Unlike **5**, allylic couplings between the C-19 exomethylene protons (δ 4.92 and 4.87) and H-7 (δ 4.39) were observed in the COSY spectrum of **8**. On the basis of these combined data the identity and location of the substituents around the cembranolide ring as well as the unsaturation pattern were assumed to be intact in **8**. Comparison of ^{1}H-^{1}H COSY and nmr data between **5** and **8** revealed subtle differences, however, that were associated with a change in **8** in its relative configuration at C-7. NOe measurements for **8** were consistent with a relative stereochemistry at C-7 opposite to that of **5**. The absence of any nOe between H-7 and Me-18 and the large nOes observed between H-7 and both H-3 and H-13, provided experimental evidence for the relative spatial arrangement shown at chiral centers C-3, C-4, C-7, and C-13. We envision the known

7-*epi*-uprolide C acetate [**4**] (16) as a precursor for **8** upon transannular back-side attack of the C-7 hydroxyl group at C-4 of the epoxide. Indeed, isomerization of **4** upon addition of PTSA in C_6H_6 at 25° yielded a product which proved identical by ^{1}H-nmr, ^{13}C-nmr, low-resolution eims, and tlc retention time with **8**, hence confirming the compound as the epimer of **5** at C-7. This chemical interconversion also allowed us to correlate directly the relative stereochemistry at positions C-3, C-4, and C-7 with the C-1, C-12, C-13, C-14 array in both **5** and **8**.

Hreims established a molecular formula for uprolide F diacetate [**9**] of $C_{24}H_{36}O_8$, two carbons, four hydrogens, and two oxygen atoms (i.e., CH_3CO_2H) more than in the molecular formula of **5**. Like **5**, compound **9** showed ir absorptions (3467, 1765, 1738, and 1665 cm^{-1}) indicating the presence of -OH, γ-lactone, ester, and olefin functionalities. Comparison of the ^{1}H- and ^{13}C-nmr spectra of **9** with those of **5** confirmed the structural similarity of these compounds and revealed some features unique to **9**. The γ-lactone moiety, the oxolane ring, and the common functionality in the 14-membered ring of **5** were assumed to be intact in **9** on the basis of similar ir, uv, and nmr data. Compound **9**, however, had two acetate groups, as indicated by ^{1}H-nmr signals at δ 1.87 (3H, s) and 1.99 (3H, s), and only one exocyclic methylene group (vs. two in **5**) as indicated by ^{13}C-nmr signals at δ 139.2 (s) and 116.4 (t). Moreover, two signals for methyl groups on oxygen-bearing carbons were observed at δ 1.64 (3H, s) and 1.14 (3H, s) in the ^{1}H-nmr spectrum and the signal ascribed to H-7 [δ 3.35 (1H, br d, J=12.0 Hz)] appeared shifted upfield with respect to that found in **5**. The combined ms and nmr spectral data and the close similarity of the rest of the ^{1}H- and ^{13}C-nmr spectra of **9** with those of **5**, led to the assignment of **9** as the 8-acetate derivative of **5**. Assuming the α-configuration for H-7 as in **5**, the Me-19 group was assigned the β-configuration on the basis of the strong nOe between Me-19 and the signal at δ 2.77 (H-5β) and the lack of an nOe between Me-19 and H-7. Other nOes observed for **9** were similar to those observed in **5**.

Compound **10**, uprolide G acetate, was isolated in trace amounts by silica hplc. Hrfabms established a molecular formula for **10** of $C_{23}H_{36}O_7$, one carbon, four hydrogens, and one oxygen (i.e., CH_3OH) more than in the molecular formula of **5**. The ^{1}H- and ^{13}C-nmr spectra of **10** were remarkably similar to those of **9**, suggesting that the two compounds had the same skeletal arrangement and oxygenation pattern. The ^{1}H-nmr spectrum of **10** differed from that of **9** in having only one acetyl methyl [δ 1.87 (3H, s)] and a methoxy signal at δ 3.24 (3H, s). These data led to the assignment of **10** as the 8-methoxy derivative of **5**. Placement of the Me-19 group in a β-configuration was supported by the nOe observed between Me-19 (δ 1.32) and the signals at δ 3.26 (H-3) and 2.16 (H-5β) plus the absence of an nOe between H-7 and Me-19. The remaining relative stereochemistry of **10** was identical with that of **9** on the basis of nmr (^{1}H and ^{13}C) and nOe data.

Some cembranolides from *E. mammosa* possess certain structural features so far unreported in the literature for compounds of this type. Uprolides **5**, **7**, **9**, and **10** have a 4*S*,7*R* oxolane ring while **8** has a 4*S*,7*S* ether bridge across the 14-membered ring. With the possible exception of the known furano-cembranoid pachyclavulariadiol [**11**] and its monoacetate and diacetate derivatives (17), the new uprolides reported here appear to be the first cembranolide diterpenes possessing a 4,7-oxa-bridge moiety. The uprolide derivatives **5**, **7**, and **8** are also unique among the compounds in this series in having an exocyclic C-8, C-19 double bond instead of an endocyclic C-7, C-8 double bond. In contrast to the majority of compounds isolated from *E. mammosa* (5–16), the five new cembranolides reported here do not possess the typical 3,4-epoxy moiety. Some of the new uprolides displayed moderate cytotoxicity against HeLa cells: **5** (IC_{50}=2.5 μg/ml), **7** (IC_{50}=5.0 μg/ml), **8** (IC_{50}=3.0 μg/ml), and **9** (IC_{50}=5.1 μg/ml). Uprolide D-

acetate [**5**] also displayed cytotoxicity against the following human tumor cell lines: CCRF-CEM T-cell leukemia (IC_{50}=7.0 μg/ml), HCT 116 colon cancer (IC_{50}=7.0 μg/ml), and MCF-7 breast adenocarcinoma (IC_{50}=0.6 μg/ml).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet 600 Ft-ir spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a General Electric Multinuclear QE-300 nmr spectrometer; ^{1}H-nmr chemical shifts are recorded with respect to the residual $CHCl_3$ signal (7.26 ppm) and ^{13}C-nmr chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). Optical rotations were determined on a Perkin-Elmer model 243B polarimeter. Lreims were recorded on a Hewlett-Packard 5995A spectrometer, and hreims, hrfabms, and lrfabms were determined at the Midwest Center for Mass Spectrometry of the University of Nebraska-Lincoln. Cc was performed on Analtech Si gel (35–75 mesh) and tlc analyses were carried out using Analtech glass-packed precoated Si gel plates. All solvents used were either spectral grade or were distilled from glass prior to use.

ANIMAL MATERIAL.—As described previously (16). A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico.

EXTRACTION AND ISOLATION.—The $CHCl_3$ extract of the Caribbean gorgonian *E. mammosa* was generated as described previously (16). Si gel (100 g) cc of fraction 7 (2.86 g) with 40% EtOAc in hexane gave fractions 1 through 9. Fraction 6 (395 mg) was subsequently purified by successive hplc [(a) Partisil 10/50 Si gel with 10% *i*-PrOH in hexane and (b) Partisil 10/50 Si gel in 30% *i*-PrOH in hexane] to yield 25 mg (0.006% dry wt) of uprolide D acetate [**5**] and 14 mg (0.001% dry wt) of uprolide E acetate [**8**]. Fraction 8 was also purified by hplc [Partisil 10/50 Si gel with 15% *i*-PrOH in hexane] to give 14 mg (0.001% dry wt) of uprolide D [**7**], 18 mg (0.001% dry wt) of uprolide F diacetate [**9**], and 13 mg (0.001% dry wt) of uprolide G acetate [**10**].

Uprolide D acetate [**5**].—Colorless oil: $[\alpha]^{25}D$ +45.62° (*c*=0.69, $CHCl_3$); uv λ max (MeOH) 208 nm (ε 18,700); ir (neat) ν max 3485, 2965, 2935, 2879, 1770, 1743, 1660, 1371, 1260, 1237, 1102, 1028, 961 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hreims *m/z* $[M]^+$ 392.22136 (15) ($C_{22}H_{32}O_6$ requires 392.21987), 374 (2), 348 (14), 109 (100).

Uprolide D [**7**].—Colorless oil: $[\alpha]^{25}D$ −19.86° (*c*=0.71, $CHCl_3$); uv λ max (MeOH) 212 nm (ε 16,500); ir (neat) ν max 3403, 2962, 2930, 2872, 1749, 1271, 1109, 1065, 1023, 1004 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hreims *m/z* $[M]^+$ 350.20956 (2) ($C_{20}H_{30}O_5$ requires 350.20938), 332 (3), 261 (4), 141 (18), 109 (21), 85 (82).

Uprolide E acetate [**8**].—Colorless oil: $[\alpha]^{25}D$ +68.34° (*c*=0.75, $CHCl_3$); uv λ max (MeOH) 208 nm (ε 9,900); ir (neat) ν max 3498, 2971, 2966, 2922, 1750, 1737, 1670, 1644, 1371, 1240, 1167, 1092, 1043, 1021, 958 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hreims *m/z* $[M]^+$ 392.22141 (10) ($C_{22}H_{32}O_6$ requires 392.21987), 348 (6), 314 (2), 274 (3), 231 (3), 203 (7), 109 (54), 81 (40).

Uprolide F diacetate [**9**].—Colorless oil: $[\alpha]^{25}D$ +145.7° (*c*=0.88, $CHCl_3$); uv λ max (MeOH) 210 nm (ε 6,900); ir (neat) ν max 3467, 2969, 2934, 2876, 1765, 1738, 1665, 1370, 1240, 1100, 1030 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hreims *m/z* $[M]^+$ 452.24077 (0.3) ($C_{24}H_{36}O_8$ requires 452.24099), 434 (0.2), 392 (34), 332 (5), 289 (2).

Uprolide G acetate [**10**].—Colorless oil: $[\alpha]^{25}D$ +125.0° (*c*=0.66, $CHCl_3$); uv λ max (MeOH) 210 nm (ε 8,700); ir (neat) ν max 3437, 2964, 2930, 2870, 1766, 1742, 1456, 1374, 1262, 1238, 1109, 1039, 1022 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hrfabms *m/z* $[M+H]^+$ 425.25420 (100) ($C_{23}H_{37}O_7$ requires 425.25390).

Acetylation of uprolide D acetate [**5**].—A solution of **5** (15 mg, 0.038 mmol) in a mixture of Ac_2O-pyridine (2:1) was stirred at 25° for 14 h. After concentration, 7.3 mg of a homogeneous compound was isolated whose nmr spectra conclusively identified it as **6**. Acetylation of **7** gave diacetate **6** under identical experimental conditions.

ISOMERIZATION OF **4** TO **8**.—A solution of **4** (22 mg, 0.056 mmol) in C_6H_6 (15 ml) was stirred at 25° for 40 min with PTSA (5.1 mg). After concentration followed by cc on Si gel, 13.6 mg of a homogeneous compound was obtained whose tlc retention time, eims and nmr spectra (^{1}H- and ^{13}C-) conclusively identified it as **8**.

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