Calyculaglycosides D and E, Novel Cembrane Glycosides from the Caribbean Gorgonian Octocoral *Eunicea* Species and Structural Revision of the Aglycon of Calyculaglycosides A−C

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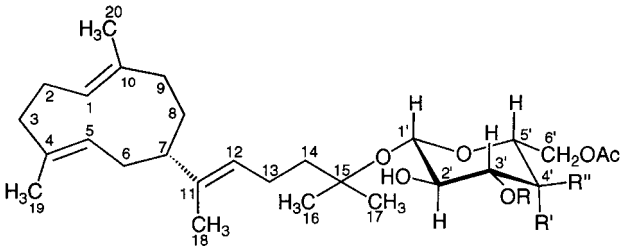
A recent investigation of the constituents of *Eunicea* sp. from Colombia and Puerto Rico has provided two novel cembrane glycosides, calyculaglycosides D (**6**) and E (**7**), and a new cembranoid diterpene in free form, (+)-nephthenol (**4**). Metabolites **6** and **7** possess a glycosyl-fused cembrane skeleton with a 1*S* configuration. Biogenetic considerations have led to a revision of the previously assigned dilophol skeleton of calyculaglycosides A−C, which was confirmed by extensive 2D NMR investigation and a chemical degradation study. Consequently, the true structures for calyculaglycosides A−C are **8**−**10**, respectively, not **1**−**3**. This is the first report of the occurrence of marine diterpene glycosides having a cembrane aglycon.

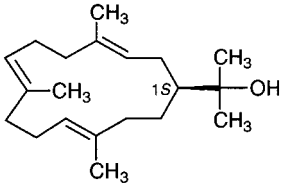
Calyculaglycosides A−C, a group of biologically active diterpenoid hexose glycosides, were isolated by our group from the Colombian gorgonian octocoral *Eunicea* sp. and assigned structures **1**−**3**. We reported that these compounds possessed significant cytotoxic and antiinflammatory activities in 1997, a finding that motivated us to undertake the acquisition of additional quantities for further biological evaluation.[1] In the course of purifying larger quantities of the hexane extract of *Eunicea* sp. collected in Colombia and Puerto Rico, we isolated and identified (3*E*,7*E*,11*E*)-(1*S*)-cembra-3,7,11-trien-15-ol (**4**), the (+)-antipode of the known metabolite nephthenol (**5**),



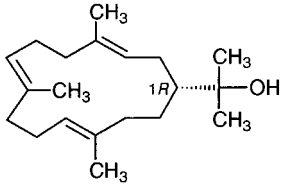
1 R= Ac; R'= OH; R"= H

2 R= H; R'= H; R"= OAc

3 R= H; R'= OAc; R"= H

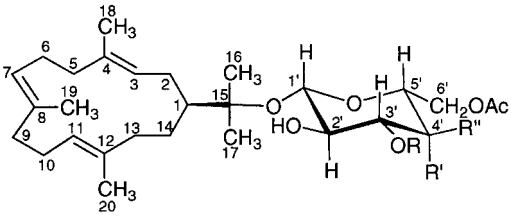


(+)-nephthenol (**4**)



(—)-nephthenol (**5**)

and two structurally related monoacetylated hexose glycosides, named calyculaglycosides D (**6**) and E (**7**).[2] When we compared the NMR spectra of cembrane alcohol **4** with the NMR data ascribed to the aglycon of glycosides **6** and **7**, they were almost indistinguishable.[3] Consequently, we considered that calyculaglycosides D and E originated from glycosidation of **4**. More importantly, the NMR data of **4** were also found to be quite similar to the NMR data arising from the aglycon portion of the calyculaglycosides A−C, suggesting that the latter group of glycosides possess the same aglycon.[4] To confirm our assumption, we carefully evaluated the spectroscopic data of **6** and **7** and reinterpreted the data reported earlier for compounds **1**−**3**. In this paper, we present the analytical data for calyculaglycoside D (**6**) and calyculaglycoside E (**7**) and reveal that the true structures for calyculaglycosides A−C are **8**−**10**, respectively, not **1**−**3**.[5] Unambiguous confirmation of this was achieved by careful acid hydrolysis of glycoside **6** with HCl, which afforded an aglycon identified as (+)-nephthenol (**4**) by comparison of the GLC and TLC retention times, $[\alpha]_D$, and mass spectra with those of an authentic sample. Revised 1D and 2D NMR data for calyculaglycosides A−C (**8**−**10**) are provided in Tables 1 and 2. Compounds **6** and **7** are currently undergoing biological evaluation.



calyculaglycoside D (**6**) R= H; R'= OH; R"= H

calyculaglycoside E (**7**) R = H; R'= H; R"= OH

calyculaglycoside A (**8**) R= Ac; R'= OH; R"= H

calyculaglycoside B (**9**) R= H; R'= H; R"= OAc

calyculaglycoside C (**10**) R= H; R'= OAc; R"= H

Results and Discussion

The specimens of *Eunicea* sp. used in this chemical study were collected in March 1996 near Santa Marta Bay, Colombia, and in October 2000 off the coast of Cabo Rojo,

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Table 1. Revised NMR, COSY, NOESY, and HMBC Spectral Data for Calyculaglycoside B (**9**)

position	δ_H, mult., intrg. (*J* in Hz)[a]	δ_C (mult.)[b]	COSY[c]	NOE[c]	HMBC[d]
1	1.46, m, 1H (3.4,3.4,7.2,7.2)	47.7 (d)	H-2α*β*, H-14α*β*	H-3	H-2α*β*, Me-16, Me-17
2α	2.14, m, 1H	28.2 (t)	H-1, H-2*β*, H-3	H-2*β*, H-14α, Me-18	H-14
2*β*	1.87, m, 1H (7.5,7.6,15.0)		H-1, H-2α, H-3	H-2α	
3	5.07, br t, 1H (6.6)	125.8 (d)	H-2α*β*, Me-18	H-1, H-5	H-2α*β*, Me-18
4		133.5 (s)			H-2α*β*, Me-18
5	2.08, m, 2H	38.8 (t)		H-3, Me-18	H-3, Me-18
6	2.14, m, 2H	24.7 (t)	H-7	H-7, Me-19	H-7
7	4.93, br t, 1H (6.6)	125.7 (d)	H-6, Me-19	H-6	H-9, Me-19
8		133.1 (s)			H-6α*β*, Me-19
9	2.00, m, 2H	37.8 (t)			H-11, Me-19
10	2.10, m, 2H	24.0 (t)	H-11	Me-20	H-11
11	5.00, br t, 1H (6.9)	125.1 (d)	H-10, Me-20		H-13, Me-20
12		134.1 (s)			H-14α*β*, Me-20
13	2.00, m, 2H	39.4 (t)	H-14α*β*		Me-20
14α	1.24, m, 1H (6.2,6.2,13.3)	28.3 (t)	H-1, H-13, H-14*β*	H-2α, H-14*β*	H-2α*β*
14*β*	1.62, m, 1H		H-1, H-13, H-14α	H-14α	
15		81.6 (s)			Me-16, Me-17, H-1′
Me-16	1.22, s, 3H	24.0 (q)[e]		H-1′	Me-17
Me-17	1.22, s, 3H	24.5 (q)[e]		H-1′	Me-16
Me-18	1.56, s, 3H	15.6 (q)	H-3	H-2α, H-5	H-3, H-5
Me-19	1.56, s, 3H	15.3 (q)	H-7	H-6	H-7, H-9
Me-20	1.56, s, 3H	15.5 (q)	H-11	H-10	H-11, H-13
1′	4.45, d, 1H (7.8)	96.7 (d)	H-2′, H-3′	Me-16, Me-17, H-3′, H-5′	H-2′, H-5′
2′	3.41, t, 1H (8.7)	74.5 (d)	H-1′, H-3′, H-4′	H-4′	H-1′, H-3′
3′	3.68, t, 1H (9.3)	74.7 (d)	H-1′, H-2′, H-4′	H-1′	H-1′, H-2′, H-4′
4′	4.87, t, 1H (9.9)	70.9 (d)	H-2′, H-3′, H-5′, H-6′a,b	H-2′	H-3′, H-5′, H-6′a,b
5′	3.59, ddd, 1H (2.7,6.0,9.7)	71.6 (d)	H-4′, H-6′a,b	H-1′, H-6′a,b	H-1′, H-3′, H-4′, H-6′b
6′a	4.10, dd, 1H (2.4,12.0)	62.8 (t)	H-4′, H-5′, H-6′b	H-5′, H-6′b	H-4′
6′b	4.19, dd, 1H (6.0,12.0)		H-4′, H-5′, H-6′a	H-5′, H-6′a	H-4′
OAc-4′	2.12, s, 3H	20.9 (q)			
		170.6 (s)			H-4′, OAc-4′
OAc-6′	2.06, s, 3H	20.7 (q)			
		170.7 (s)			H-6′a, OAc-6′
OH-2′	2.38, br s, 1H, exchangeable[e]				
OH-3′	2.65, br s, 1H, exchangeable[e]				

[a] The ^{1}H NMR spectrum was recorded at 500 MHz in $CDCl_3$ at 25 °C. Assignments were aided by spin-decoupling experiments. Chemical shifts are given in δ units downfield from Me_4Si. [b] The ^{13}C NMR spectrum was recorded in $CDCl_3$ at 125 MHz. Numbers of attached protons were determined by DEPT experiments. Assignments were aided by HMQC and HMBC experiments. [c] ^{1}H−^{1}H COSY, RCT COSY, and 2D-PSNOESY were performed in $CDCl_3$ at 300 MHz. [d] The HMBC spectrum was recorded at 500 MHz in $CDCl_3$. Protons correlated to carbon resonances in the ^{13}C NMR column. Parameters were optimized for $J_{CH} = 6$ and 8 Hz. [e] Signals within a column may be reversed.

Puerto Rico. The gorgonian specimens were stored frozen, freeze-dried, and extracted separately with 1:1 MeOH−$CHCl_3$. Repetitive silica gel chromatography and HPLC analysis of the hexane extracts yielded (+)-nephthenol (**4**), together with samples of pure calyculaglycosides D (**6**) and E (**7**). Interestingly, we did not detect the presence of diacetylated hexose glycosides (such as calyculaglycosides A−C) in the specimen of *Eunicea* sp. from Puerto Rico.

(+)-Nephthenol (**4**), $[\alpha]^{22}_D$ +22.8°, was obtained as a UV-inactive colorless oil that corresponded to a molecular formula of $C_{20}H_{34}O$ on the basis of its ^{13}C NMR and HREIMS ($[M]^+$ *m/z* 290.2612, Δ 0.2 mmu). Its IR spectrum displayed strong bands at 3414 cm^{-1} (broad, hydroxyl group) and 1667 cm^{-1} (sharp, olefin groups). A careful literature survey revealed that the NMR (^{1}H and ^{13}C), IR, and mass spectra of **4**, as well as the magnitude of its specific rotation, appeared identical with those reported for (−)-nephthenol (**5**).[2,3] However, solutions of the two cembrane alcohols in $CHCl_3$ showed opposite signs of rotation. Therefore, (−)-nephthenol (**5**) and **4** are clearly enantiomeric. Since a series of chemical degradation and correlation studies by Tursh et al. aimed at establishing the absolute configuration of (−)-nephthenol (**5**) indicated the 1*R* configuration, so **4** must possess the 1*S* configuration.[2b] Detailed analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC NMR data confirmed the structure of (+)-nephthenol (**4**) and confirmed the assignment of all ^{1}H and ^{13}C NMR resonances.[3]

Calyculaglycoside D (**6**) was isolated as a colorless viscous oil after successive silica gel column chromatography and HPLC analysis. Data from HRFABMS and ^{13}C NMR spectrometry established a molecular formula of $C_{28}H_{46}O_7$. The ^{1}H NMR spectrum contained one methyl signal at δ 2.06 (s, 3H) and one carbon signal at δ 171.0 (s) in the ^{13}C NMR spectrum, which, together with an intense IR absorption at 1742 cm^{-1}, suggested that **6** was a monoacetate. While the presence of five other methyl resonances [three overlapped at δ_H 1.56 (s, 9H) and two at δ_H 1.22 (s, 6H)], in addition to the acetate methyl, was consistent with a diterpenoid carbon skeleton, this observation did not correlate with the molecular formula, indicating a larger molecule. A ^{13}C NMR resonance at δ 97.2 (d) assigned to a sugar anomeric carbon and five carbons in the region between δ_C 62 and 74 suggested that **6**, like calyculaglycosides A−C, was a hexose glycoside. The presence of an acetal and the numerous oxygenated carbons in **6**, together with the molecular formula indicating an eight-carbon fragment in excess of a diterpenoid molecule, were strong indications that calyculaglycoside D contained a monoacetylated hexose sugar. Interpretation of the LRFAB (3-NBA) mass spectral fragmentation pattern of **6**, showing a $C_8H_{14}O_7Na$ (*m/z* 245) prominent peak [monoacetylated hexose unit; intensity = 100%], supported this assignment. A ^{1}H−^{1}H COSY NMR experiment, which revealed an isolated spin system of seven protons in the region of δ 3.7−4.4, confirmed that **6** was indeed a hexose glycoside and allowed the confident assignment of all of the sugar protons. Once the presence of the hexose residue in calyculaglycoside D (**6**) was confirmed, attention was

Table 2. Revised ^{1}H and ^{13}C NMR Data for Calyculaglycosides A (**8**) and C (**10**)

	calyculaglycoside A (**8**)[a,b]		calyculaglycoside C (**10**)[a,b]	
position	δ_H, mult., intrg. (*J* in Hz)	δ_C (mult.)	δ_H, mult., intrg. (*J* in Hz)	δ_C (mult.)
1	1.45, m, 1H (4.0,7.4,11.3)	47.8 (d)	1.47, m, 1H (3.8,7.5,11.3)	47.7 (d)
2α	2.14, m, 1H	28.2 (t)	2.17, m, 1H	28.2 (t)
2*β*	1.87, m, 1H (6.9,7.5,15.3)		1.88, m, 1H (7.3,7.5,15.1)	
3	5.06, br t, 1H (6.3)	125.8 (d)	5.07, br t, 1H (6.6)	125.8 (d)
4		133.5 (s)		133.6 (s)
5	2.07, m, 2H	38.9 (t)	2.08, m, 2H	38.8 (t)
6	2.10, m, 2H	24.7 (t)	2.13, m, 2H	24.7 (t)
7	4.94, br t, 1H (6.6)	125.7 (d)	4.93, br t, 1H (6.7)	125.7 (d)
8		133.1 (s)		133.1 (s)
9	1.97, m, 2H	37.9 (t)	2.00, m, 2H	37.8 (t)
10	2.06, m, 2H	24.0 (t)	2.08, m, 2H	24.0 (t)
11	4.99, br t, 1H (6.4)	125.0 (d)	5.00, br t, 1H (6.4)	125.0 (d)
12		134.2 (s)		134.1 (s)
13	1.99, m, 2H	39.4 (t)	2.03, m, 2H	39.4 (t)
14α	1.22, m, 1H	28.2 (t)	1.23, m, 1H	28.2 (t)
14*β*	1.62, m, 1H		1.61, m, 1H	
15		81.7 (s)		81.6 (s)
Me-16	1.22, s, 3H[c]	23.8 (q)[c]	1.22, s, 3H[c]	23.8 (q)[c]
Me-17	1.23, s, 3H[c]	24.8 (q)[c]	1.23, s, 3H[c]	24.8 (q)[c]
Me-18	1.54, s, 3H	15.6 (q)	1.56, s, 3H	15.6 (q)
Me-19	1.56, s, 3H	15.3 (q)	1.56, s, 3H	15.3 (q)
Me-20	1.54, s, 3H	15.6 (q)	1.56, s, 3H	15.6 (q)
1′	4.51, d, 1H (7.8)	97.1 (d)	4.44, d, 1H (7.8)	97.2 (d)
2′	3.45, t, 1H (9.0)	72.2 (d)	3.71, dd, 1H (7.6,9.6)[d]	72.0 (d)
3′	4.93, dd, 1H (3.3,9.3)	78.0 (d)	3.45, dd, 1H (3.6,9.7)[d]	72.3 (d)
4′	3.49, br s, 1H	69.7 (d)	5.30, dd, 1H (0.9,3.6)	69.1 (d)
5′	3.50, br s, 1H	73.9 (d)	3.81, dt, 1H (1.2,6.9)	70.8 (d)
6′a	4.34, br s, 1H	63.5 (t)	4.11, br s, 1H	62.1 (t)
6′b	4.34, br s, 1H		4.13, br s, 1H	
OAc-3′ or -4′	2.16, s, 3H	21.0 (q)	2.14, s, 3H	20.8 (q)
		172.4 (s)		171.1 (s)
OAc-6′	2.08, s, 3H	20.8 (q)	2.03, s, 3H	20.7 (q)
		171.3 (s)		170.5 (s)
OH's	3.06, br s, 2H[e]		2.57, br s, 1H;[e] 2.63, br s, 1H[e]	

[a] ^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$ at 25 °C. Assignments were aided by ^{1}H−^{1}H COSY, RCT COSY, 2D-PSNOESY, and spin-decoupling experiments. Chemical shifts are given in δ units (downfield from Me_4Si). [b] ^{13}C NMR spectra were recorded in $CDCl_3$ at 75 MHz. Numbers of attached protons were determined by DEPT experiments. Assignments were aided by HMQC and HMBC experiments. [c] Signals within a column may be reversed. [d] Peak broadening in $CDCl_3$ did not allow for an assignment of multiplicity and *J* data. Therefore, these values are reported in C_6D_6 solution. [e] D_2O exchangeable.

directed toward determining the complete structures of the aglycon and sugar components.

A combination of ^{1}H−^{1}H COSY, ^{13}C NMR, HMQC, and HMBC data revealed the aglycon component of calyculaglycoside D to possess the same C_{20} hydrocarbon skeleton as (+)-nephthenol (**4**). The geometries of the double bonds were assigned on the basis of the shielded vinyl methyl carbon resonances at δ_C 15.6 (Me-18), 15.3 (Me-19), and 15.5 (Me-20).[6] The chemical shift of the C-15 carbon [δ_C 81.4 (s)], showing strong HMBC correlations to Me-16 (δ_H 1.22), Me-17 (δ_H 1.22), and the anomeric proton H-1′ (δ_H 4.40), clearly indicated that the sugar unit was bound to the cembranoid aglycon by a glycosidic linkage at C-15. Linking these partial structures to the cembrane ring system was accomplished by HMBC experiments, which revealed the long-range coupling between C-1 (δ_C 47.7), the only sp^3 tertiary carbon of the macrocyclic triene component, and the geminal C-16 and C-17 methyl protons. Unequivocal identification of the aglycon in calyculaglycoside D stemmed from mineral acid hydrolysis of **6** with 2 N HCl at 75 °C, which afforded (+)-nephthenol (**4**) by co-TLC, $[\alpha]_D$, and GLC-MS comparison with an authentic sample.[7]

The connectivities between the protons of the hexose component in **6** were determined by a ^{1}H−^{1}H COSY experiment, and the carbon and the C−H correlation assignments by HMQC and HMBC experiments. Chemical shifts for the protons at C-6′ indicated that the hydroxyl group on this carbon was acetylated. The anomeric proton at C-1′ was apparent as the lowest field sugar methine proton (δ 4.40). This proton was a doublet with $J = 7.2$ Hz, which confirmed it as an axial proton, thus showing the hemiacetal linkage as equatorial or *β*. H-2′ (3.56 ppm) was split into a doublet of doublets ($J = 7.2$, 9.4 Hz), which indicated two axial vicinal protons. Most conspicuous was the very small coupling constant between the C-3′ and C-4′ methine protons. Since C-3′ was clearly an axial proton, C-4′ must be equatorial and, therefore, the C-4′ hydroxyl must be axial. NOESY correlations from C-5′ to C-1′ and C-3′ established the cis 1,3-diaxial relationship between these protons. The absolute stereochemistry of the galactose residue was assumed to be D on the basis of a series of chiral GC−MS studies performed on the acid hydrolysates of calyculaglycoside A (**8**) and C (**10**), each possessing a di-*O*-acetyl-*β*-D-galactose residue.[1] These results demonstrated that the hexose component of calyculaglycoside D (**6**) is 6′-*O*-acetyl-*β*-D-galactopyranose.

Calyculaglycoside E (**7**) was isolated as a colorless oil, which also analyzed for $C_{28}H_{46}O_7$ by HRFABMS and ^{13}C NMR spectrometry. Spectral data from this compound were highly comparable with those from the monoacetate **6**. The chemical shifts of many signals in the ^{1}H and ^{13}C NMR spectra of **7** were very similar to those from calyculaglycoside D (**6**) (see Experimental Section). Using a combination of ^{1}H−^{1}H COSY, HMQC, and HMBC data all of the proton-bearing carbons, their protons, and the quaternary carbons in calyculaglycoside E were precisely matched. Thus, it was concluded with confidence that **7** has the same

diterpenoid aglycon component found in **6** and that the minor differences seen in their NMR spectra were due to the presence of a distinct sugar residue in each glycoside. The highly comparable ^{13}C NMR spectral data confirmed that monoacetates **6** and **7** indeed possess the same diterpenoid aglycon. Through the use of $^{1}H-^{1}H$ COSY, HMQC, HMBC, and NOESY experiments, the sugar in **7** was identified as monoacetylated glucose in a β-pyranoside configuration. The low-field shifts of the C-6′ protons (δ 4.33 and 4.29) indicated that the acetate ester was at the sugar C-6′ position. The relative stereochemistry of the monosaccharide was determined by NOE experiments and coupling constants. The large coupling constants (>7 Hz) observed between the C-1′, C-2′, C-3′, C-4′, and C-5′ methine protons suggested that all protons were axial and all hydroxyl groups equatorial. A NOESY correlation from the C-2′ and C-4′ methine protons and those from C-1′ with C-3′ and C-5′ confirmed the axial conformation of these protons, as was suggested by the coupling constants. The stereochemistry of the glycosidic bond was determined as β on the basis of $^{3}J_{H-1'H-2'}$ values (7.6 Hz) and the fact that the anomeric oxygen was cis to CH_2OAc-6′. Since analysis by GC−MS using a chiral capillary column for the acid hydrolysate of calyculaglycoside B (**9**) had indicated the common D-configuration of the sugar, it follows that the hexose portion of calyculaglycoside E (**7**) is a 6′-*O*-acetyl-β-D-glucopyranose unit.[1] An HMBC correlation between the anomeric proton at δ_H 4.43 (H-1′) to a carbon at δ_C 81.4 (s, C-15) connected the monosaccharide to the macrocyclic diterpene system. The strong NOE response between H-1′ and the geminal Me-16/Me-17 pair confirmed this spatial relationship and yielded the final structure, **7**.

In the present study two minor diterpene glycosides, **6** and **7**, have been isolated from the hexane extracts of *Eunicea* sp. collected in Colombia and Puerto Rico. Moreover, the structures of calyculaglycosides A−C have been revised from the previously reported **1**−**3**, with a dilopholtype aglycon, to structures **8**−**10**, displaying a glycosyl-fused (3*E*,7*E*,11*E*)-(1*S*)-cembra-3,7,11-triene skeleton. On careful acid hydrolysis, calyculaglycoside D (**6**) liberated (+)-nephthenol (**4**), thus indicating that compounds **6**−**10** must have an identical aglycon composition. Revised NMR data for calyculaglycosides A−C (**8**−**10**) appear in Tables 1 and 2. This is the first report of the occurrence of marine diterpene glycosides having a cembrane aglycon.[8]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer model 243B polarimeter. IR spectra were recorded on a FT-IR spectrophotometer. ^{1}H NMR, ^{13}C NMR, $^{1}H-^{1}H$ COSY, 2D-NOESY, HMQC, and HMBC spectra were recorded on a 300 MHz spectrometer. ^{1}H NMR chemical shifts were recorded with respect to internal Me_4Si, and ^{13}C NMR chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). High-resolution EIMS and FABMS were recorded at the Materials Characterization Center (MCC) of the University of Puerto Rico. Column chromatography was carried out on silica gel (35−75 mesh) and TLC performed using glass packed precoated silica gel plates. HPLC was performed using a 10 μm silica gel Partisil 10 semipreparative column (9.4 mm × 50 cm). All solvents used either were spectral grade or were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the crude hexane extract. The external appearance and general characteristics of the *Eunicea* sp. specimens have been described elsewhere.[1] Specimens of *Eunicea* sp. under the name Sánchez USNM 97733 are on deposit in the octocoral collection, Smithsonian Institution, Washington, DC, under the curatorship of Dr. Frederick M. Bayer.

Collection and Extraction of *Eunicea* sp. from Puerto Rico. Minced and freeze-dried specimens of *Eunicea* sp. (780 g) collected off the coast of Cabo Rojo, Puerto Rico, were extracted exhaustively with $CHCl_3$−MeOH (1:1) (4 × 3 L). After filtration the crude extract was evaporated under vacuum to yield a brown residue (66.0 g) that was suspended in water and then partitioned between *n*-hexane (3 × 1 L) and $CHCl_3$ (3 × 1.5 L). The hexane extract was concentrated in vacuo to yield 38.5 g of a green oily residue, which was fractionated by silica gel (455 g) column chromatography (gradient elution; 0−100% EtOAc in hexane, then 100% acetone followed by 100% MeOH), yielding 11 tertiary fractions, designated I−XI. Fraction II (ca. 8.2 g) was chromatographed successively over (1) silica gel (16 g) with 20:1 hexane−EtOAc and (2) silica gel (1.8 g) with 10:1 hexane−EtOAc, yielding (+)-nephthenol (**4**, 4.8 mg, 0.012% of the total extract). After repeated column chromatography on (1) silica gel (15 g) using 8:1 hexane−2-propanol and (2) silica gel (1.0 g) with 10:1 hexane−2-propanol, fraction IX (10.6 g) yielded 25.5 mg of calyculaglycoside D (**6**) (0.065% of the extract).

Collection and Extraction of *Eunicea* sp. from Colombia. The original isolation procedures for calyculaglycosides A−C (**8**−**10**) have been previously described.[1] Minced and freeze-dried specimens of *Eunicea* sp. (2.1 kg) collected near Santa Marta Bay, Colombia, were extracted exhaustively with $CHCl_3$−MeOH (1:1) (8 × 1 L). After filtration the crude extract was evaporated under vacuum to yield a residue (170.5 g) that was suspended in water and then partitioned against *n*-hexane (6 × 2.5 L). The hexane extract was concentrated in vacuo to yield 72.0 g of a green oily residue, which, after filtration under vacuum in toluene solution, was fractionated by size-exclusion chromatography on a Bio-Beads SX-3 column with toluene as eluant. The combined portions (NMR and TLC guided) were concentrated to obtain three main fractions, designated A (31.7 g), B (24.3 g), and C (14.5 g). A portion of fraction B (ca. 17.3 g) was chromatographed over a silica gel column with 20% hexane−EtOAc and fractionated roughly into three subfractions on the basis of TLC and NMR analysis. From the first subfraction (5.6 g) was isolated (+)-nephthenol (**4**, 24.4 mg, 0.05% of the extract) after repetitive silica gel chromatography (gradient elution; 20−40% EtOAc in hexane followed by 1−10% $CHCl_3$ in MeOH) and HPLC using 99:1 hexane−2-propanol as eluant. The last subfraction (520 mg) was purified by successive silica gel chromatography using a 1:4 mixture of hexane−EtOAc and HPLC with 3:1 hexane−2-propanol to afford calyculaglycoside D (**6**, 53.8 mg, 0.10% of the extract) and calyculaglycoside E (**7**, 90 mg, 0.17% of the extract).

(+)-Nephthenol (4): colorless oil, $[\alpha]^{22}_D$ +25.3° (*c* 2.1, $CHCl_3$); IR, ^{1}H NMR, ^{13}C NMR, HREIMS, as previously reported.[2,3]

Calyculaglycoside D (6): colorless oil; $[\alpha]^{24}_D$ +10.0° (*c* 4.3, $CHCl_3$); IR (neat) 3406, 2925, 1742, 1368, 1235, 1055, 892, 753 cm^{-1}; UV λ_{max} (MeOH) nm (log ϵ) 204 (3.79), 230 (3.40); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (m, 1H, J = 3.3, 3.3, 7.0 Hz, H-1), 2.16 (m, 1H, H-2α), 1.88 (m, 1H, H-2β), 5.07 (br t, 1H, J = 6.2 Hz, H-3), 2.06 (m, 2H, H-5$\alpha\beta$), 2.16 (m, 2H, H-6$\alpha\beta$), 4.93 (br t, 1H, J = 6.0 Hz, H-7), 1.98 (m, 2H, H-9$\alpha\beta$), 2.10 (m, 2H, H-10), 4.99 (br t, 1H, J = 6.0 Hz, H-11), 2.00 (m, 2H, H-13$\alpha\beta$), 1.28 (m, 1H, H-14α), 1.65 (m, 1H, H-14β), 1.22 (s, 3H, Me-16), 1.22 (s, 3H, Me-17), 1.56 (br s, 3H, Me-18), 1.56 (br s, 3H, Me-19), 1.56 (br s, 3H, Me-20), 4.40 (d, 1H, J = 7.2 Hz, H-1′), 3.56 (dd, 1H, J = 7.2, 9.4 Hz, H-2′), 3.61 (d, 1H, J = 3.2, 9.4 Hz, H-3′), 3.89 (d, 1H, J = 2.2 Hz, H-4′), 3.65 (t, 1H, J = 6.4 Hz, H-5′), 4.32 (dd, 2H, J = 3.4, 6.9 Hz, H-6′ab), 2.06 (s, 3H, $OCOCH_3$), 2.36 (br s, 1H, OH-2′), 2.81 (br s, 1H, OH-3′), 2.61 (br s, 1H, OH-4′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.7 (d, C-1), 28.1 (t, C-2), 125.9 (d, C-3), 133.5 (s, C-4), 38.8 (t, C-5), 24.7 (t, C-6), 125.7 (d, C-7), 133.1 (s, C-8), 37.8 (t, C-9), 24.0 (t, C-10), 125.0 (d, C-11), 134.2 (s, C-12), 39.4 (t, C-13), 28.2 (t, C-14), 81.4 (s, C-15), 23.8 (q, C-16), 24.8 (q, C-17), 15.6 (q, C-18), 15.3 (q, C-19), 15.5 (q, C-20), 97.2 (d, C-1′), 72.2 (d, C-2′), 73.3 (d,

C-3′), 68.3 (d, C-4′), 71.9 (d, C-5′), 62.8 (t, C-6′), 20.8 (q, OCO*C*H$_3$), 171.0 (s, O*C*OCH$_3$); LRFABMS (3-NBA) *m/z* [M + Li]$^+$ 501 ($C_{28}H_{46}O_7Li$), [sugar + Li]$^+$ 229 ($C_8H_{14}O_7Li$); HR-FABMS (3-NBA) *m/z* [M + Li]$^+$ obsd 501.3400, $C_{28}H_{46}O_7Li$ required 501.3404.

Calyculaglycoside E (7): colorless oil; $[\alpha]^{24}_D$ +11.5° (*c* 2.5, $CHCl_3$); IR (neat) 3406, 2919, 1737, 1368, 1240, 1050, 907, 753 cm^{-1}; UV λ_{max} (MeOH) nm (log ϵ) 204 (3.97), 220 (3.72); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (m, 1H, H-1), 2.19 (m, 1H, H-2α), 1.87 (m, 1H, H-2β), 5.06 (t, 1H, *J* = 6.5 Hz, H-3), 2.12 (m, 2H, H-5$\alpha\beta$), 2.17 (m, 2H, H-6$\alpha\beta$), 4.91 (t, 1H, *J* = 6.0 Hz, H-7), 1.95 (m, 2H, H-9$\alpha\beta$), 2.10 (m, 2H, H-10$\alpha\beta$), 4.99 (t, 1H, *J* = 6.1 Hz, H-11), 2.02 (m, 2H, H-13$\alpha\beta$), 1.27 (m, 1H, H-14α), 1.63 (m, 1H, H-14β), 1.21 (s, 3H, Me-16), 1.21 (s, 3H, Me-17), 1.54 (s, 3H, Me-18), 1.54 (s, 3H, Me-19), 1.54 (s, 3H, Me-20), 4.43 (d, 1H, *J* = 7.6 Hz, H-1′), 3.32 (t, 1H, *J* = 8.4 Hz, H-2′), 3.54 (t, 1H, *J* = 8.9 Hz, H-3′), 3.38 (t, 1H, *J* = 9.1 Hz, H-4′), 3.44 (ddd, 1H, *J* = 2.6, 5.5, 8.9 Hz, H-5′), 4.33 (dd, 1H, *J* = 5.2, 12.0 Hz, H-6′a), 4.29 (dd, 1H, *J* = 1.9, 12.0 Hz, H-6′b), 2.07 (s, 3H, OCOC*H*$_3$), 2.90 (br s, 1H, OH-2′), 3.90 (br s, 1H, OH-3′), 4.09 (br s, 1H, OH-4′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.8 (d, C-1), 28.2 (t, C-2), 125.9 (d, C-3), 133.4 (s, C-4), 38.9 (t, C-5), 24.7 (t, C-6), 125.7 (d, C-7), 133.0 (s, C-8), 37.9 (t, C-9), 24.0 (t, C-10), 124.8 (d, C-11), 134.3 (s, C-12), 39.4 (t, C-13), 28.2 (t, C-14), 81.4 (s, C-15), 24.1 (q, C-16), 24.6 (q, C-17), 15.6 (q, C-18), 15.3 (q, C-19), 15.5 (q, C-20), 96.8 (d, C-1′), 73.9 (d, C-2′), 76.5 (d, C-3′), 70.5 (d, C-4′), 73.4 (d, C-5′), 63.8 (t, C-6′), 20.8 (q, OCO*C*H$_3$), 171.4 (s, O*C*OCH$_3$); LRFABMS (3-NBA) *m/z* [M + Li]$^+$ 501 ($C_{28}H_{46}O_7Li$), [sugar + Li]$^+$ 229 ($C_8H_{14}O_7Li$); HRFABMS (3-NBA) *m/z* [M + Li]$^+$ obsd 501.3389, $C_{28}H_{46}O_7Li$ required 501.3404.

Acid Hydrolysis of Calyculaglycoside D (6). A suspension of glycoside **6** (10.2 mg) in 2 N aqueous HCl (0.5 mL) was immersed in an oil bath preheated at 75 °C and stirred for 1 h. After this period, the reaction mixture was allowed to cool to room temperature, diluted with water, and extracted with ether (2 × 1 mL). Evaporation of the solvent followed by silica gel (0.5 g) column chromatography (eluting with 8:1 hexane−EtOAc) gave 1.5 mg of pure (+)-nephthenol (**4**) as established by comparison of the GLC-MS, $[\alpha]_D$, and co-TLC with an authentic sample. Modifications to this procedure led typically to concurrent decomposition of the aglycon.[7]

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References and Notes

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(4) When compared to (+)-nephthenol (**4**), the ^{13}C NMR signals due to the aglycon portion of glycosides **6**−**10** revealed an almost perfect match. Only those resonances assigned to C-1, C-15, C-16, and C-17 seemed to deviate slightly. Naturally, these small variations in chemical shifts can be explained by the presence in **6**−**10** of a nearby monosaccharide attached to the aglycon through C-15.

(5) In our first analysis of the HMBC spectra for calyculaglycoside B (CLG-B) we mistakenly reported a 3-bond heterocorrelation between C-7 (δ_H 47.7) in structure **2** and the Me-18 protons appearing at δ_H 1.56 (see ref 1). On reexamination of the HMBC data, such a correlation turned out to be artifactual. Furthermore, we failed to notice the strong HMBC correlations between C-7 and the very distant geminal methyl protons labeled as Me-16 and Me-17 (each at δ_H 1.22) in structure **2**. Clearly, the latter correlations are inconsistent with the proposed structure. In retrospect, these errors led us to an incorrect structure assignment for the aglycon portion of CLG-B. In addition, two intense ion peaks at *m/z* 109.1036 (36%) and 69.0697 (86%) in the HREIMS of CLG-B, corresponding to fragments of the molecular formulas of C_8H_{13} and C_5H_9, respectively, were erroneously attributed to a C_8 alkyl side chain such as that depicted in structures **1**−**3**.

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(7) As reported in ref 1, we had attempted to isolate the free aglycon of calyculaglycosides A−C before by HCl hydrolysis, but our method resulted in unexpected decomposition of the aglycon. In the present work, we modified our original hydrolysis protocol and established a milder procedure that allows the aglycon to be isolated without undue decomposition (see Experimental Section).

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