Isolation and Structure Elucidation of Feeding Deterrent Diterpenoids from the Sea Pansy, *Renilla reniformis*

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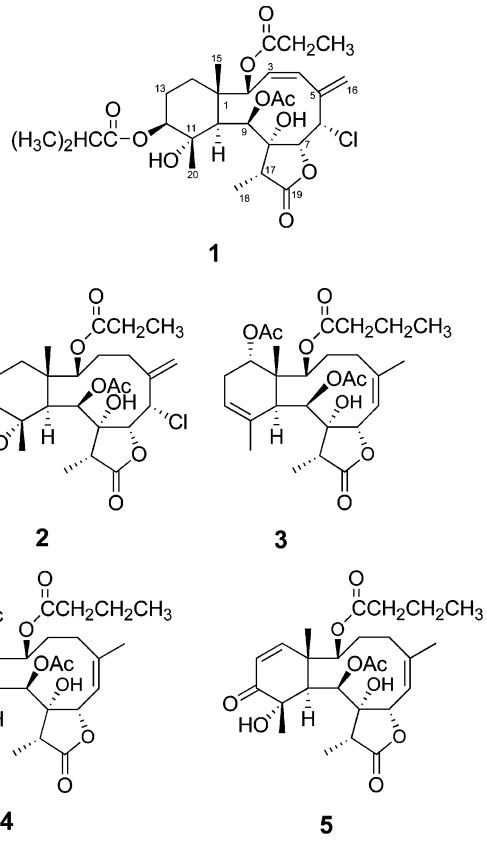
Four new briarane diterpenoids, renillins A−D (**1**−**4**), and the known compound renillafoulin C (**5**) were isolated from the sea pansy, *Renilla reniformis*. The structures and relative stereochemistry were elucidated by the interpretation of spectroscopic data. Using laboratory feeding assays, all compounds tested at natural concentrations significantly deterred feeding by the predatory lesser blue crab, *Callinectes similis*, while renillins C (**3**) and D (**4**) also deterred feeding by the predatory mummichog fish, *Fundulus heteroclitus*. Renillins A (**1**) and B (**2**) were found to possess an oxygenation pattern without precedent in this skeletal class.

Marine invertebrates of the subclass Octocorallia (phylum Cnidaria, class Anthozoa) are a rich source of briarane diterpenoids, of which over 300 compounds differing in oxidation patterns and ester substituents have been reported to date.[1,2] Many of these metabolites contain interesting biological activity that ranges from cytotoxic[3] and antiinflammatory[4] to the ability to protect their source organism from potential predators.[5,6]

The soft-bodied, umbrella-shaped sea pansy, *Renilla reniformis*, occurs inter/subtidally on the sandy beaches of the coastal Southeastern United States.[7] Sea pansies are brightly colored and exposed to predators at the sand−water interface; yet the specialist nudibranch, *Armina tigrina*, is its only documented predator.[8] While a prior study on the chemistry of a North Carolina population of *R. reniformis* resulted in the isolation of the antifouling briarane diterpenoids, the renillafoulins (e.g., **5**),[9,10] no antifeedant chemical defenses have been described for *R. reniformis*. In our continuing effort to characterize the antipredator defensive strategies of marine invertebrates,[11] we have used bioassay-guided fractionation to lead to a number of new briarane diterpenoids from *R. reniformis* that appear to protect the sea pansy from predation by fish and crabs. The isolation, structure elucidation, and antifeedant properties of four new briarane diterpenoids, renillins A−D (**1**−**4**), and one known compound, renillafoulin C (**5**), are reported below.



Results and Discussion

Sea pansies were collected by hand at low tide from exposed sand bars in Wassaw Sound, Georgia. Animals were placed in seawater and returned to the laboratory where volumes and wet masses were recorded before being frozen for storage. Frozen animals were exhaustively extracted with 2:1 methanol/dichloromethane. Fractionation of the crude extract was guided by feeding assays using the marine fish *Fundulus heteroclitus* and the crab *Callinectes similis,* which afforded an unpalatable fraction containing the known compound renillafoulin C (**5**). The structure of **5** was confirmed by low-resolution MS analysis and by comparison of its ^{1}H and ^{13}C NMR spectral data to literature values.[9] Additional metabolites in the deterrent fraction were purified by repetitive reversed-phase HPLC. Inspection of the ^{1}H NMR and low-resolution MS data of these metabolites and comparison with the data of **5** suggested that they are briarane diterpenoids but not of the renillafoulin series.

Renillin A (**1**) was isolated as a white powder that gave an $[M + Na]^+$ ion with m/z 607.2277 in the HRESIMS, appropriate for the molecular formula $C_{29}H_{41}ClO_{10}Na$. Inspection of the IR spectrum revealed absorbances indicative of a hydroxyl functionality (3400 cm^{-1}), a γ-lactone (1778 cm^{-1}), and an ester carbonyl (1729 cm^{-1}). The structure and relative stereochemistry of **1** were determined by ^{1}H, ^{13}C, COSY, HMQC, HMBC, and ROESY

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Table 1. ^{1}H NMR Chemical Shifts of Renillins A−D (**1**−**4**) (multi, *J*) at 500 MHz in DMSO-d_6

carbon no.	**1**	**2**	**3**	**4**
2	6.04 (d, *10*)	5.02 (br s)	4.94 (d, *10.0*)	4.85 (d, *6.5*)
3	5.66 (dd, *12,10*)	2.43 (m)/1.67 (m)	1.72 (td, *15,5*)/1.53 (m)	2.83 (m)/1.45 (m)
4	6.01 (d, *12*)	2.48[a]/2.30 (m)	2.63 (m)/2.47 (m)	2.43 (m)/1.78 (m)
6	5.25 (br, m)	5.03 (s)	5.36 (m)	5.42 (d, *9.5*)
7	4.95 (d, *4.0*)	5.60 (br s)	5.32 (s)	5.25 (d, *10.0*)
9	5.51 (d, *5.5*)	5.24 (br s)	5.74 (br s)	5.82 (d, *3.0*)
10	2.61 (d, *5.0*)	2.50[a]	2.94 (br s)	2.84 (d, *3.5*)
12	4.72 (br m)	4.69 (br m)	5.34 (m)	2.86
13	*β*: 1.78 (m)	1.78(m)/1.50 (m)	2.20 (m)/1.80 (m)	2.08 (m)/1.97 (m)
	α: 1.51 (m)			
14	*β*: 1.43 (m)	1.50 (m)/1.16 (m)	4.59 (s)	4.43 (m)
	α: 1.38 (m)			
15	0.90 (s)	0.99 (s)	0.85 (s)	0.84
16	*pro-E*: 5.67 (d, *2.5*)	5.60/5.38 (br s)	1.91 (s)	1.94 (s)
	pro-Z: 5.94 (br, s)			
17	2.49[a]	2.52 (m)	2.65 (q, *10.0*)	2.71 (q, *7.0*)
18	0.94 (d, *7.0*)	1.05 (d, *5.0*)	1.00 (d, *10.0*)	1.01 (d, *7.5*)
19				
20	1.37 (s)	1.29 (m)	1.89 (s)	1.49 (s)
OH-8	5.94	5.33 (s)	5.97 (s)	6.15 (s)
OH-11	6.35 (br, s)	5.74 (br s)		
C-2 ester				
	2.32 (m)	2.37 (q, *5.0*)	2.20 (m)	2.15 (m)
	1.03 (t, *7.5*)	1.05 (t, *5.0*)	1.50 (m)	1.47 (m)
			0.88 (t, *5.0*)	0.86 (t, *7.5*)
C-9 acetate				
	2.15 (s)	2.16 (s)	1.83 (s)	2.19 (s)
C-12/14 ester				
	2.47	2.51 (m)	2.15 (s)	1.81 (s)
	1.11 (d, *7.5*)[b]	1.10 (d, *5.0*)		
	1.10 (d, *7.5*)[b]	1.10 (d, *5.0*)		

[a] Under solvent peak at 2.49 ppm. [b] Interchangeable.

Table 2. ^{13}C NMR Chemical Shifts of Renillins A−D (**1**−**4**) at 100 MHz in DMSO-d_6[c]

carbon no.	**1**	**2**	**3**	**4**
1	44.0[a]	41.8 br	43.9	42.9
2	79.5	79.6	74.5	74.9
3	130.0	27.9	31.6	31.0
4	127.8	27.0	39.5[b]	28.4
5	138.0	n.o.	142.9	143.6
6	61.2	65.0 br	119.8	119.7
7	78.9	73.5	78.8	78.5
8	81.9	81.1	81.2	81.4
9	69.7	79.0 br	68.0[b]	69.2
10	42.2	42.2	39.5[b]	38.3
11	74.5	73.5	135.2	57.7
12	74.7	74.8	119.4	59.3
13	21.8	22.9	26.0	24.5
14	34.0	32.4	73.0	72.9
15	14.9	17.0 br	14.0	14.7
16	114.6[b]	118.7	27.5	27.2
17	45.5	46.0 br	42.8	43.1
18	6.9	8.0 br	6.8	6.9
19	175.5	175.2	176.3	176.3
20	23.1	23.6	24.0	26.3
C-2 ester	173.0	173.6	172.1	172.1
	27.1	27.0	35.6	35.6
	9.0	9.1	17.7	17.7
			13.5	13.5
C-9 acetate	170.7	170.2	170.1	170.6
	22.5	21.5	21.3	21.4
C-12/14 ester	174.7	176.0	170.2	169.7
	34.0	33.7	21.3	21.2
	18.7	18.8		
	18.7	18.8		

[a] From HMBC spectrum. [b] From HMQC spectrum. [c] br = broad signal. n.o.= signal not observed.

NMR spectral experiments. All protons (Table 1) were assigned to their respective carbons (Table 2) by HMQC data, leaving two hydroxyl protons at δ 6.35 and 5.94. The HMQC and ^{13}C NMR spectra displayed resonances for four carbonyls at δ 175.5, 174.7, 173.0, and 170.7; four olefinic carbons at δ 138.0, 130.0, 127.8, and 114.6; one carbon at δ 61.2, bonded to chlorine;[4,12,13] and six carbons bonded to oxygen at δ 81.9, 79.5, 78.9, 74.7, 74.5, and 69.7. The four carbonyls and two carbon−carbon double bonds accounted for all but three degrees of unsaturation required by the molecular formula, requiring a tricyclic structure for **1**. Further inspection of the one- and two-dimensional NMR data led to the identification of acetate, propionate, and isobutyrate groups. An HMBC correlation from the allylic carbinol methine proton resonance at δ 6.04 (H-2) to the carbonyl signal at δ 173.0 located the propionate group at C-2, while an HMBC correlation from the carbinol methine proton resonance at δ 5.51 (H-9) to the carbonyl signal at δ 170.7 located the acetate group at C-9. No HMBC correlation was observed to the isobutyrate carbonyl at δ 174.7; however, once the remaining structural features of **1** had been deduced, several factors made it logical to assign the isobutyrate group to the secondary carbon C-12 (δ 74.7; ^{1}H δ 4.72). First, H-7 (δ 4.95) and H-12 (δ 4.72) were likely to be attached to the two remaining ester groups due to their downfield chemical shifts, eliminating C-8 and C-11 from consideration as sites of connectivity to the esters. Second, the connectivity of carbonyl C-19 to carbinol C-7 via oxygen appeared the most logical solution, as these two carbons represented the two ends of the diterpene biosynthetic precursor and because of consistencies with previously discovered briaranes (e.g., **5**).[9] Thus, the two hydroxyl groups were assigned to the tertiary carbons C-8 (δ 81.9) and C-11 (δ 74.5), with their specific assignments established by ROESY correlations, vide infra.

The $\Delta^{3,5(16)}$ diene was assigned through HMBC and COSY correlations. Resonances for two olefinic methines at δ 5.66 (H-3) and 6.01 (H-4) coupled in the COSY spectrum and, as indicated in Figure 1, H-3 displayed an HMBC cross-peak with the quaternary carbon C-5 (δ 138.0). The substitution of the diene was established by

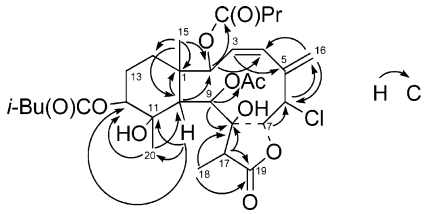

Figure 1. Selected HMBC correlations of renillin A (**1**).

an HMBC correlation from the H-16 olefinic methylene resonance (δ 5.67) to the chlorinated methine C-6 (δ 61.2) and by a COSY correlation between H-3 (δ 5.66) and the allylic propionoxy methine H-2 (δ 6.04). From the chlorinated methine H-6 (δ 5.25), a COSY correlation was observed with the carbinol methine H-7 (δ 4.95). This connection was reinforced by an HMBC correlation from H-7 to C-6 (δ 61.2).

The attachment of Me-15 to the ring juncture carbon C-1 was confirmed by an HMBC correlation from the singlet proton resonance of Me-15 (δ 0.90) to the quaternary carbon at δ 44.0 (C-1) (Figure 1). Further HMBC correlations from Me-15 to C-2 (δ 79.5), C-10 (δ 42.2), and C-14 (δ 34.0) assigned the carbon centers that flank C-1 and connected C-1 to the diene. The ring juncture C-1−C-10 was confirmed through HMBC cross-peaks between the methine H-10 (δ 2.61) and carbons C-1 (δ 44.0) and C-2 (δ 79.5). A COSY correlation between H-10 (δ 2.61) and H-9 (δ 5.51) continued the assignment of the γ-lactone-fused 10-membered ring, and an HMBC correlation from the acetoxy methine H-9 (δ 5.51) to the oxygenated tertiary carbon C-8 (δ 81.9) facilitated entry into the γ-lactone system. The doublet Me-18 proton signal at δ 0.94 (J = 7.0) and a methine resonance, which was eclipsed by the solvent peak at δ 2.49 (H-17), were coupled in the COSY spectrum and displayed HMBC correlations to the oxygenated tertiary carbon C-8 (δ 81.9) and to the carbonyl C-19 (δ 175.5). No other HMBC correlations were observed to the lactone carbonyl C-19. Once the remaining cyclohexane system was assigned, the closures of the 10-membered ring and γ-lactone were inferred from chemical shift and biosynthetic arguments that satisfied the degree of unsaturation requirements, the IR spectral data, and the ROESY data (vide infra).

From the ring juncture methine resonance at δ 2.61 (H-10), HMBC correlations were observed to the oxygenated tertiary carbon C-11 (δ 74.5), the isobutyroxy methine C-12 (δ 74.7), and the remaining methyl group Me-20 (δ 23.1). The Me-20 singlet at δ 1.37 showed HMBC correlations to C-10 (δ 42.2) and to the isobutyroxy methine C-12 (δ 74.7), which logically attached Me-20 to the tertiary C-11. From H-12 (δ 4.72), a COSY correlation was observed to both methylene protons at δ 1.78 and 1.51 (H-13β and H-13α, respectively). In turn, these methylene resonances showed COSY correlations with the methylene protons H-14β (δ 1.43) and H-14α (δ 1.38). The cyclohexane moiety was completed through a HMBC correlation from the Me-15 protons (δ 0.90) to the methylene C-14 (δ 34.0), completing the gross structure of **1**.

Analysis of ROESY data (Figure 2) determined the relative stereochemistry of **1**. As per convention when analyzing the relative stereochemistry of briarane diterpenoids, Me-15 was assigned to the β-face, anchoring the relative stereochemical analysis. The olefin Δ^3 was assigned the Z configuration due to a characteristic $^3J_{H-H}$ coupling constant of 12 Hz and a dipolar correlation between the olefinic proton resonances at δ 5.66 (H-3) and 6.01 (H-4). On the β-face, ROESY correlations were observed between

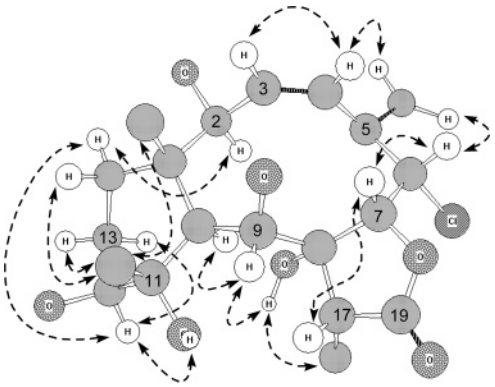

Figure 2. 3D stereochemical representation of renillin A (**1**) with selected ROESY correlations. Ester groups and methyl-15, 18, and 20 protons have been removed for clarity.

the Me-15 proton resonance at δ 0.90 and the methyl group Me-20 (δ 1.37), which correlated with methylene resonances H-13β (δ 1.78) and H-14β (δ 1.43). On the α-face, OH-11 (δ 6.35) showed a ROESY correlation with the adjacent methine H-12 (δ 4.72), which likewise displayed correlations with methylene resonances H-13α (δ 1.51) and H-14α (δ 1.38). These correlations established a gauche relationship between the oxygenated substituents of C-11 and C-12 and led to the attachment of the remaining hydroxyl group OH-8 (δ 5.94) at C-8. The OH-8 assignment was supported by α-face ROESY correlations between the ring juncture methine resonance H-10 (δ 2.61) and the acetoxy methine resonance at δ 5.51 (H-9), which further displayed dipolar coupling with OH-8 (δ 5.94), itself coupled with the methyl resonance at δ 0.94 (Me-18). Support for the γ-lactone and 10-membered ring closures was obtained through the β-face ROESY correlations between H-17 (δ 2.49) and H-7 (δ 4.95) and between H-7 and H-6 (δ 5.25). ROESY cross-peaks between the olefinic methylene resonance at δ 5.67 (H-16) and the olefinic methine resonance at δ 6.01 (H-4) and between δ 5.94 (H-16) and H-6 assigned the signal at δ 5.67 to the *pro-E* position and the signal at δ 5.94 to *pro-Z* at C-16. A final correlation in the ROESY spectrum between the allylic propionoxy methine H-2 (δ 6.04) and methylene resonance H-14α (δ 1.38) completed the relative stereochemistry assignment of **1**.

Renillin B (**2**) gave an $[M + Na]^+$ ion in the HRESIMS with m/z 609.2470 appropriate for the molecular formula of $C_{29}H_{43}ClO_{10}Na$, two protons in excess of **1**. Comparison of the ^{1}H NMR spectrum of **2** with **1** revealed the conspicuous lack of two vicinally coupled methine signals between 5.0 and 6.0 ppm and the addition of extra methylene resonances between 1.1 and 2.4 ppm. The HMQC showed olefinic methylene resonances at δ 5.60 and 5.38 (H_2-16) that correlated to the same carbon C-16 (δ 118.7), which taken together with the latter observations suggested the reduction of the $\Delta^{3,5(16)}$ diene of **1** to the $\Delta^{5(16)}$ exocyclic olefin of **2**. Inspection of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) along with the two-dimensional data confirmed the presence of the acetate, propionate, and isobutyrate groups, and other shared structural feature of **2** with **1**. The broad resonances observed in both the ^{1}H and ^{13}C NMR spectra at 298 K strongly suggested a slow conformational interconversion of **2**; however, increasing the temperature to 333 K resulted in minimal peak sharpening. The inability to detect the C-5 olefinic center in the ^{13}C and HMBC spectra of **2** is attributed to the slow interchange between conformers, a phenomenon seen with other conformationally flexible briarane compounds.[14] The reduction of Δ^3 in **2** was further supported by a COSY correlation between the propionoxy methine H-2 (δ 5.02) and two methylene

resonances H-3 (δ 2.43, 1.67) that correlated to the same carbon C-3 (δ 27.9) in the HMQC spectrum. The latter methylene signals were further coupled in the COSY spectrum to another set of methylene resonances at δ 2.48 and 2.30 (H_2-4), which in turn were correlated in the HMQC spectrum to a single carbon resonance at δ 27.0 (C-4). The presence of the exocyclic olefin $\Delta^{5(16)}$ was supported by allylic couplings between the methylene signals at δ 2.48 and 2.30 (H_2-4) and the olefinic methylene resonances at δ 5.60 and 5.38 (H_2-16), respectively, which likewise were both allylically coupled to H-6 (δ 5.03). No HMBC correlations were observed between the carbinol methine protons and the ester carbonyls, and the ester attachments of **2** were assumed to be equivalent to that of **1**. Isomeric briarane diterpenoids based on ester interchange are rare; however, literature examples[11,14] reveal that such an interchange would lead to concerted proton chemical shift differences between the interchanged esters, a phenomenon not observed with **1** and **2**, which have esters with similar proton chemical shifts; thus, the acylation pattern and, by extension, the relative stereochemistry of **2** were hypothesized to mirror that seen in **1**. Despite the structural diversity observed within the briarane family, to our knowledge all briarane diterpenoids reported to date have in common an oxidized C-14 center. Renillins A (**1**) and B (**2**), with fully reduced C-14 centers, possess an oxidation pattern of the cyclohexane system without precedent in this skeletal class.

Renillin C (**3**) gave an $[M + Na]^+$ ion in the HRESIMS with m/z 543.2562, appropriate for the molecular formula of $C_{28}H_{40}O_9Na$. Relative to the ^{1}H NMR spectra of **1**, **2**, and **5**, the resonances of **3** suggested substantial differences in the skeletal oxidation pattern. The IR spectrum confirmed the presence of the γ-lactone (1770 cm^{-1}), and all protons were assigned to their respective carbons through the inspection of the HMQC data (Tables 1 and 2), revealing the presence of one hydroxyl group (δ 5.97) and four carbons not bonded to hydrogen (δ 142.9, 135.2, 81.2, 43.9). Inspection of the ^{13}C NMR spectrum indicated four carbonyls (δ 176.3, 172.1, 170.2, 170.1) that, through further analysis of the COSY and HMBC data, were assigned to the γ-lactone, two acetate moieties, and an *n*-butyrate group. The presence of four deshielded ^{13}C resonances (δ 142.9, 135.2, 119.8, and 119.4) and, in the ^{1}H NMR spectrum, two vinyl methyl groups (δ 1.91 and 1.89) and two vinyl protons (δ 5.34 and 5.36) suggested the presence of two trisubstituted olefins in **3**. Together, the olefins, carbonyls, and a tricyclic briarane skeleton accounted for all degrees of unsaturation.

The oxidation pattern of **3** was assigned by chemical shift data and analysis of the COSY and HMBC spectra. HMBC correlations from the methyl proton resonance at δ 1.91 (Me-16) to C-5 (δ 142.9) and to C-6 (δ 119.8) and further correlations from the second deshielded methyl proton signal at δ 1.89 (Me-20) to C-11 (δ 135.2) and to C-12 (δ 119.4) established the olefins. The methylene resonances at δ 1.72 and 1.53 (H_2-3) displayed HMBC cross-peaks with the quaternary olefinic center C-5 (δ 142.9), thereby positioning one of the trisubstituted olefins within the 10-membered ring, while a HMBC correlation from the ring juncture methine H-10 (δ 2.94) to the quaternary olefinic center C-11 (δ 135.2) positioned the other trisubstituted olefin in the cyclohexane system. The acylation pattern of **3** was anchored by HMBC correlations from the ring junction resonances to the carbinol methines. The methyl protons at δ 0.85 (Me-15) showed HMBC cross-peaks with resonances at δ 73.0 (C-14) and 74.5 (C-2), and an HMBC correlation was observed from the ring junction methine H-10 (δ 2.94) to C-9 (δ 68.0). Further HMBC correlations positioned the ester groups. The acetoxy methine H-14 (δ 4.59) displayed a HMBC correlation to a carbonyl at δ 170.2, while the butyroxy methine H-2 (δ 4.94) showed a HMBC correlation to the carbonyl at δ 172.1. The second acetate was attached to carbinol methine C-9 (δ 68.0), as in **1**. A strong ROESY correlation from the acetoxy methine H-14 (δ 4.59) to the β-face methyl group protons Me-15 (δ 0.85) led to the assignment of H-14 to the β-face, mirroring the stereochemistry observed in **1**, **2**, and **5**, thus completing the structure of **3**. The gross structure of **3** was found to be similar to that of an unnamed minor constituent of the sea pen *Stylatulide* sp., which contained an acetate group at C-2 as opposed to the *n*-butyrate of **3**.[12]

Renillin D (**4**) gave an $[M + H]^+$ ion in the HRESIMS with m/z 537.2717 appropriate for the molecular formula $C_{28}H_{41}O_{10}$, one oxygen atom more than the constitution of **3**. A comparison between the ^{1}H NMR spectra of **3** and **4** revealed the loss of the deshielded singlet at δ 5.34, the addition of a proton at δ 2.86, and a Me-20 proton shift from δ 1.89 in **3** to 1.49 in **4**. The IR spectrum supported the identification of the γ-lactone (1779 cm^{-1}). All protons were assigned to their respective carbons through the inspection of the HMQC experiment (Tables 1 and 2), which revealed the presence of one hydroxyl group (δ 6.15), and four carbons not bonded to hydrogen (δ 143.6, 81.4, 57.7, 42.9). Inspection of the ^{13}C NMR spectrum indicated four carbonyls (δ 176.3, 172.1, 170.6, 169.7) that through further analysis of the COSY and HMBC data could be assigned to the γ-lactone, two acetate moieties, and an *n*-butyrate group. The ^{13}C NMR spectrum also revealed the presence of only one set of olefinic resonances (δ 143.6 and 119.7) and the addition of two resonances suggestive of an epoxide (δ 59.3 and 57.7). The olefin, carbonyls, and tricyclic system accounted for all but one degree of unsaturation, attributed to the presence of an epoxide. Assignment of the epoxide was undertaken through the HMBC correlations observed from the ring junction methine H-10 (δ 2.84) to an oxygenated tertiary carbon C-11 (δ 57.7) and from the Me-20 proton at δ 1.49 to the second oxygenated tertiary carbon C-12 (δ 59.3), incorporating the epoxide in the cyclohexane system. HMBC correlations attached the acyl substituents to C-2 (butyrate), C-9 (acetate), and C-14 (acetate). The stereochemistry of the chiral centers in the γ-lactone-fused 10-membered ring mirrored that seen in **3**, from inspection of the ROESY spectrum. In particular, a strong ROESY correlation between the Me-20 proton resonances at δ 1.49 and H-9 (δ 5.82) led to the assignment of the 11,12-epoxide with the α,α-configuration. 11α,12α-Epoxides in briarane diterpenoids typically display characteristic features shared by **4**, such as epoxide carbon chemical shifts below 60 ppm and deshielded ring juncture proton signals.[4,12,13]

Although they are generally hypothesized to act as chemical defenses,[6,7] not all of the briarane diterpenes of *R. reniformis* were unpalatable to a predatory fish. Only compounds **3** and **4** deterred feeding by the mummichog fish, *F. heteroclitus*. When tested in artificial foods at natural concentrations, each reduced feeding by 40% ($p < 0.05$).[15] However, all isolated compounds deterred the lesser blue crab *C. similis*, with **1** and **2** each reducing food consumption by 70% ($p < 0.01$), and **3**, **4**, and **5** reducing food consumption by 50% ($p < 0.05$), 40% ($p < 0.05$), and 100% ($p < 0.001$), respectively. When each compound was assayed at a common concentration of 0.060 mM, a similar predator-specific pattern was observed, with only **3** and **4** deterring feeding by the predatory fish, whereas all five

compounds deterred the crab.[15] The generalist predators *F. heteroclitus* and *C. similis* were chosen as test predators due to their overlapping distribution with *R. reniformis*. *R. reniformis*, while conspicuous at the sand−water interface, is known to have only one predator, the specialist nudibranch *Armina tigrina*,[8] suggesting that the chemical defenses discovered herein are likely to be effective at protecting *R. reniformis* from generalist predators in its natural habitat.

Octocorals are a rich source of briarane diterpenoids. Much of this chemical diversity can be attributed to intraspecific variation. In this study, four new briarane diterpenoids, including two with novel oxygenation patterns for their skeletal class, were discovered from a Georgia population of the sea pansy, *R. reniformis*. These four compounds and a fifth previously identified diterpenoid all deterred feeding by the lesser blue crab at natural concentrations, whereas only two of the five compounds deterred the predatory mummichog fish. As compounds **3**, **4**, and **5** share the same oxidation and substitution pattern of their γ-lactone-fused 10-membered rings, the differences in deterrence are apparently caused by differences in the cyclohexane moiety. These results highlight the importance of testing multiple predator types and assaying at natural concentrations to understand more completely the potential defensive function of specific metabolites. Future studies that investigate how ecological function varies with the natural structural diversity of structural families are required to further our understanding of secondary metabolism.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Jasco P-1010 polarimeter. IR spectra were acquired on a 8400s Shimadzu FTIR. ^{1}H and two-dimensional NMR data were acquired on a Bruker Avance DRX 500 MHz spectrometer, while ^{13}C NMR spectra were acquired on a Bruker AMX-400 spectrometer at 100 MHz. NMR spectra were acquired in DMSO-d_6 and referenced to DMSO-d_5 (δ^1H 2.49; δ^{13}C 39.5). High-resolution mass spectra were acquired on a QSTAR-XL hybrid QqTOF tandem mass spectrometer. HPLC separation was performed with a Waters 2690 separations module coupled to a Waters 996 photodiode array detector, utilizing a Zorbax SB-C18 column (9.4 mm × 250 mm). The 3D stereochemical representation of renillin A (**1**) was constructed with ChemBats3D Pro 6.0, which performs MM2 energy minimizations with a modified Allinger force field. Figure 2 represents a local energy minimum for **1** and was generated through a MM energy minimization procedure (291 iterations; no solvent parameters specified), followed by multiple perturbations with subsequent minimizations. The 3D structure was created from an initial structure of **1** containing the hypothesized relative stereochemistry as supported by the ROESY data, and thus it represents one thermodynamically stable population of **1** for which the observed ROESY correlations are feasible.

Animal Material. During June and July 2002 and 2003, the sea pansy, *Renilla reniformis,* was collected by hand at low tide from exposed sand bars off Wassaw (31°54′ N; 80°55′ W), Little Tybee (31°57′ N; 80°56′ W), and South Cabbage Islands (31°57′ N; 80°58′ W), Georgia. *R. reniformis* is the only sea pansy that inhabits the Southeastern United States, and it was readily identified by comparison to literature descriptions.[7] A voucher specimen (REN052002) is held at the School of Biology, Georgia Institute of Technology.

Bioassay. Aquarium feeding assays using the predatory mummichog fish *Fundulus heteroclitus* and lesser blue crab *Callinectes similis* were performed as previously described,[16,17] with extracts, fractions, and compounds tested at the concentrations at which they were isolated. Pure compounds were also tested at their natural concentrations as determined by HPLC[15] and at 0.060 mM. Extracts, fractions, or compounds were presented to consumers in artificial foods containing squid meat at a concentration that nutritionally mimicked the sea pansy. Consumption data for treated versus control foods were analyzed by Fisher's exact test.

Extraction and Isolation. Frozen specimens of *R. reniformis* (500 g wet weight) were extracted three times in 600 mL of methanol/dicholoromethane (2:1) for 24 h. The extract was filtered and concentrated in vacuo, and 1.8 g (of 15.9 g extract) was subjected to solvent partitioning (25 mL of methanol/water (95:5) vs 3 × 50 mL of ethyl acetate). The combined ethyl acetate extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo to give a dark orange fraction (425 mg) that was loaded onto a Sephadex LH-20 column (40:10:4 EtOAc/methanol/water) and separated into five fractions (A−E), pooled by common TLC characteristics. Fraction C (172.5 mg) was chromatographed over a C_{18} silica Waters Sep-Pak (10 g) utilizing a step gradient from methanol/water (1:1) to 100% methanol. The 7:3 and 4:1 eluants were combined (61 mg) based on common TLC properties and subjected to repetitive isocratic reversed-phase HPLC with methanol/water mixtures, monitoring at 209 nm, to yield renillin A (**1**) (2.0 mg; 0.015% of dry weight; 0.08 mM), renillin B (**2**) (1.5 mg; 0.011% of dry weight; 0.060 mM), renillin C (**3**) (1.5 mg; 0.011% of dry weight; 0.060 mM), renillin D (**4**) (1.7 mg; 0.013% of dry weight; 0.060 mM), and renillafoulin C (**5**) (4.7 mg; 0.036% of dry weight; 0.20 mM).

Renillin A (1): amorphous white powder; $[\alpha]^{25}_D$ −92° (*c* 0.02 g/100 mL, $CHCl_3$); IR (thin film) ν_{max} 3400, 1778, and 1729 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data, Tables 1 and 2, respectively; LRESIMS *m/z* 607.3 $[M + Na]^+$ (35); HRESIMS $[M + Na]^+$ *m/z* 607.2277 (calcd for $C_{29}H_{41}ClO_{10}Na$, 607.2286).

Renillin B (2): amorphous white powder; $[\alpha]^{25}_D$ −52° (*c* 0.015 g/100 mL, $CHCl_3$); IR (thin film) ν_{max} 3356, 1777, and 1726 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data, Tables 1 and 2, respectively; LRESIMS *m/z* 609.2 $[M + Na]^+$ (15); HRESIMS $[M + Na]^+$ *m/z* 609.2470 (calcd for $C_{29}H_{43}ClO_{10}Na$, 609.2442).

Renillin C (3): amorphous white powder; $[\alpha]^{25}_D$ −89° (*c* 0.015 g/100 mL, $CHCl_3$); IR (thin film) ν_{max} 3443, 1770, and 1732 cm^{-1};^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data, Tables 1 and 2, respectively; LRESIMS *m/z* 543.3 $[M + Na]^+$ (90); HRESIMS $[M + Na]^+$ *m/z* 543.2562 (calcd for $C_{28}H_{40}O_9Na$, 543.2570).

Renillin D (4): amorphous white powder; $[\alpha]^{25}_D$ −42° (*c* 0.017 g/100 mL, $CHCl_3$); IR (thin film) ν_{max} 3394, 1779, and 1729 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data, Tables 1 and 2, respectively; LRESIMS *m/z* 537.3 $[M + H]^+$ (15); HRESIMS $[M + H]^+$ *m/z* 537.2717 (calcd for $C_{28}H_{41}O_{10}$, 537.2700).

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Supporting Information Available: A table of HMBC correlations for renillins A−D (**1**−**4**) along with ^{1}H, ^{13}C, COSY, HMQC, HMBC, and ROESY NMR spectra of **1** and ^{1}H and ^{13}C NMR spectra of **2**−**4** are available free of charge via the Internet at http://pubs.acs.org.

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