Terpenoids from the Sponge *Aplysilla glacialis* and Specimens of the Nudibranch *Cadlina luteomarginta* Found on the Sponge

Mark Tischler and Raymond J. Andersen*

Departments of Chemistry and Oceanography, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

M. Iqbal Choudhary† and Jon Clardy*

Department of Chemistry-Baker Laboratory, Cornell University, Ithaca, New York 14853

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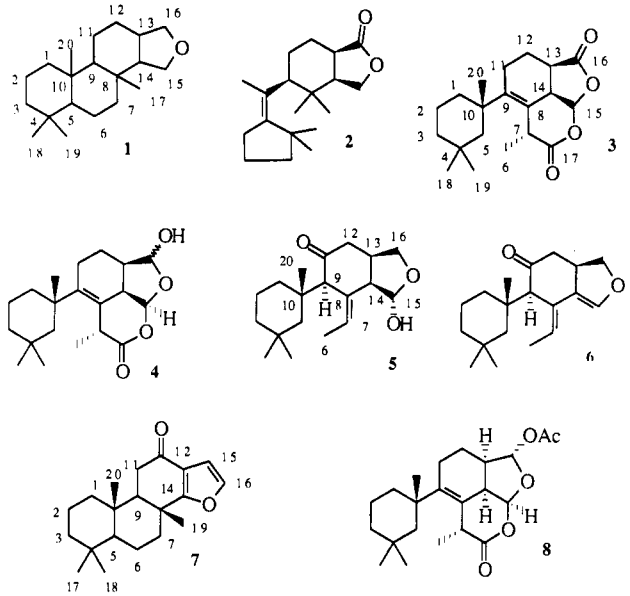
Four new rearranged and/or degraded "spongian" terpenoids and one new "marginatane" diterpenoid have been isolated from the marine sponge *Aplysilla glacialis*. The structure of cadlinolide A (3) was solved by X-ray diffraction analysis, and the structures of cadlinolide B (4), aplysillolide A (5), aplysillolide B (6), and marginatone (7) were determined by extensive spectroscopic analysis and chemical interconversions. Glaciolide (2), cadlinolide A (3), and tetrahydroaplysulphurin-1 (8) were isolated from skin extracts of specimens of the dorid nudibranch *Cadlina luteomarginata* found feeding on *A. glacialis*.

A fascinating collection of highly functionalized terpenoids with intact spongian (1) carbon skeletons,[1] or with rearranged and/or degraded carbon skeletons that are biogenetically attainable from hypothetical spongian diterpenoid precursors, have been isolated from marine sponges[1-3,7] and nudibranches.[4,7] The distribution of spongian-derived metabolites spans many species in a number of sponge genera including several species in the genus *Aplysilla*.[3] Most of the nudibranchs from which spongian-derived terpenoids have been isolated are sponge-eating tropical species belonging to the genus *Chromodoris*.[3b,4a-c]

We have a continuing interest in the skin chemistry of the northeastern Pacific dorid nudibranch *Cadlina luteomarginata*,[5] a close relative of tropical nudibranchs in the genus *Chromodoris*. *C. luteomarginata* sequesters metabolites from sponges that make up its diet and stores them in glands on its dorsum where they are used for defensive purposes.[5,6] The skin chemistry of *C. luteomarginata* varies rather dramatically from collecting site to collecting site, reflecting the variation in species composition and secondary metabolite content of its sponge diet.[5,6] We recently collected a small number of specimens of *C. luteomarginata* in a surge channel on Sanford Island, Barkley Sound, B.C., where they were found feeding on the encrusting sponge *Aplysilla glacialis*. The structure of glaciolide (2), a major constituent of extracts from the Sanford Island collection of both *C. luteomarginata and A. glacialis*, has already been communicated.[7] We now report the isolation of four new spongian-derived terpenoids, cadlinolide A (3), cadlinolide B (4), aplysillolide A (5), and aplysillolide B (6), and one marginatane diterpenoid, marginatone (7), from the Sanford Island population of *A. glacialis*, as well as the isolation of glaciolide (2), cadlinolide A (3), and tetrahydroaplysulphurin-1 (8) from the specimens of *C. luteomarginata* found on the sponge.

A. glacialis and specimens of *C. luteomarginata* were collected by hand using scuba in an exposed surge channel (0 to −3 m) on the west side of Sanford Island. Freshly collected sponge and the nudibranchs were immediately immersed in methanol in separate containers. The methanol extracts from *A. glacialis* were concentrated in vacuo, and the residue was partitioned between brine and ethyl acetate. Concentration of the ethyl acetate layers in vacuo gave a dark green oil that was fractionated by



†On leave from H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan.

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Table I. ^{1}H NMR Data (400 MHz, $CDCl_3$) for Cadlinolide A (3), Cadlinolide B (4), and Tetrahydroaplysulphurin-1 (8) (Chemical Shifts in ppm from Internal TMS)

	cadlinolide A (3)			cadlinolide B (4)			tetrahydroaplysulphurin-1 (8)	
proton	δ	COSY correlations	NOE's[a]	δ	COSY Correlations	NOE's	δ	COSY correlations
H5	1.72							
H5′	1.78							
Me6	1.48, d, J = 7.4	H7	H7, H14, H15	1.41, 4, J = 7.4	H7		1.42, d, J = 7.4	H7
H7	4.28, q, J = 7.4	Me6		4.20, 1, J = 7.4	Me6	Me6, Me20	4.21, 1, J = 7.4	Me6
H11	2.35, bd, J = 7.9	H11′, H12, H12′		2.36, m	H11′, H12, H12′		2.36, m	H11′, H12, H12′
H11′	2.19, m	H11, H12, H12′, H14		2.04, m	H11, H12, H12′		2.09, m	H11, H12, H12′, H14
H12	2.06, m	H11, H11′, H12′, H13		1.92, m	H11, H11′, H12′, H13		1.90, m	H11, H11′, H12′, H13
H12′	1.69, m	H11, H11′, H12, H13		1.20, m	H11, H11′, H12, H13		1.28, m	H11, H11′, H12, H13
H13	3.12, dt, J = 7.9, 4.6	H12, H12′, H14	H14, H15	2.40, m	H12, H12′, H14, H16		2.52, m	H12, H12′, H14, H16
H14	3.48, m	H11′, H13, H15	H13, H15, Me6	3.23, m	H13, H15	H15, H13, Me6	3.22, m	H11′, H13, H15
H15	6.16, d, J = 5.3	H14	H13, H14	6.05, d, J = 6.2	H14	H14	6.00, d, J = 6.2	H14
H16	–	–	–	5.39, d, J = 3.9	H13		6.18, d, J = 2.4	H13
Me18	0.77, s*	Me19		0.77, s			0.78, s*	
Me19	0.92, s*	Me18		0.92, s*			0.91, s*	
Me20	1.13, s			1.13, s			1.13, s	
OAc							2.08 s	

[a] Resonance in proton column irradiated. * Interchangable.

repeated silica gel chromatography to give pure samples of terpenoids 2[7] to 7.

Cadlinolide A (3), isolated as optically active colorless crystals from hexane (mp 126–7 °C, 0.006% dry wt), gave a parent ion in the EIHRMS at m/z 332.1983 Da appropriate for a molecular formula of $C_{20}H_{28}O_4$ (ΔM –0.5 mmu). Well-resolved resonances for all 20 carbon atoms were apparent in the ^{13}C NMR spectrum of cadlinolide A (see the Experimental Section) and an APT experiment[8] indicated that all 28 hydrogen atoms were attached to carbon (4 × CH_3, 6 × CH_2, 4 × CH, 6 × C). ^{13}C NMR resonances at δ 170.0 and 173.3, in conjunction with IR bands at 1789 and 1760 cm^{-1}, revealed that the four oxygen atoms in cadlinolide A were present as two ester functionalities. The frequency of one of the ester carbonyl stretching vibrations (1789 cm^{-1}) suggested the presence of a γ-lactone. A deshielded ^{13}C NMR resonance observed at δ 99.4 (CH) in the spectrum of 3 was assigned to a ketal functionality. Since cadlinolide A contained only four oxygen atoms, the alkoxy oxygens of the two esters had to be attached to the ketal carbon. The final unsaturated functionality that could be identified from the ^{13}C NMR data of 3 was a tetrasubstituted double bond (δ 118.8 (C), 147.3 (C)). Four rings had to be incorporated into the structure of cadlinolide A to account for the remaining sites of unsaturation required by the molecular formula.

The ^{1}H NMR spectrum of cadlinolide A (3) was well dispersed and extremely informative (Table I). Even though a number of additional fragments could be confidently identified from the ^{1}H NMR data, it was not possible to unambiguously assign a structure to cadlinolide A using only spectroscopic arguments. Therefore, the structure of cadlinolide A was solved by single-crystal X-ray diffraction analysis. A computer generated perspective drawing of the final X-ray model is presented in Figure 1. Hydrogens have been omited for clarity, and since the X-ray experiment did not define the absolute configuration, the enantiomer shown represents an arbitrary choice. There were two molecules in the asymmetric unit, and as they did not differ significantly in conformation, only one is shown. The cyclohexane ring has a chair conformation with C18 and C9 in axial positions. The cyclohexene ring adopts a half chair conformation, while the δ-lactone has a chair conformation with C6 axial. With the structure of cadlinolide A (3) in hand, it was a routine operation to assign the ^{1}H NMR data as indicated in Table I.

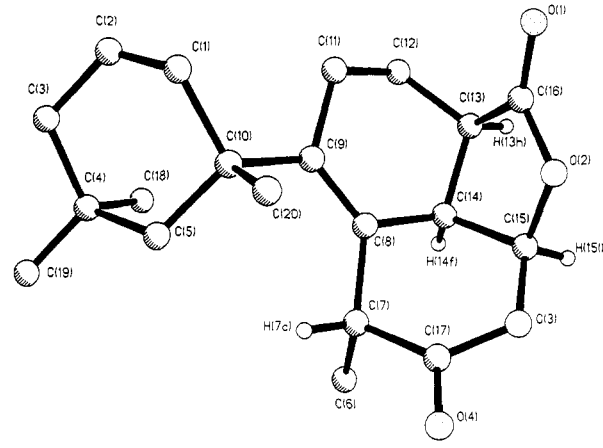

Figure 1. A computer-generated perspective drawing of the final X-ray model of cadlinolide A (3). No absolute configuration is implied.

Cadlinolide B (4), isolated as a colorless oil, gave a parent ion at m/z 334.2152 Da in the EIHRMS appropriate for a molecular formula of $C_{20}H_{30}O_4$ (ΔM +0.8 mmu) containing two hydrogen atoms more than the molecular formula of cadlinolide A (3). Examination of the ^{13}C NMR, ^{1}H NMR, and IR data obtained from cadlinolide B (4)

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Table II. ^{1}H NMR Data (400 MHz, $CDCl_3$) for Aplysillolide A (5) and Aplysillolide B (6) (Chemical Shifts in ppm from Internal TMS)

	aplysillolide A (5)			aplysillolide B (6)		
proton	δ	COSY correlations	NOE's[a]	δ	COSY correlations	NOE's[a]
Me6	1.65, dd, J = 6.8, 2.4	H7, H14	H7, H9	1.65, d, J = 7.2	H7	H7, H9
H7	5.80, dd, J = 2.3, 6.8	Me6	Me6, H15	5.75, q, J = 7.2	Me6	Me6, H15
H9	3.11, s			3.11, s		Me6, Me20
H12	2.18, dd, J = 11.5, 16.6	H12′, H13	H12′, H13, H16	2.73, dd, J = 13.3, 6.3	H12′, H13	H12′, H13
H12′	2.36, dd, J = 5.5, 16.6	H12, H13	H12, H13	2.49, dd, J = 13.6, 11.9	H12, H13	H12
H13	2.88, m	H12, H12′, H14, H16, H16′	H12, H12′, H16	3.27, m	H12, H12′, H14, H16, H16′	H12, H16
H14	3.04, m	Me6, H13, H15				
H15	5.63, d, J = 2.3	H14	H7	6.33, d, J = 2.4	H13	
H16	4.23, dd, J = 6.4, 8.7	H13, H16′	H13, H16′	4.60, t, J = 9.3	H16′, H13	H13, H16′
H16′	3.54, dd, J = 3.9, 8.7	H13, H16	H12, H12′, H15, H16	3.92, dd, J = 9.1, 10.9	H16, H13	H16, H12′
Me18	0.88, s*			0.88, s*		
Me19	0.97, s*			0.96, s*		
Me20	1.13, s			1.12, s		H9, H12′, H15

[a] Resonance in proton column irradiated. * Interchangable.

revealed that it was simple derivative of cadlinolide A (3); the C16 γ-lactone carbonyl in 3 having been reduced to a lactol functionality in 4. The ^{13}C NMR spectrum of 4 (see the Experimental Section) showed only a single ester carbonyl resonance at δ 171.7 and two ketal carbon resonances at δ 101.8 and 102.6, while the IR spectrum of 4 showed only a single carbonyl stretching band at 1730 cm^{-1} and a strong OH stretching band at 3369 cm^{-1}. Many of the resonances in the ^{1}H NMR spectrum of cadlinolide B (4) showed minor shadow peaks attributed to the existence of an equilibrium mixture of two epimers at C16. Resonances at δ 6.05 (H15) and 5.39 (H16) were assigned to the ketal protons in the major epimer. Correlations in the COSY spectrum of 4 provided connectivities from one ketal resonance (H16, δ 6.05) around the ring to the second one (H15, δ 5.39) through two intervening methine resonances (δ 3.23, H14, and δ 2.40, H13) in accord with the presence of the γ-lactol. NOE experiments (Table I) confirmed that the relative stereochemistries at C7, C13, C14, and C15 were identical with those in cadlinolide A (3). Determination of the relative configurations at C16 in the major and minor epimers proved to be problematic. NOE experiments, involving irradiation of the H16 protons in both epimers, failed to show enhancements that could be used to define the stereochemistry at C16. In addition, the magnitude of the H13/H16 vicinal coupling constant in the two epimers (5.4 and 3.9 Hz) was so similar that it proved impossible to base an assignment of configuration on this information.

Treatment of cadlinolide B (4) with acetic anhydride and pyridine converted it into a single monoacetate 8 that was identical with the known compound tetrahydroaplysulphurin-1.[2a] Comparison of the ^{1}H and ^{13}C NMR data that we collected on cadlinolide B acetate to the data reported for tetrahydroaplysulphurin-1 revealed a nearly exact correspondence in the ^{13}C data but substantial differences in the ^{1}H data. Examination of an authentic ^{1}H NMR spectrum of tetrahydroaplysulphurin-1, kindly provided by Professor Cambie, showed that the two molecules were identical and that the reported ^{1}H NMR data for tetrahydroaplysulphurin-1[2a] was erroneous and needed be corrected as listed in Table I.

Aplysillolide A (5), isolated as an optically active colorless oil, gave a $M^+ + 1$ ion at m/z 307 Da in the CIMS and a $M^+ - H_2O$ ion at m/z 288.2088 Da ($C_{19}H_{28}O_2$ ΔM −0.1 mmu) in the EIHRMS appropriate for a molecular formula of $C_{19}H_{30}O_3$. The ^{13}C NMR spectrum of 5 showed resonances for the 19 carbons (see the Experimental Section) indicated by the mass spectrum and an APT experiment revealed that 29 of the protons in the molecule were attached to carbon atoms (4 × CH_3, 6 × CH_2, 5 × CH, 4 × C).[8] An OH stretching band at 3420 cm^{-1} in the IR spectrum confirmed that the remaining proton was part of a hydroxyl. Resonances which could be assigned to trisubstituted olefin (δ 125.3 (CH), 132.1 (C)) and saturated ketone (δ 212.8 (C)) functionalities were present in the ^{13}C NMR spectrum of aplysillolide A. The absence of evidence for additional unsaturated functional groups implied that the molecule had to be tricyclic in order to satisfy its unsaturation number.

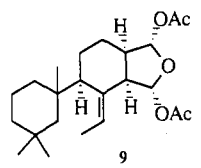
The nature of the functional group incorporating the two non-ketone oxygen atoms of 5 was identified from the ^{13}C NMR spectrum. A resonance at δ 102.6 (CH) was assigned to a ketal carbon and a resonance at δ 71.1 (CH_2) was assigned to a methylene carbon singly bonded to an oxygen atom. Since there were only three oxygen atoms total in 5, both the hydroxyl oxygen and the oxygen attached to the methylene carbon had to be attached to the ketal carbon to form a hemiketal functionality. A resonance at δ 5.63 (H15) in the ^{1}H NMR spectrum of 5 was assigned to the methine proton on the hemiketal carbon and a pair of resonances at δ 4.23 and 3.54 (H16, H16′) were assigned to the geminal protons on the methylene carbon singly bonded to the hemiketal oxygen atom (Table II). Correlations in the COSY spectrum of 5 linked the ketal methine resonance (H15) to the methylene proton resonances (H16, H16′) through two intervening methine resonances that were observed at δ 3.04 (H14) and 2.88 (H13) (Table II). These COSY correlations indicated that the hemiketal functionality was part of a γ-lactol. Vicinal coupling between an olefinic methyl resonance (δ 1.65 Me6) and an olefinic proton resonance (δ 5.80 H7) indicated that the methyl and the olefinic proton were geminal substituents on the trisubstituted olefin in aplysillolide A. Homoallylic coupling observed in the COSY spectrum between the olefinic methyl protons (δ 1.65 Me6) and the methine proton resonating at δ 3.04 (H14) required that the fully substituted carbon of the trisubstituted olefin functionality had to be attached to the methine carbon (C14). This attachment of the trisubstituted olefin to the

γ-lactol in **5** gave a substructure which closely resembled the ring C and D functionality in the previously reported metabolites 9,11-dihydrogracilin A (**9**)[2d] and gracilin A (**10**).[2i]

The remaining functionality indicated in the spectral data of aplysillolide A (**5**) could also be accommodated on the basic tricyclic framework present in **9** and **10**. COSY correlations showed that the γ-lactol methine proton resonating at δ 2.88 (H13) was further coupled to a pair of geminal methylene proton resonances at δ 2.18 and 2.36, which were assigned to H12 and H12′ (Table II). The lack of additional coupling into the H12 and H12′protons, and their downfield chemical shifts, located the ketone functionality at C11. A singlet resonance at δ 3.11, assigned to the H9 methine proton, was in accord with the placement of the ketone at C11. The chemical shifts of the resonances assigned to the carbon atoms (δ 37.4 (C1), 18.9 (C2), 38.9 (C3), 31.1 (C4), 49.1 (C5), 40.6 (C10), 27.4 (Me), 35.9 (Me), 23.9 (Me)) and the methyl protons (δ 0.88 (Me), 0.97 (Me), 1.13 (Me)) in ring A of aplysillolide A (**5**) were in excellent agreement with the carbon (δ 36.2 (C1), 19.2 (C2), 39.0 (C3), 31.1 (C4), 50.3 (C5), 39.0 (C10), 27.5 (Me18), 36.0 (Me19), 24.0 (Me20)) and proton (δ 0.89 (Me), 0.96 (Me), 1.03 (Me)) assignments reported for 9,11-dihyrogracilin A (**9**).[2d] NOE enhancements (Table II) were consistent with the relative configurations shown at C13, C14, and C15 in **5** and with the *Z* configuration of the $\Delta^{7,8}$ double bond.

It was not possible to directly determine the relative configurations at C9 and C10 in aplysillolide A (**5**) by spectroscopic means. In particular, the shape of the cyclohexane ring containing the C11 ketone and $\Delta^{7,8}$ olefin functionalities prevented the use of NOE to establish the configuration at C9 relative to C13 and C14. In an attempt to circumvent this problem, aplysillolide A (**5**) was reduced with LAH, and the resulting triols were acetylated and chromatographed to give the major triacetate **11**. COSY and NOE experiments were used to establish the structure of **11** and to assign its ^{1}H NMR spectrum (see the Experimental Section). However, NOE experiments also failed to unambiguously determine the relative stereochemistry at C9 in **11**.

Aplysillolide B (**6**) was obtained in very low yield as an oil that gave a parent ion in the EIHRMS at *m/z* 288.2043 Da consistent with a molecular formula of $C_{19}H_{28}O_2$ (ΔM +0.5 mmu), differing from the molecular formula of aplysillolide A (**5**) simply by loss of H_2O. The ^{1}H NMR spectrum of aplysillolide B (**6**) (Table II) readily confirmed that it was simply the dehydration product of aplysillolide A (**5**). An olefinic methyl resonance at δ 1.65 and an olefinic methine resonance at δ 5.75 could be routinely assigned to the Me6 and H7 protons by comparison of their chemical shifts with the corresponding resonances in **5**. A five spin system identified in the COSY spectrum of **6** was assigned to H12, H12′ (δ 2.73, 2.49), H13 (δ 3.27) and H16, H16′ (δ 4.60, 3.92). The resonance assigned to H13 (δ 3.27) in **6** failed to show vicinal coupling into an aliphatic resonance corresponding in chemical shift to the H14 resonance in aplysillolide A (**5**); however, it did show a long-range allylic coupling into an olefinic resonance at δ 6.33 (d, *J* = 2.4 Hz), which was assigned to H15. A strong NOE observed between H7 and H15 confirmed the assignments and established that the 7,8 olefin also had the *Z* configuration in aplysillolide B (**6**). Resonances assigned to the H9, Me18, Me19, and Me20 protons in the ^{1}H NMR spectrum of **6** had chemical shifts virtually identical with those of their counterparts in the spectrum of **5** (Table III). A weak NOE enhancement was observed in H15 upon



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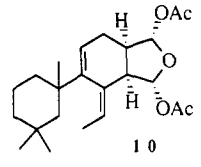

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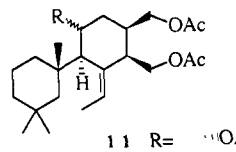
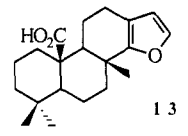


11 R= OAc

12 R= OAc



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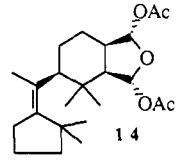

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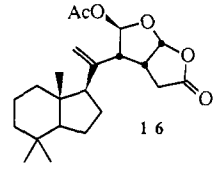

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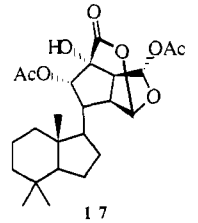

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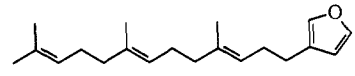

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irradiation of the Me20 resonance. Drieding models indicated that this result was only possible if ring C in aplysillolide B (**6**) adopted a chairlike conformation in which H13 and C10 had axial orientations and, therefore, H9 and H13 had the cis relative stereochemistry expected from a spongian biogenetic origin. We have assumed that the relative configurations at C9 and C13 are identical in aplysillolides A (**5**) and B (**6**) as shown because the chemical shifts of H9 and the ring A methyl protons are nearly identical in the two compounds (Table II).

Marginatone (**7**), obtained as an optically active colorless oil, gave a parent ion in the EIHRMS at *m/z* 300.2093 Da corresponding to a molecular formula of $C_{20}H_{28}O_2$ (ΔM +0.4 mmu). Resonances at δ 106.2 (CH), 118.2 (C), 142.2 (CH), and 161.7 (C) in the ^{13}C NMR spectrum (see the Experimental Section) and at δ 6.59 (d, *J* = 2 Hz) and 7.26 (d, *J* = 2 Hz) in the ^{1}H NMR spectrum of **7** (Table III) were assigned to a disubstituted furan ring. The relative chemical shifts of the two furan protons, in conjunction with the observation of NOE's and a 2-Hz scalar coupling between them, demonstrated that they were α (δ 7.26) and β (δ 6.59) protons on adjacent carbons and, therefore, that the furan was 2,3-disubstituted. A ^{13}C NMR resonance at δ 195.2 (C) and an IR band at 1680 cm^{-1} were assigned to an α,β-unsaturated ketone functionality in **7**. The ketone had to be conjugated to the furan ring because there was no ^{13}C NMR evidence for additional olefinic functionalities in marginatone. Subtracting the four sites of unsaturation accounted for by the furan ring and the ketone carbonyl from the seven sites required by the molecular formula of **7** revealed that the molecule was tetracyclic.

The requirement of incorporating a 1,3-disubstituted furan ring into a tetracyclic diterpenoid metabolite could readily be satisfied by assuming that marginatone (**7**) had the marginatane carbon skeleton first encountered in the metabolite, marginatafuran (**12**), isolated from specimens

Table III. ^{1}H NMR Data (400 MHz, C_6D_6) for Marginatone (7) (Chemical Shifts in ppm from Internal TMS)

proton	δ	COSY correlations	NOE's[a]
$H6_{eq}$	1.38, m		
$H6_{ax}$	1.19, m		
$H7_{ax}$	1.35, m	H7′	
$H7'_{eq}$	2.06, dt, J = 12.2, 3.1	H7	Me19
H9	1.44, dd, J = 3.1, 13.5	H11, H11′, Me20	
$H11_{eq}$	2.45, dd, J = 3.1, 16.9	H9, H11′	
$H11'_{ax}$	2.21, dd, J = 13.6, 16.9	H9, H11	Me19, Me20
H15	6.63, d, J = 1.9	H16	H16
H16	6.78, d, J = 1.9	H15	
Me17	0.62, s		
Me18	0.70, s		
Me19	0.95, s		Me20
Me20	0.75, s		Me17, Me19

[a] Resonance in proton column irradiated.

of *C. luteomarginata* collected in the Queen Charlotte Islands.[5c] The ^{1}H NMR spectrum of **7** was in complete agreement with a marginatane type structure. Resonances at δ 1.90 (dd, J = 3.8, 12.9 Hz), 2.46 (dd, J = 12.9, 17.2 Hz), and 2.54 (dd, J = 3.8, 17.2 Hz) were assigned to a three spin system encompassing the protons at H9, $H11_{ax}$, and $H11_{eq}$, respectively. NOE's from the $H11_{ax}$ resonance at δ 2.46 and from a second resonance at δ 2.28 (dt, J = 12.8, 3.1 Hz), assigned to $H7_{eq}$, to a methyl singlet at δ 1.29 facilitated the assignment of the methyl resonance to the Me 19 protons. A NOE from $H11_{ax}$ (δ 2.46) to a second methyl singlet at δ 0.99 identified that signal as the Me20 resonance. A correlation between the resonances at δ 0.99 and 1.90 in the long-range COSY spectrum of **7** was attributed to *W* coupling between the Me20 protons and H9, providing support for the assignments. Irradiation of the Me20 resonance gave an NOE enhancement in a methyl resonance at δ 0.86, assigned to $Me17_{ax}$. The observed NOE's from $H11_{ax}$ to Me19 and Me20, the trans diaxial coupling constant observed between $H11_{ax}$ and $H9_{ax}$ (J = 12.9), the *W* coupling between H9 and Me20, and the observed NOE between Me20 and $Me17_{ax}$, established the relative stereochemistries at the centers C5, C10, C9, and C8 to be trans–anti–trans as shown in **7**.

The skin extracts of the *C. luteomarginata* specimens were concentrated, partitioned between water and ethyl acetate, and fractionated by silica gel flash chromatography in a manner identical with that described above for the purification of the *A. glacialis* metabolites. Only three terpenoids, glaciolide (**2**),[7] cadlinolide A (**3**), and tetrahydroaplysulphurin-1 (**8**) were found in the *C. luteomarginata* extracts. Glaciolide (**2**) and cadlinolide A (**3**) are major metabolites of *A. glacialis*; however, we were unable to detect any trace of **8** in the sponge extracts.

Our chemical studies of *A. glacialis* have shown it to be a very rich source of interesting new terpenoid chemistry. One of its major metabolites, glaciolide (**2**),[7] has the new rearranged and degraded "glaciane" carbon skeleton first encountered in metabolite **13** isolated from the Mediterranean sponge *Spongionella gracialis*.[9] Another *A. glacialis* metabolite, marginatone (**7**), is only the second sponge diterpenoid known to have the marginatane carbon skeleton.[10] The discovery of marginatone (**7**) in *A. Glacialis* points to a sponge in the genus *Aplysilla* as the most likely dietary source of the marginatafuran (**12**) originally isolated from *C. luteomarginata*.[5c] Aplysillolides A (**5**) and B (**6**) are notable for the presence of the ketone at C11, a center that becomes oxidized to a carboxylic acid functionality in several rearranged spongian derived terpenoids such as norrisolide (**14**)[4b] and chromodorolide A (**16**).[4a]

An intriguing question that arises from the co-occurrence of spongian and marginatane type diterpenoids in *A. glacialis*, is whether or not the furan residue of an acyclic precursor such as ambliofuran (**17**) intercepts an intermediate cation in the cyclization step to form the tetracyclic skeletons. Trapping with the unsubstituted β-carbon of the furan in **17** would lead to the spongian skeleton, while trapping with the α-carbon immediately adjacent to the side chain would lead to the marginatane skeleton. Compounds having the marginatane skeleton have been synthesized by cation-initiated cyclization of a derivative of ambliofuran (**17**);[11] however, no products containing the spongian skeleton were detected in the reaction. The exclusive formation of marginatane type products in the laboratory synthesis could be attributed to the much greater stability of the final cation formed by alkylation at the α–carbon of the furan. Enzymatic control might promote attack at the β-carbon to give the spongian skeleton or alternatively some 2,5-dihydrofuran derivative may terminate the cyclization in the biosynthesis of the spongians.

Our investigations of the predator/prey pair *C. luteomarginata* and *A. glacialis* have provided another example of the selective concentration of dietary sponge metabolites by a dorid nudibranch. However, the origin of the tetrahydroaplysulphurin-1 (**8**) found in the skin extracts of *C. luteomarginata* remains unclear. We have looked carefully for this metabolite in the extracts of several collections of *A. glacialis* from the Sanford Island surge channel, but we have been unable to find any trace of it. One possible explanation would be that *C. luteomarginata* has the ability to acetylate alcohols and that the nudibranch is simply converting cadlinolide B (**4**) into its acetate **8**. We are currently exploring this possibility.

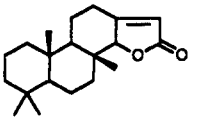
Experimental Section

Collection Data. *A. glacialis* was collected during all seasons in exposed surge channels of Sanford Island, Barkley Sound, B.C., at depths of 0 to −3 m. Immediately after collection, the sponge was immersed in methanol and stored at room temperature for up to three days. If the sponge was not worked up immediately, it was stored a low temperatures (−5 °C) until used (typically within 2 weeks). Intact specimens of *C. luteomarginata* found grazing on *A. glacialiis* were immersed in methanol immediately after collection and stored at −5 °C until needed.

Extraction and Chromatographic Separation. The methanol extract (4 L) of homogenized *A. Glacialis* tissue 1600 g (dry weight after extraction) was concentrated in vacuo and partitioned between brine (200 mL) and ethyl acetate (4 × 250 mL). The combined ethyl acetate layers were dried over an-

(9) Mayol, L.; Piccialli, V.; Sica, D. *Gaze. Chim. Ital.* **1988**, *118*, 559. We were not aware of this publication when we initially reported that glaciolide (**2**) was the first terpenoid having this new carbon skeleton.[7] Neither compound **13** or its carbon skeleton were given trivial names; therefore, we propose to continue using the name "glaciane" for the skeleton found in **2** and **13**.

(10) Compound **15** was recently reported from the sponge *A. polyrhaphis*, and it was described as the first example of a diterpenoid with the "isospongian" skeleton.[3d] The "isospongian" skeleton is identical with the "marginatane" skeleton first encountered in marginatafuran (**12**).[5c]



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(11) (a) Nishizawa, M.; Yamada, H.; Hayashi, Y. *Tetrahedron Lett.* **1986**, *27*, 187. (b) Nishizawa, M.; Yamada, H.; Hayashi, Y. *J. Org. Chem.* **1987**, *52*, 4878.

hydrous Na_2SO_4 and evaporated in vacuo to give 12.4 g (0.78%) of a crude oil. Flash chromatography (40 mm diameter column, 15 cm of silica gel, step gradient 100% hexanes to 100% ethyl acetate) yielded fractions containing fats, pigments, and terpenoids (red to bright pink charring TLC spots: vanillin–H_2SO_4 spray reagent). Combinations of repeated silica gel flash chromatography, silica gel radial chromatography, and normal-phase HPLC using hexane/ethyl acetate mixtures as eluents gave pure samples of the new terpenoids glaciolide (**2**), cadlinolide A (**3**), cadlinolide B (**4**), aplysillolide A (**5**), aplysillolide B (**6**), and marginatone (**7**).

The methanol extracts of *C. luteomarginata* were worked up in an identical manner to yield glaciolide (**2**), cadlinolide A (**3**), and tetrahydroaplysulphurin-1 (**8**).

Cadlinolide A (3): clear colorless needles; 94.3 mg (0.006% dry wt) recrystallized from hexane at 2 °C; mp 126–127 °C; $[\alpha]_D$ +19° ($CHCl_3$, *c* 8.0); IR (film) ν_{max} 2948, 2874, 1789, 1760, 1147, 984, 756 cm^{-1}; MS (EI) *m/z* (relative intensity) 332 (M^+, 3), 317 (1), 304 (2), 303 (2), 289 (2), 259 (4), 243 (4), 231 (4), 223 (4), 203 (4), 195 (5), 191 (4), 189 (4), 177 (7), 175 (4), 166 (5), 163 (5), 147 (10), 145 (8), 135 (9), 133 (11), 125 (12), 122 (9), 121 (13), 119 (14), 110 (13), 109 (25), 105 (20), 95 (23), 93 (15), 91 (26), 85 (30), 83 (45), 81 (19), 79 (17), 77 (15), 69 (65), 67 (19), 57 (17), 55 (48); ^1H NMR see Table I; ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.68 (d), 19.94 (t), 20.57 (t), 23.25 (t), 28.14 (s), 31.31 (t), 31.38 (d), 31.89 (d), 35.07 (d), 38.20 (t), 38.90 (d), 39.19 (t), 39.90 (d), 50.15 (t), 99.43 (d), 118.85 (s), 147.29 (s), 169.89 (s), 173.26 (s) ppm; EIHRMS *m/z* calcd for $C_{20}H_{28}O_4$ 332.1982, found 332.1983.

Cadlinolide B (4): clear colorless oil; yield 5.4 mg (0.0003% dry wt); IR (film) ν_{max} 3369, 2931, 1730, 1457, 1028, 606 cm^{-1}; MS (EI) *m/z* (relative intensity) 334 (M^+, 1), 316 (15), 301 (4), 262 (4), 206 (35), 178 (27), 177 (33), 175 (10), 163 (12), 149 (35), 147 (16), 137 (10), 135 (14), 133 (15), 125 (28), 124 (14), 121 (16), 109 (52), 95 (26), 91 (22), 83 (31), 81 (30), 69 (100), 67 (30); ^1H NMR see Table I; ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.53, 20.71, 24.20, 25.62, 28.06, 29.71, 31.33, 31.57, 32.65, 39.11, 39.36, 39.55, 40.81, 43.81, 50.99, 101.81, 102.62, 122.96, 146.28, 171.66 ppm; EIHRMS calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2152.

Aplysillolide A (5): colorless glass; yield 24.3 mg (0.002% dry wt); $[\alpha]_D$ +30° ($CHCl_3$, *c* 2.4); IR (film) ν_{max} 3421, 1701 cm^{-1}; MS (EI) *m/z* (relative intensity) 288 ($M^+ - H_2O$, 9), 182 (89), 164 (72), 136 (81), 121 (47), 107 (28), 91 (37), 83 (43), 69 (100), 55 (69); ^1H NMR see Table II; ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.69 (q), 18.94 (t), 23.86 (q), 27.35 (q), 31.08 (s), 35.37 (q), 35.86 (d), 37.41 (t), 38.88 (t), 40.62 (s), 42.74 (t), 49.07 (t), 50.41 (d), 62.47 (d), 71.24 (t), 102.60 (d), 125.28 (d), 132.07 (s), 212.64 (s) ppm; HRMS calcd for $C_{19}H_{28}O_2$ ($M^+ - H_2O$) 288.2090, found 288.2088.

Aplysillolide B (6): colorless oil; yield 15.6 mg (0.002% dry wt); IR (film) ν_{max} 2928, 2868, 1699, 1462, 1338, 1365, 1231, 1097, 911 cm^{-1}; MS (EI) *m/z* (relative intensity) 288 (M^+, 2), 164 (100), 134 (16), 121 (9), 69 (47); ^1H NMR Table II; MS EIHRMS *m/z* calcd for $C_{19}H_{28}O_2$ 288.2089, found 288.2084.

Marginatone (7): white solid; yield 9.5 mg (0.001% dry wt); $[\alpha]_D$ −16° ($CHCl_3$, *c* 0.4); IR (film) ν_{max} 2925, 2866, 1680, 1440, 1387, 1262, 1046, 719, 644, 617 cm^{-1}; MS (EI) *m/z* (relative intensity) 300 (M^+, 38), 285 (26), 258 (19), 243 (9), 203 (13), 201 (12), 189 (14), 187 (11), 176 (23), 175 (14), 164 (38), 163 (83), 162 (40), 161 (84), 150 (36), 149 (82), 148 (45), 147 (100), 137 (73), 136 (14), 135 (47), 133 (19), 127 (27), 121 (29), 119 (26), 109 (72), 108 (16), 107 (19), 95 (58), 93 (24), 91 (59), 83 (17), 81 (60), 79 (36), 77 (44), 69 (77), 69 (39), 65 (17), 44 (36); ^1H NMR see Table III; ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.06 (q), 17.94 (t), 18.26 (t), 20.52 (q), 21.30 (q), 33.24 (q), 35.32 (t), 35.31 (t), 37.42 (s), 39.32 (t), 41.83 (t), 56.03 (d), 56.48 (d), 106.18 (d), 118.18 (s), 142.25 (d), 161.73 (s), 195.19 (s) ppm; EIHRMS *m/z* calcd for $C_{20}H_{28}O_2$ 300.2090, found 300.2093.

Cadlinolide B Acetate (Tetrahydroaplysulphurin-1) (8). (i) Material isolated from *C. luteomarginata*: colorless oil; yield 12.8 mg (0.009% dry wt of nudibranch); $[\alpha]_D$ +65° ($CDCl_3$, *c* 1.5) [lit. value $[\alpha]_D$ +169° (*c* 1.0)[2a]]; IR (film) ν_{max} 2944, 1750, 1458, 1372, 1230, 995, 557 cm^{-1}; MS (DCI^-, NH_3) *m/z* (relative intensity) 394 ($M^+ + NH_4^+$, 79), 334 (100), 317 (71), 288 (16), 272 (30), 225 (7), 180 (18), 163 (45), 147 (24), 109 (12), 69 (10); ^1H NMR see Table I; ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.74 (q), 20.73 (t), 21.19 (q), 23.99 (t), 25.03 (t), 28.28 (q), 31.07 (q), 31.58 (s), 32.52 (q), 38.05 (d), 39.03 (t), 39.51 (t), 39.73 (s), 40.63 (d), 42.06 (d), 50.88 (t), 100.57 (d), 102.71 (d), 121.25 (s), 146.48 (s), 169.87 (s), 170.94 (s) ppm; EIHRMS calcd for $C_{22}H_{32}O_5$ 376.2250, found 376.2248. (ii) Treatment of cadlinolide B (**4**) with acetic anhydride and pyridine at room temperature overnight gave a quantitative yield of a single acetate that was identical by TLC and spectroscopic comparison with the tetrahyroaplysulphurin-1 (**8**) isolated from *C. luteomarginata*.

Reduction of Aplysillolide A (5). Aplysillolide A (**5**) (14.5 mg, 0.047 mmol) was dissolved in dry diethyl ether (1 mL) and added to a suspension of lithium aluminum hydride (15 mg) in dry diethyl ether (2 mL) at room temperature. After 0.5 h, the reaction was quenched by the addition of ethyl acetate (3 mL) and 0.5 N hydrochloric acid (2 mL). The solution was extracted with ethyl acetate (4 × 10 mL), and the combined ethyl acetate layers were dried over anhydrous sodium sulfate. Filtration and evaporation of the ethyl acetate yielded a white solid (13.2 mg) which was immediately dissolved in a mixture of pyridine (1 mL) and acetic anhydride (2 mL). After 14 h at 25 °C, the excess pyridine and acetic anhydride were removed in vacuo to give a mixture of triacetates **11** and its C11 epimer that were separated on preparative silica gel TLC. **Triacetate 11** (10.5 mg, 0.025 mmol, 53%): colorless oil; IR (film) ν_{max} 2947, 1741, 1444, 1369, 1235, 1034, 976, 605 cm^{-1}; MS (DCI^+, NH_3) *m/z* (relative intensity) 454 ($M^+ + NH_4^+$, 100); ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3 H), 0.98 (s, 3 H), 1.15 (s, Me20), 1.65 (d, *J* = 6.8 Hz, Me6), 2.02 (s, 3 H), 2.05 (s, 3 H), 2.07 (s, 3 H), 2.17 (dd, *J* = 13.5, 6.5 Hz, H12), 2.36 (dd, *J* = 11.9, 5.9 Hz, H13), 2.71 (d, *J* = 6.3 Hz, H9), 2.98 (m, H14), 3.89 (dd, *J* = 11.2, 6.8 Hz, H16), 4.00 (dd, *J* = 11.2, 7.1 Hz, H16′), 4.21 (dd, *J* = 11.2, 7.9 Hz, H15), 4.30 (dd, *J* = 11.2, 7.7 Hz, H15′), 5.15 (m, H11), 5.32 (q, *J* = 6.7 Hz, H7) ppm; EIHRMS calcd for $C_{23}H_{36}O_4$ ($M^+ - CH_3CO_2H$) 376.2613, found 376.2605.

X-ray Analysis of Cadlinolide A (3). Cadlinolide A (**3**) crystallized in the monoclinic crystal system with *a* = 8.819 (2), *b* = 11.857 (3), and *c* = 17.754 (3) Å, and β = 90.20 (2)° as determined from a least-squares fit of 30 diffractometer measured 2θ values. The systematic extinctions, optical activity, and crystal density were uniquely consistent with space group $P2_1$ with two molecules of composition $C_{20}H_{28}O_4$ forming the asymmetric unit (*Z* = 4). All unique diffraction maxima with $2\theta \leq 112°$ were collected using $2\theta - \theta$ scans and Cu Kα radiation. Of the 2567 reflections collected in this fashion, 2395 (93%) were judged observed ($|F_o| \geq 5\sigma(|F_o|)$ after coredtion for Lorentz, polarization, and background effects. The structure was solved without difficulty using direct methods. All non-hydrogen atoms were located on electron density synthesis following tangent formula refinement, and hydrogens were included at appropriate positions. Full-matrix least-squares refinements with anisotropic heavy atoms and fixed isotropic riding hydrogens have converged to a conventional crystallographic residual of 6.26% (wR = 6.61%). Additional crystallographic details are available as supplementary material.

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Registry No. **2**, 125482-27-1; **3**, 130246-91-2; **4**, 130221-21-5; **5**, 130221-22-6; **6**, 130221-23-7; **7**, 130221-24-8; **8**, 91236-89-4.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond lengths, bond angles, and torsional angles from the X-ray analysis of cadlinolide A (**3**) and difference NOE spectra on aplysillolide B (**6**) (9 pages). Ordering information is given on any current masthead page.