

**GLACIOLIDE, A DEGRADED DITERPENOID WITH A NEW CARBON SKELETON FROM
THE NUDIBRANCH *CADLINA LUTEOMARGINATA* AND THE SPONGE *APLYSILLA
GLACIALIS***

Mark Tischler and Raymond J. Andersen*
Departments of Chemistry and Oceanography
University of British Columbia
Vancouver, B.C.
CANADA V6T 1W5

Abstract *The structure of glaciolide (1), a degraded and rearranged diterpenoid having the new glaciene carbon skeleton, has been solved by extensive spectroscopic analysis of the parent compound and some simple chemical derivatives.*

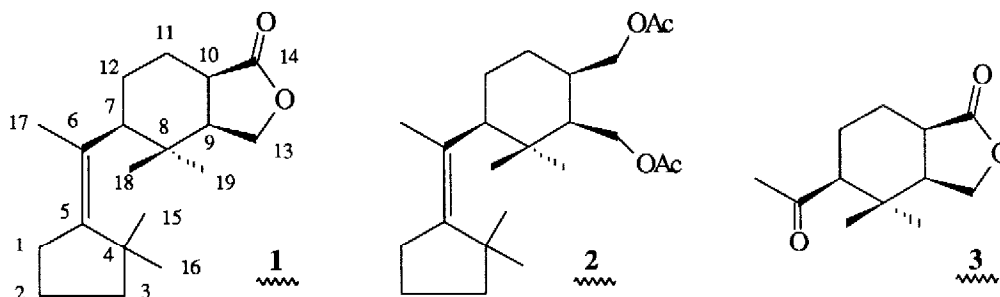
The dorid nudibranch *Cadlina luteomarginata* is relatively abundant in both intertidal and subtidal habitats along the west coast of North America from the Queen Charlotte Islands, British Columbia, in the north to Punta Eugenia, Baja California, in the south. *Cadlina* sequesters secondary metabolites from sponges in its diet and concentrates them in glands on its dorsum, where they are used to thwart predation.^{1,2} Consequently, the skin chemistry of *Cadlina* varies from one collecting site to another reflecting differences in the sponge fauna at various locations.

We have continued to make collections of *C. luteomarginata* in an attempt to learn more about the variation of its skin chemistry with locale. A collection of 27 individuals was recently made in an exposed surge channel (0 to -3m) on Sanford Island, Barkley Sound, B.C., where the nudibranchs were found feeding on the sponge *Aplysilla glacialis*. We now wish to report the structure of glaciolide (1), a rearranged and degraded spongian diterpenoid, that was found to be a major component in the skin extracts of the Sanford Island specimens of *C. luteomarginata* and also in the extracts of *A. glacialis*.

Freshly collected nudibranchs were immediately immersed in methanol. Concentration of the decanted methanol *in vacuo* gave an oily residue that was partitioned between water and ethyl acetate. Fractionation of the ethyl acetate soluble material by sequential application of silica gel flash (gradient: hexane to EtOAc) and silica gel radial thin layer (gradient: hexane to EtOAc/hexane 1:3) chromatographies gave pure glaciolide (1) (4.8mg; colorless needles from hexane, mp 102-103°C; $[\alpha]_D^{25} +18.9^\circ$ (c 0.14, Ethanol)).

Glaciolide (1) gave a parent ion in the HREIMS at m/z 290.2248 Da corresponding to a molecular formula of $C_{19}H_{30}O_2$ ($\Delta M +0.2$ mmu). The ^{13}C nmr spectrum of 1 showed resolved resonances for all of the nineteen carbon atoms indicated by the mass spectrum and an APT experiment³ demonstrated that all 30 protons were attached to carbon atoms (CH_3 X 5; CH_2 X 6; CH X 3; C X 5).⁴ A band at 1776 cm^{-1} in the IR spectrum of 1 and a ^{13}C nmr resonance at δ 178.9 (C) suggested that the two oxygen atoms were present as a γ lactone. Only two

olefinic carbon resonances were observed in the ^{13}C nmr spectrum of **1** (δ 145.2(C), 128.5(C)). This evidence established that the molecule contained a single tetrasubstituted olefin functionality and that the remaining three sites of unsaturation required by the molecular formula had to be rings.



The ^1H nmr spectrum of glaciolide (**1**), recorded in either benzene- d_6 or CDCl_3 (Table 1), was highly dispersed and extremely informative. A pair of deshielded methylene protons (δ 4.15 and 4.26), which had to be attached to the γ carbon (^{13}C nmr: δ 68.0 (CH_2)) of the lactone, served as a starting point from which one spin system (H13, H13' to H7) in a major fragment of glaciolide (**1**) (Figure 1) could be unravelled via the COSY data (Table 1). Double resonance experiments confirmed the assignments shown for this spin system and the coupling constants observed for H7 ($J=2.3, 12.0$ Hz) suggested that it was a methine proton having an axial orientation on a cyclohexane ring occupying a chair conformation. Long range COSY correlations observed between a methyl resonance at δ 0.93 (Me18) and the H7 (δ 2.48) and H9 (δ 2.23) resonances were assigned to W coupling, indicating that a quaternary carbon bearing an axial methyl had to be situated between the C7 and C9 carbons of this fragment. The observation of nOes between H7 and Me19, between H13 (δ 4.26) and Me18 and Me19, and between Me19 and H9 were consistent with the placement of a gem-dimethyl at C8 (Table 1). A long range coupling observed in the COSY spectrum between H10 and H12_{eq} was also assigned to W coupling, implying that H10 was equatorial. This assignment, in conjunction with the observation of a nOe between H7 and H9, fixed the relative stereochemistry about the cyclohexane ring as shown in Figure 1.

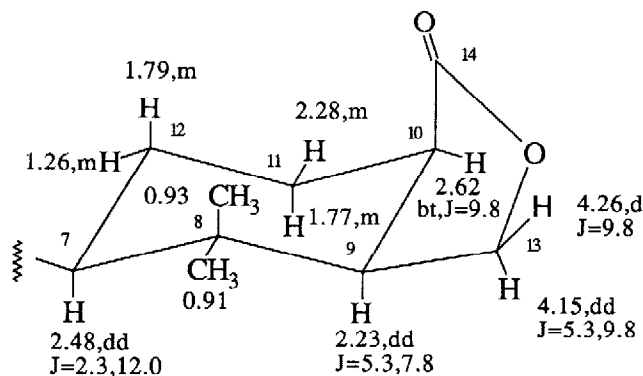
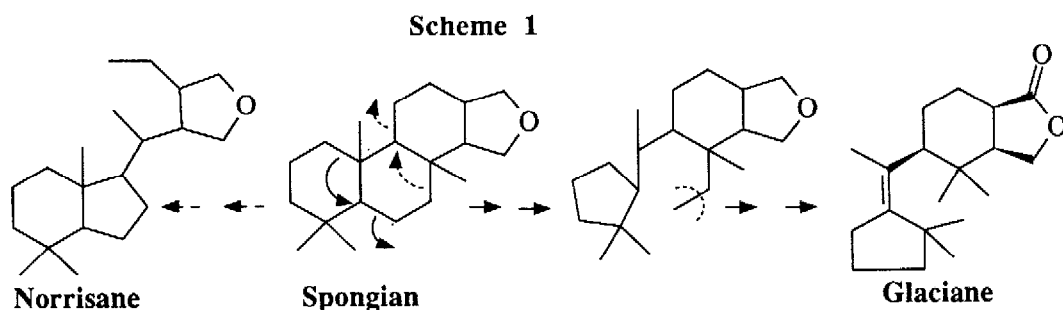


Figure 1

Support for the structure of the major fragment came from the transformation of glaciolide (**1**) into the diacetate **2**⁵ via LAH reduction followed by acetylation. The COSY spectrum of **2** (benzene-*d*₆) contained evidence for the presence of two acetoxymethyl groups (δ 4.32,dd(4.9,11.3) H13: 4.19,dd(2.0,11.3) H13': 4.24,bm H14 & H14': 1.76,s(AcO): 1.70,s(AcO)) which were linked by two methine carbons (δ 2.35,m H10: 1.76,m H9) as expected from the reduction of the γ lactone in **1**. Irradiation of the Me19 protons (δ 0.88) in **2** induced nOes in the Me18 protons (δ 0.70) and in H14 and H14' (δ 4.24bm) supporting the geminal relationship of Me18 and Me19 and also confirming the axial orientation of C14 as indicated in Figure 1.

The remaining portion of glaciolide (C₉H₁₅) had to contain a tetrasubstituted olefin (δ 145.2(C), 128.5(C)), an olefinic methyl (δ 1.49), two tertiary aliphatic methyls (δ 1.17,s; 1.23,s), a quaternary carbon, three methylenes and a single ring. Only two allylic methylene protons could be identified in the ¹H nmr spectrum of **1** (δ 2.2-2.3,m: H1,H1') and irradiation of a complex multiplet centered at δ 1.56 (H2,H2'; H3,H3') converted the allylic proton resonances into a pair of broad doublets typical of an AB spin system. The COSY spectrum of **1** showed strong correlations, assigned to homoallylic coupling, between the allylic protons (H1,H1') and both the Me17 protons and H7. Therefore, the olefinic methyl (Me17) and C7 of the major fragment (Figure 1) had to be geminal substituents on one of the olefinic carbons. Since there was evidence for only one pair of allylic methylene protons, the second substituent on the other olefinic carbon had to be the quaternary carbon bearing the two aliphatic tertiary methyls. Linking the allylic methylene to the quaternary carbon with the two remaining methylene carbons led to the constitution shown in **1** for glaciolide. The stereochemistry about the tetrasubstituted olefin was shown to be *Z* by the observation of intense nOes between H7 and the Me15 and Me16 protons, and between the Me17 protons and the allylic protons on C1. Final support for the proposed structure of glaciolide (**1**) came from its reaction with RuO₄ (CCl₄, rt, 2h) to give the degradation product **3**⁶ in excellent yield.



Glaciolide (**1**) has a new terpenoid carbon skeleton whose biogenesis from a spongian precursor can be envisaged as shown in Scheme 1. It is interesting to note that the cleavage (ring B) and contraction (ring A) steps proposed in Scheme 1 for the formation the "glaciolide" skeleton (solid arrows) mimic the cleavage (ring C) and contraction (ring B) steps (dashed arrows) proposed for the formation of the previously reported norrisane skeleton⁷ also from a spongian precursor. Spongian derived diterpenoids have previously been isolated from the skin extracts of many species of tropical nudibranchs, most of which belong to the genus *Chromodoris*.^{7,8} The discovery of glaciolide (**1**) in *C. luteomarginata* extends the known occurrence of these interesting metabolites to cold temperate nudibranchs.

Table 1. ¹ H nmr data for glaciolide (1). Recorded in CDCl ₃ at 400 MHz.			
Proton	δ(ppm from TMS)	COSY Correlations	nOes (resonance in Proton column irradiated)
H1	2.2-2.3,m	H2,H7,Me17	
H2	1.56,m	H1	H1,Me15,Me16
H3	1.56,m	-	
H7	2.48,dd(12.0,2.3)	H2,H12 _{ax} ,H12 _{eq}	H9,H11 _{ax} ,Me15,Me16,Me19
H9	2.23,dd(7.8,5.3)	H10,H13,H13'	
H10	2.62,bt(7.8)	H9,H11 _{ax} ,H12 _{eq}	H9,H13',H11 _{ax}
H11 _{ax}	1.77,m	H10,H11 _{eq}	
H11 _{eq}	2.28,m	H10,H11 _{ax} ,H12 _{eq}	
H12 _{ax}	1.79,m	H11 _{eq} ,H7,H12 _{eq}	
H12 _{eq}	1.26,m	H7,H10,H11 _{eq} ,H12 _{ax}	
H13	4.26,d(9.8)	H9,H13'	Me18,Me19,H13'
H13'	4.15,dd(9.8,5.3)	H9,H13	H10,H9,H13
Me15	1.17,s		
Me16	1.23,s		H7
Me17	1.49,t(1.3)	H1	Me18,H1
Me18 _{ax}	0.93,s		
Me19 _{eq}	0.91,s		H9

Acknowledgement The authors wish to thank Mike LeBlanc and the staff of the Bamfield Marine Station for assisting the collection of *C. luteomarginata*. Financial support was provided by NSERC and Rohm and Haas Co..

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4. Glaciolide (1): ¹³C nmr (75MHz, CDCl₃) δ 18.2(CH₃), 18.5(CH₃), 21.5(CH₂), 22.7(CH₂), 23.8(CH₂), 27.9(CH₃), 29.1(CH₃), 29.6(CH₃), 34.3(CH₂), 35.2(C), 37.8(CH), 41.4(C), 46.6(CH), 46.7(CH₂), 48.7(CH), 68.0(CH₂), 128.5(C), 145.2(C), 178.9(C); LREIMS m/z M⁺290, 275, 163(100%), 147, 135, 121, 107; ¹H nmr (400MHz, C₆D₆) δ 0.62,s(Me), 0.82,s(Me), 1.10,s(Me), 1.17,s(Me), 1.33,t (J=1Hz, Me), 3.44,dd (J=5.3,9.7Hz), 3.74,d (J=9.7Hz).
5. Diacetate 2: HREIMS M⁺ m/z 378.2765 (C₂₃H₃₈O₄ Δ M -0.5mmu); ¹H nmr (C₆D₆) δ 0.70,s(Me), 0.88,s(Me), 1.13,s(Me), 1.17,s(Me), 1.38,s(Me), 1.70,s(Me), 1.76,s(Me).
6. Compound 3: HREIMS M⁺ m/z 210.1254 (C₁₂H₁₈O₃ Δ M -0.2mmu); ¹H nmr (CDCl₃) δ 0.93,s(Me), 1.05,s(Me), 2.18,s(Me), 2.61,dt(J=7.4, 2.8Hz), 4.17,dd(J=5.8, 9.7Hz), 4.36,dd(J=1.7, 9.7Hz); IR (film) 1762, 1688 cm⁻¹.
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(Received in USA 30 June 1989)