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Ansellone A, a Sesterterpenoid Isolated from the Nudibranch *Cadlina luteomarginata* and the Sponge *Phorbas* sp., Activates the cAMP Signaling Pathway

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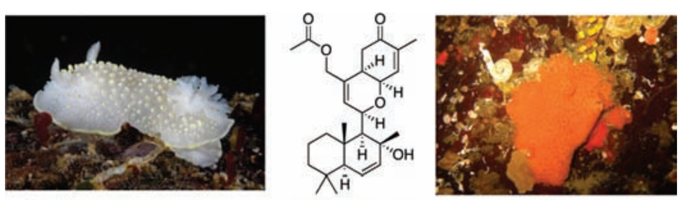
ABSTRACT



Cadlina luteomarginata Ansellone A *Phorbas* sp.

Ansellone A (1) has been isolated from the dorid nudibranch *Cadlina luteomarginata* and the sponge *Phorbas* sp. It has the new "ansellane" sesterterpenoid carbon skeleton, and it activates the cAMP signaling pathway.

Dorid nudibranchs are missing the hard shell used by most of their molluscan relatives for protection, leaving their chemosensory rhinophores and oxygen-harvesting branchial plumes exposed on their dorsums. They have a large foot that provides locomotion, but only at speeds that are slow relative to potential predators. Nudibranchs tend to be found in shallow water habitats, where they frequently sit out in the open blatantly advertising their vulnerability. Despite their apparent lack of physical attributes and behavioral patterns suited for defensive purposes, nudibranchs have few documented predators.[1] Astute field observations and some simple antifeedant experiments led marine biologists to propose that chemicals provide an invisible protective armor for these soft bodied molluscs.

Cadlina luteomarginata is a common nudibranch in the rocky intertidal and subtidal habitats of British Columbia. More than 35 terpenoids representing 21 different carbon skeletons have been isolated from *C. luteomarginata* skin and egg mass extracts.[2] The 21 carbon skeletons include monoterpenoid, sesquiterpenoid, diterpenoid, sesterterpenoid,

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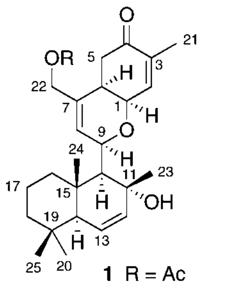
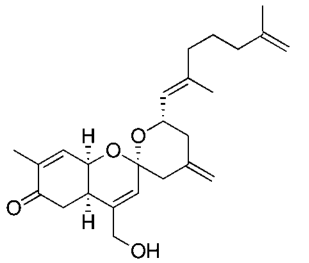
degraded diterpenoid, and degraded sesterterpenoid representatives. Four of these, the marginatane,[3] glaciane,[4] cadlinalane,[2a] and luteane skeletons,[5] were first encountered in *C. luteomarginata* metabolites. The terpenoids obtained from *C. luteomarginata* represent a range of biosynthetic diversity of secondary metabolites rarely encountered in a single living organism. Most of the terpenoids isolated from *C. luteomarginata* are known to be sequestered from the nudibranch's sponge diet,[2] but a subset are biosynthesized de novo by the nudibranch.[6]



1 R = Ac
3 R = H



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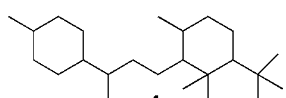

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As part of our ongoing investigations of the skin chemistry and biosynthetic capabilities of *C. luteomarginata*,[2a,6] specimens of the nudibranch and the sponge *Phorbas* sp. that the nudibranchs were feeding on were collected in Howe Sound, B.C. Independent fractionation of the nudibranch and sponge crude extracts led to the identification of ansellone A (**1**) as the major component in both. Ansellone A (**1**) is a new sesterterpenoid with the unprecedented tricyclic "ansellane" carbon skeleton. It is biogenetically related to the previously reported alotaketal A (**2**)[7] and phorbaketals A−C,[8] and like the alotaketals, it activates the cAMP signal transduction pathway without a ligand/receptor binding event. Details of the isolation, structure elucidation, and biological activity of ansellone A (**1**) are presented below.

The sponge *Phorbas* sp. (400 g) and two individuals of *C. luteromarginata* that were found feeding on the sponge were collected by hand using scuba at −10 m off Ansell Point, B.C.[9] Fresh sponge tissue was cut into small pieces and extracted twice with MeOH. Live nudibranchs were immersed whole in MeOH (5 mL), and after the original solvent was decanted, they were extracted once more with fresh MeOH (5 mL). The combined *Phorbas* sp. MeOH extracts were concentrated in vacuo to give an orange gum (400 mg) that was partitioned between H_2O (50 mL) and EtOAc (3 × 10 mL). Concentration of the combined EtOAc layers in vacuo and chromatography of the resulting gum on silica gel eluting with a step gradient from 100% hexanes to 3:7 hexanes/EtOAc gave a pure sample of ansellone A (**1**) (8 mg). The combined MeOH extracts from the *C. luteomarginata* specimens yielded 0.6 mg of pure ansellone A (**1**) when fractionated in an identical manner.

Ansellone A (**1**) was isolated as an optically active oil ($[\alpha]_D$ −15.4, MeOH) that gave a $[M + Na]^+$ ion at *m/z* 465.2621 in the HRESIMS consistent with a molecular formula of $C_{27}H_{38}O_5$ (calcd for $C_{27}H_{38}O_5Na$, 465.2617), requiring nine sites of unsaturation. The ^{13}C NMR spectrum recorded for ansellone A in C_6D_6 showed 27 well-resolved resonances in agreement with the HRESIMS, and the HSQC data identified 37 hydrogen atoms attached to carbon (6 × CH_3, 5 × CH_2, 9 × CH). A low-resolution ESIMS recorded with CD_3OD as the injection solvent gave a $[M + Na]^+$ ion at *m/z* 466 confirming the presence of one exchangeable hydrogen atom that together with the 37 hydrogen atoms attached to carbon accounted for the 38 hydrogen atoms indicated by the HRESIMS measurement.

Downfield resonances in the ^{13}C NMR spectrum could be assigned to six alkene carbons [δ 128.0 (C-13), 131.4 (C-7), 134.8 (C-8), 135.7 (C-12), 138.6 (C-3), and 139.4 (C-2)], one $\alpha\beta$-unsaturated ketone carbonyl (δ 197.7, C-4), and an ester or carboxylic acid carbonyl (δ 170.2, C-26), which together accounted for five sites of unsaturation. The absence of ^{13}C NMR evidence for additional unsaturated funtionality indicated that ansellone A (**1**) was tetracyclic.

Detailed analysis of the COSY and HMBC data obtained for ansellone A (**1**) identified the three fragments A, B, and C shown in Figure 1. COSY correlations defined a linear ^1H spin system starting with a two-proton doublet at δ 2.67 (H_2-5) and continuing in sequence to a methine at δ 2.10 (H-6), an oxymethine at δ 3.44 (H-1), an olefinic methine at δ 6.14 (H-2), and ending with an olefinic methyl at δ 1.69 (H_3-21) as shown in fragment A in Figure 1. HMBC correlations observed between each of the olefinic methyl (δ 1.69, H_3-21), olefinic methine (δ 6.14, H-2), and methylene (δ 2.67, H_2-5) ^1H resonances and the ketone carbonyl resonance at δ 197.7 (C-4) revealed that the linear ^1H spin system and the conjugated ketone were part of a cyclohexenone ring.

A second linear ^1H spin system shown in fragment B in Figure 1 was also revealed by the COSY data. A pair of resonances at δ 4.27 (H-22) and 4.42 (H-22′), assigned to diastereotopic geminal oxymethylene protons via HSQC data, showed COSY correlations to each other. Both of the oxymethylene proton resonances [δ 4.27 (H-22) and 4.42 (H-22′)] showed COSY correlations to the olefinic methine resonance at δ 5.70 (H-8). The H-22/H-22′ to H-8 correlations were assigned to allylic coupling on the basis of their small coupling contants ($J < 1$ Hz). COSY correlations between the olefinic methine resonance (δ 5.70, H-8) and an oxymethine resonance at δ 4.77 (H-9), and between the oxymethine (H-9) and a methine at δ 1.46 (H-10), were both

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attributed to vicinal coupling. Homoallylic coupling between the geminal methylene protons at δ 4.27 (H-22) and 4.42 (H-22′) and the oxymethine proton at δ 4.77 (H-9) was also observed in the COSY spectrum.

HMBC correlations between the carbonyl resonance at δ 170.2 (C-26) and a deshielded methyl resonance at δ 1.67 (Me27) and both of the H-22 and H-22′ resonances (δ 4.27 and 4.42) demonstrated that the oxymethylene carbon (C-22) was acetylated. Additional HMBC correlations between the olefinic methine resonance at δ 5.70 (H-8) and carbon resonances at δ 66.1 (C-22), 131.4 (C-7), and 76.9 (C-9), and between the oxymethine resonance at δ 4.77 (H-9) and carbon resonances at δ 131.4 (C-7), 134.8 (C-8), and 63.8 (C-10) were consistent with the proposed structure of fragment B (Figure 1).

A third ^{1}H spin system encompassing three contiguous methylene groups (C-18: δ ^{1}H 1.04, 1.29, ^{13}C 41.7; C-17: δ ^{1}H 1.33, 1.53, ^{13}C 18.9; C-16: δ ^{1}H 1.02, 1.82, ^{13}C 38.9) and a fourth linear ^{1}H spin system comprising a disubsituted alkene (C-12: δ ^{1}H 5.52, ^{13}C 135.7; C-13: δ ^{1}H 5.59, ^{13}C 128.0) and an aliphatic methine (C-14: δ ^{1}H 1.71, ^{13}C 57.0) were also identified from the HSQC and COSY data.

A pair of aliphatic methyl resonances at δ 0.78 (Me-25, δ ^{13}C 22.2) and 0.86 (Me-20, δ ^{13}C 33.6) each showed HMBC correlations to methine, methylene, and quarternary carbon resonances at δ 57.0 (C-14), 41.7 (C-18), and 33.1 (C-19), respectively, demonstrating that the methyl groups were geminal substituents on a quaternary carbon and the quarternary carbon was attached to one end of the contiguous three methylene chain and to the aliphatic methine carbon as shown in substructure C in Figure 1. Another methyl resonance at δ 1.20 (Me-24, δ ^{13}C 16.8) showed HMBC correlations to a quaternary carbon resonance at δ 42.1 (C-15), a methylene resonance at δ 38.9 (C-16), assigned to the carbon at other end of the methylene chain, and to the methine carbon resonanace at δ 57.0 (C-14), revealing that C-14 to C-19 were part of a cyclohexane ring. A relatively deshielded methyl singlet at δ 1.50 (Me-23, δ ^{13}C 26.5) showed HMBC correlations to a carbon resonance at δ 73.1 (C-11), assigned to an oxygenated tertiary carbon, and to the alkene carbon at δ 135.7 (C-12), indicating that the oxygenated carbon was the second substituent on the disubstituted $\Delta^{12,13}$ alkene. In DMSO-d_6, an exchangeable ^{1}H resonance at δ 4.40 and a methyl resonance at δ 1.24 (Me-23) both showed a HMBC correlations to an nonprotonated oxygenated carbon resonance at δ 71.1, assigned to C-11, indicating that there was a tertiary alcohol at this position.

HMBC correlations between the fragment B oxymethylene and alkene proton resonances at δ 4.27 (H-22), 4.42 (H-22′), and 5.70 (H-8), respectively, and the fragment A methine carbon resonance at δ 34.8 (C-6) showed that the fragment A cyclohexenone ring was the third substituent on the fragment B alkene as illustrated on **I** in Figure 2. Connection between the terminal methine carbon (C-10) in fragment B and the two carbons with unsatisfied valences in fragment C (C-11 and C-15) was supported by the observation of HMBC correlations between the methyl

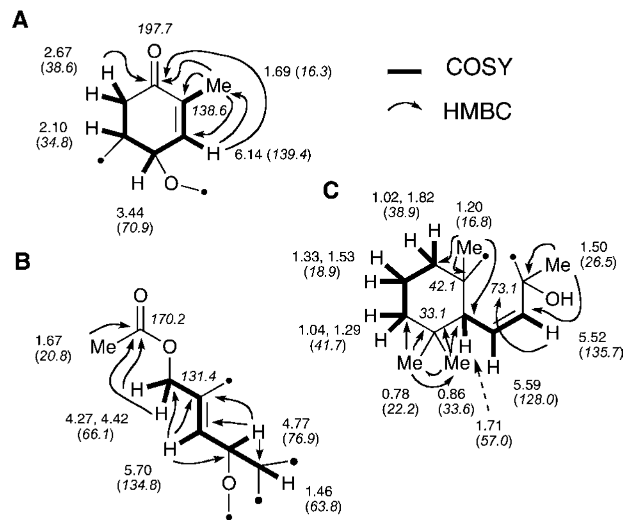

Figure 1. Fragments of ansellone A (**1**) elucidated from COSY and HMBC data.

resonances at δ 1.20 (Me-24) and 1.50 (Me-23) and the methine carbon resonance at δ 63.8 (C-10), completing the partial structure **I**. The molecular formula of ansellone A (**1**) contains only five oxygen atoms, and the molecule must have four rings. Therefore, the oxymethine carbons in **I** (C-1 and C-9) had to be linked by an ether to give the constitution shown in **II** (Figure 2) for ansellone A (**1**).

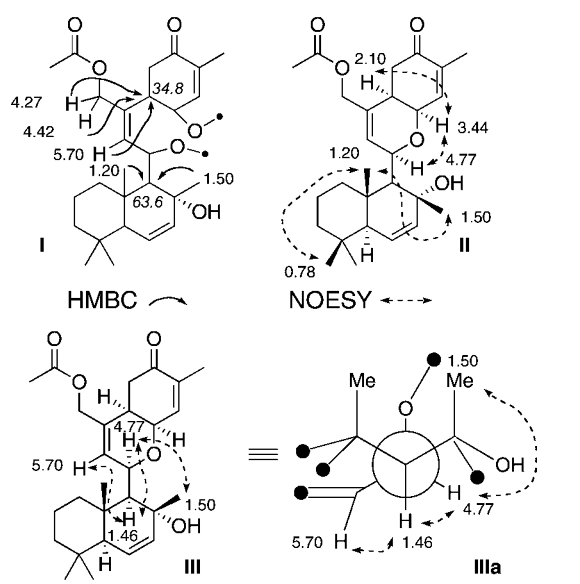

Figure 2. HMBC and NOESY correlations observed for ansellone A (**1**).

There was no HMBC evidence for the C-1 to C-9 ether linkage, but 1D NOESY experiments showed a strong NOE between H-1 (δ 3.44) and H-9 (δ 4.77) that was consistent with the ether linkage and established the *cis* relationship between H-1 and H-9. A strong 1D NOESY correlation between H-1 and H-6 (δ 2.10) revealed that the cyclohexenone and dihydropyran rings were *cis* fused as shown on **II** in Figure 2. 1D NOESY correlations between the Me-24 (δ 1.20) and both of the Me-23 (δ 1.50) and Me-25 (δ 0.78) resonances revealed that the C-10 to C-19 decalin ring system was *trans* fused with all three methyl groups in the axial orientation. A 1D NOESY correlation between Me-23 (δ 1.50) and H-9 (δ 4.77) required H-10 to be axial as shown in **III**. The relative configuration between the conjoint bicyclic ring systems in **1** was uniquely defined by the observation of 1D NOESY correlations between H-8 (δ 5.70) and H-10 (δ 1.46), between H-9 (δ 4.77) and H-10, and between Me-23 (δ 1.50) and H-9 that could only be accommodated by the C-9*R**/C-10*S** relative configurations shown in the Newman projection **IIIa** in Figure 2.

Ansellone A (**1**) contains the 4a,5-dihydro-2*H*-chromen-6(8a*H*)-one ring system present in alotaketal A (**2**).[7] We previously assigned the absolute configuration of alotaketal A (**2**) as 1*R*,6*S* from the CD spectrum using Snatzke's rules[10] to predict the sign of the Cotton effect arising from the enone $n \rightarrow \pi^*$ transition. Subsequently, Rho's group assigned the absolute configuration of the closely related phorbaketal as 1*S*,6*R* using Mosher ester methodology. The limited amounts of the alotaketals available to us prohibited the use of the Mosher ester approach to check our CD assignment for alotaketal A (**2**). Ansellone A (**1**) has a CD spectrum that is similar to that of alotaketal A (**2**)[7] (Supporting Information), with both having a positive Cotton effect for the $n \rightarrow \pi^*$ transition, showing that their enone substructures have the same absolute configuration.

Hydrolysis of ansellone A (**1**) gave the deacetylated product **3** that produced crystals suitable for X-ray diffraction analysis. Figure 3 shows an ORTEP diagram for **3** that confirms the constitution and relative configuration assigned to **1** as described above. The X-ray structure of **3** made use of differences in anomalous dispersion using Cu radiation to unambiguously assign the absolute configuration 1*S*,6*R*,9*R*,10*S*,11*R*,14*S*,15*S* shown in the ORTEP drawing. The refined Flack parameter is $-0.01(16)$.[11] This agrees with Rho's configurational assignment for the phorbaketals, and it requires that the absolute configuration of alotaketal A be reassigned as 1*S*,6*R*,9*S*,13*S* as shown in **2**.

The presence of the 4a,5-dihydro-2*H*-chromen-6(8a*H*)-one substructure in both ansellone A (**1**) and alotaketal A (**2**) suggested that the two compounds might have similar biological activities. Indeed, ansellone A (**1**) also activates cAMP signaling in HEK293 cells in the absence of hormone binding with an EC_{50} of 14 μM (Supporting Information). It is much less active than alotaketal A (**2**) (EC_{50} 0.018 μM) in the same assay but is only slightly less active than the diterpenoid forskolin (EC_{50} 3.0 μM) that is widely used as a cell biology tool to stimulate cAMP signaling in cells.[12]

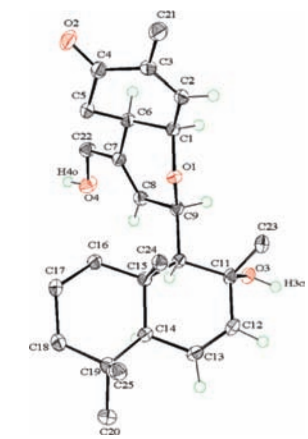

Figure 3. ORTEP diagram for desacetylansellone A (**3**).

Ansellone A (**1**) is a new sesterterpenoid with the unprecedented "ansellane" carbon skeleton **4** that is sequestered by the dorid *C. luteomarginata* from its *Phorbas* sp. sponge diet. The occurrence of **1** in the skin extracts of *C. luteomarginata* further expands the truly remarkable structural diversity of terpenoid natural products that are deployed by this shell-less mollusc on its dorsum, presumably for defensive purposes. Ansellone A (**1**) provides new SAR data for an emerging cAMP-activating terpenoid pharmacophore.

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Supporting Information Available: 1D and 2D NMR spectra and a table of NMR assignments for **1**. Experimental data including bioassay and a taxonomic description of the *Phorbas*. This material is available free of charge via the Internet at http://pubs.acs.org.

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