ORGANIC LETTERS

2009
Vol. 11, No. 22
5166−5169

Alotaketals A and B, Sesterterpenoids from the Marine Sponge *Hamigera* Species that Activate the cAMP Cell Signaling Pathway

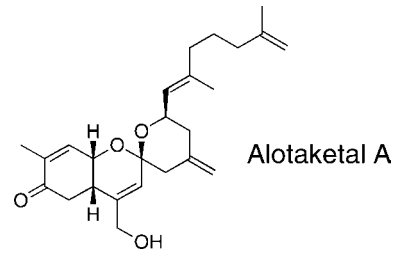
Roberto Forestieri,[†] Catherine E. Merchant,[‡] Nicole J. de Voogd,[§] Teatulohi Matainaho,[||] Timothy J. Kieffer,[*,‡] and Raymond J. Andersen[*,†]

Departments of Chemistry and Earth & Ocean Sciences, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1, Departments of Cellular & Physiological Sciences and Surgery, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3, Naturalis, National Museum of Natural History, Leiden, The Netherlands, and University of Papua New Guinea, Port Moresby, Papua New Guinea

randersn@interchange.ubc.ca

Received September 5, 2009

ABSTRACT



Alotaketal A

The new sesterterpenoids alotaketals A (1) and B (2) have been isolated from extracts of the marine sponge *Hamigera* sp. collected in Papua New Guinea. Their chemical structures were elucidated by analysis of spectroscopic data. Alotaketals A and B have the unprecedented alotane carbon skeleton, and they activate the cAMP cell signaling pathway with EC_{50}'s of 18 and 240 nM, respectively.

Sutherland's Nobel Prize winning discovery of cAMP as an intracellular second messenger has been fundamental to our understanding of cell signaling.[1] The cAMP pathway is typically turned on by the binding of a hormone to a G-protein coupled receptor imbedded in the cell membrane.[1b] When a hormone binds to its G-protein coupled receptor, it triggers activation of adenylyl cyclase, an enzyme that catalyzes the conversion of ATP to cAMP. The cAMP formed by this reaction binds to cAMP-dependent protein kinase (PKA), a central downstream component in the signaling pathway, and activates its capacity to catalyze the reversible phosphorylation of substrate proteins that regulate a wide array of cellular events including transcription.[2] Roughly half of all drugs currently in clinical use target G-protein coupled receptors, an indication of the importance of cAMP signaling.[3]

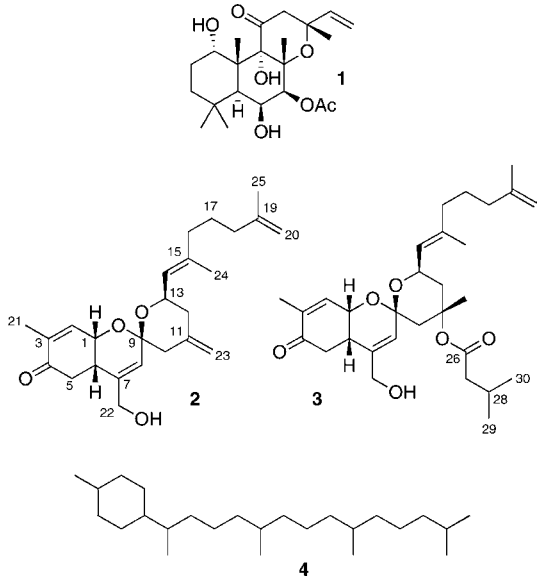
Small molecules that can selectively modulate signaling pathways in whole cells are important tools for cell biology research and potential lead compounds for drug develop-

[†] Chemistry and EOS, UBC.
[‡] Cellular & Physiological Sciences and Surgery, UBC.
[§] National Museum of Natural History, Leiden.
[||] University of Papua New Guinea.

(1) (a) Sutherland, E. W.; Rall, T. W. *J. Am. Chem. Soc.* **1957**, *79*, 3608. (b) Sutherland, E. W. *Science* **1972**, *177*, 401–408.
(2) Krebs, E. G. *Curr. Top. Cell. Regul.* **1972**, *5*, 99–133.
(3) Bridges, T. M.; Lindsley, C. W. *ACS Chem. Biol.* **2008**, *3*, 530–541.

10.1021/ol902066e CCC: $40.75
Published on Web 10/29/2009

ment.[4] Forskolin (**1**), a labdane diterpenoid isolated from the plant *Coleus forskohlii* found in India, is a potent activator of adenylyl cyclase.[5] It is widely used in cell biology research to raise intracellular cAMP levels in the absence of hormone stimulation, and it has attracted some attention as a drug candidate.[6]



As part of our ongoing interest in discovering new marine natural products that modulate cell signaling,[7] we have used a cell-based luciferase reporter assay to screen a library of crude sponge extracts for compounds that activate the cAMP pathway (the assay is described in detail in the Supporting Information). Crude extracts of the marine sponge *Hamigera* sp. showed promising activity in the screen (Supporting Information). Assay-guided fractionation of the extract led to the identification of the sesterterpenoids alotaketals A (**2**) and B (**3**)[8] that are activators of cAMP signaling at submicromolar concentrations. Details of the isolation, structure elucidation, and biological activities of alotaketals A (**2**) and B (**3**) are presented below.

Specimens of *Hamigera* sp. were collected by hand using SCUBA at a depth of 20 m on reefs in Milne Bay, Papua New Guinea.[9] Freshly collected sponge was frozen on site and transported to Vancouver frozen over cold packs. Thawed sponge samples (24 g) were cut into small pieces that were extracted exhaustively with MeOH, and the combined MeOH extracts were concentrated in vacuo to give a bioactive gum (280 mg). A portion of this gum (38 mg) was subjected to Sephadex LH20 chromatography eluting with MeOH. The bioactive fractions were combined and fractionated further using C_{18} reversed-phase HPLC (eluant: 8:2 acetonitrile/H_2O) to give pure samples of alotaketals A (**2**) (5.3 mg) and B (**3**) (2.1 mg).

Alotaketal A (**2**) was isolated as an optically active amorphous white solid that gave a $[M + Na]^+$ ion at *m*/*z* 421.2346 in the HRESIMS appropriate for a molecular formula of $C_{25}H_{34}O_4$ (calcd for $C_{25}H_{34}O_4Na$ 421.2355), requiring nine sites of unsaturation. The ^{13}C NMR spectrum of **2** recorded in C_6D_6 (Supporting Information) showed 25 well-resolved resonances, and the DEPT spectrum identified 33 protons attached to carbon (3 × CH_3; 9 × CH_2; 6 × CH). A LRESIMS spectrum run in CD_3OD gave a $[M + Na]^+$ ion at *m*/*z* 422 demonstrating that the final proton in the molecule was exchangeable and, therefore, had to be part of an OH functionality. Ten of the ^{13}C NMR resonances had chemical shifts between δ 110 and 145 ppm appropriate for alkene carbons, and a resonance at δ 197.7 could be assigned to an $\alpha\beta$ unsaturated ketone. The five olefin and single ketone functionalities identified by the ^{13}C NMR data accounted for only 6 of the 9 sites of unsaturation required by the molecular formula, indicating that alotaketal A (**2**) contained three rings.

The DEPT and HSQC data identified two olefinic methylenes (δ_C 110.9, δ_H 4.80/4.81 C-20; δ_C 111.4, δ_H 4.87/4.89, C-23) and three olefinic methines (δ_C 125.1, δ_H 5.56, C-8; δ_C 126.8, δ_H 5.48, C-14; δ_C 139.6, δ_H 6.32, C-2) in **2**. In the COSY spectrum of **2**, it was possible to start at the olefinic methylene proton resonances at δ 4.81 (H-20a) and 4.80 (H-20b) and trace in sequence correlations that identified the spin system that ends in H-10a (δ 2.34) and H-10b (δ 2.29) as shown in Fragment **A** in Figure 1. HSQC correlations provided assignments for the protonated carbons in Fragment **A** and HMBC correlations from the methyl singlets at δ 1.62

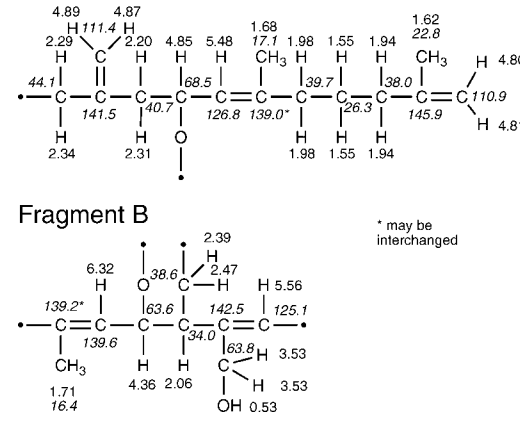


Figure 1. Fragments of alotaketal A (**2**) identified from COSY, HSQC, and HMBC data.

(4) (a) Kawasumi, M.; Nghiem, P. *J. Invest. Dermatol* **2007**, *127*, 1577–1584. (b) Spring, D. R. *Chem. Soc. Rev.* **2005**, *34*, 472–482,(c) Walsh, D. P.; Chang, Y.-T. *Chem. Rev.* **2006**, *106*, 2476–2530.

(5) (a) Bhat, S. V.; Bajwa, B. S.; Dornauer, H.; de Souza, N. J.; Fehlhaber, H. W. *Tetrahedron Lett.* **1977**, 1669–1672. (b) Seamon, K. B.; Padgett, W.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3363–3367.

(6) Gonzalez-Sanchez, R.; Trujillo-Hernandez, B.; Vasquez, C.; Huerta, M.; Elizalde, A. *J. Intl. Med. Res.* **2006**, *34*, 200–207.

(7) (a) Yang, L.; Williams, D. E.; Mui, A.; Ong, C.; Krystal, G.; van Soest, R.; Andersen, R. J. *Org. Lett.* **2005**, *7*, 1073–1076. (b) Marion, F.; Williams, D. E.; Patrick, B. O.; Hollander, I.; Mallon, R.; Kim, S. C.; Roll, D. M.; Feldberg, L.; van Soest, R.; Andersen, R. J. *Org. Lett.* **2006**, *8*, 321–324.

(8) *Hamigera* sp. was collected during an expedition that departed from Alotau, Papua New Guinea.

(9) A voucher sample (RMNH por. 4827) has been deposited at Naturalis, the National Museum of Natural History in Leiden.

(Me-25) and 1.68 (Me-24) to C-19 (δ 145.9) and C-15 (δ 139.2), respectively, and from the olefinic methylene resonances at δ 4.89/4.87 (H-23a/H-23b) to C-11 (δ 141.5) provided assigments for the nonprotonated olefinic carbons. The connectivity through the nonprotonated olefinic carbons C-19, C-15, and C-11 in the linear chain (C-20 to C-10) in Fragment **A**, identified in the COSY data by allylic couplings, was confirmed by the observation of HMBC correlations between Me-25 (δ 1.62) and C-20 (δ 110.9) and C-18 (δ 38.0), between Me-24 (δ 1.68) and C-16 (δ 39.7) and C-14 (δ 126.8), and between H-23a/H-23b (δ 4.89/4.87) and C-12 (δ 40.7) and C-10 (44.1) as shown in Figure 2.

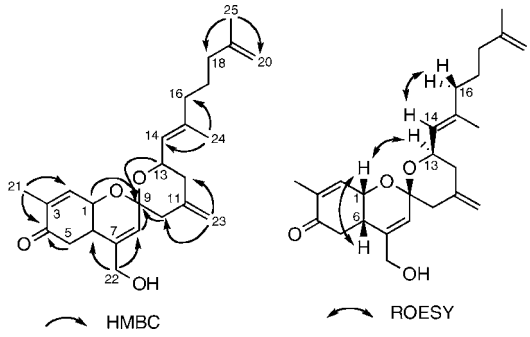


Figure 2. Selected HMBC and ROESY correlations observed for alotaketal A (**2**).

A second proton spin system, Fragment **B** shown in Figure 1, could also be identified from the COSY data obtained for **2**. Once again, HSQC correlations provided assignments for the protonated carbons in **B** as shown, and HMBC correlations observed between the H_2-22 methylene proton resonance at δ 3.53 and C-7 (δ 142.5) and between the Me-21 resonance at δ 1.71 and C-3 (δ 139.2) provided assignments for the nonprotonated olefinic carbons. HMBC correlations between the H_2-22 resonance (δ 3.53) and C-6 (δ 34.0) and C-8 (δ 125.1) and between the Me-21 resonance (δ 1.71) and C-2 (δ 139.6) as shown Figure 2 confirmed the connectivities through the nonprotonated carbons in **B** that were indicated in the COSY data only by allylic couplings.

Fragments **A** and **B** accounted for 23 of the 25 carbon resonances in the ^{13}C NMR spectrum of **2**. The remaining resonances at δ 97.2 (C-9) and 197.7 (C-4) could be assigned to a nonprotonated ketal carbon and an $\alpha\beta$ unsaturated ketone, respectively. HMBC correlations between Me-21 (δ 1.71) and H-2 (δ 6.32) and the ketone resonance at δ 197.7 (C-4), in conjunction with the deshielded chemical shift of H-2, showed that the ketone was attached to C-3. Additional HMBC correlations between the H-5a (δ 2.47) and H-5b (δ 2.39) geminal methylene resonances and the C-4 ketone (δ 197.7) demonstrated that the methylene carbon (C-5) was also bonded to the ketone to form a cyclohexenone ring in **2**.

The ketal resonance at δ 97.2 (C-9) showed HMBC correlations to H-8 (δ 5.56), H-10a (δ 2.34), and H-10b (δ 2.29) indicating that Fragments **A** and **B** were linked to the ketal though C-10 and C-8, respectively, to give the regular isoprenoid carbon skeleton of **2**. Additional HMBC correlations between the ketal carbon (δ 97.2) and both H-1 (δ 4.36) and H-13 (δ 4.85) identified the presence of ether linkages between the ketal and both oxygenated carbons C-1 and C-13, which generated a spiro ketal at C-9 and the final two rings required by the molecular formula of **2** (Figure 2).

ROESY, 1D NOESY, CD, and scalar coupling constant data revealed the relative and absolute configurations of **2**. A ROESY correlation between H-1 (δ 4.36) and H-6 (δ 2.06) showed that the cyclohexenone A ring and the dihydropyran B ring were *cis* fused (Figure 2). Additional ROESY correlations between H-1 (δ 4.36) and both H-13 (δ 4.85) and Me-24 (δ 1.68) required that the C ring pyran oxygen atom bridging C-9 and C-13 was *cis* to H-1 and the B ring pyran oxygen atom bridging C-9 and C-1 was *cis* to H-13 as shown in **2**. H-1 and the C-ring pyran oxygen atom must adopt pseudoaxial orientations on the B ring, and H-13 must be in a pseudoaxial orientation *trans* to C-8 on the C ring for H-1 to be close enough to H-13 and Me-24 to give the observed NOEs. The 12.6 Hz coupling observed between H-12b (δ 2.20) and H-13 (δ 4.85) is consistent with a pseudoaxial orientation for H-13. ROESY correlations between Me-24 (δ 1.68) and H-13 (δ 4.85) and between H-14 (δ 5.48) and H_2-16 (δ 1.98) indicated that the $\Delta^{14,15}$ olefin had the *E* configuration. All of the ROESY correlations used to assign the relative configurations were also observed in 1D NOESY experiments.

A COSY correlation observed between H-1 (δ 4.36) and H-5a (δ 2.47) was assigned to W coupling, requiring that H-1, C-1, C-6, C-5, and H-5a were coplanar. H-6 (δ 2.06) and H-5b (δ 2.39) had a 15.6 Hz scalar coupling demonstrating that they were antiperiplanar. The above evidence showed that the A ring of **2** was in a half chair conformation with C-1, C-2, C-3, C-4, and C-5 in the same plane and C-6 out of the plane. The CD spectrum obtained for **2** has a positive Cotton effect for the $n \rightarrow \pi^*$ transition centered at $\approx \lambda$ 330 nm (Supporting Information). Applying Snatzke's sector rules[10] for planar enones to **2** as shown in Figure 3 predicts

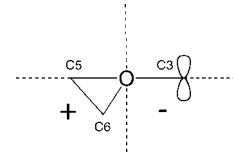


Figure 3. Application of Snatzke's enone sector rules[10] for prediction of the sign of the $n \rightarrow \pi^*$ transition in CD spectra to alotaketal A (**2**).

that the absolute configuration of **2** is 1*S*,6*S*,9*R*,13*R*,14*E* as drawn.

Alotaketal B (**3**) was also isolated as an optically active amorphous white solid that gave a $[M + Na]^+$ ion at *m*/*z*

(10) (a) Lightner, D. A.; Gurst, J. E. *Organic Conformational Analysis and Stereochemistry from Circular Dichrosim Spectroscopy*; Wiley-VCH: New York, 2000; Chapter 11. (b) Snatzke, G. *Tetrahedron* **1965**, *21*, 421–438.

523.3024 in the HRESIMS appropriate for a molecular formula of $C_{30}H_{44}O_6$ (calcd for $C_{30}H_{44}O_6Na$ 523.3036), requiring nine sites of unsaturation. Comparison of the ^1H and ^{13}C NMR data obtained for **3** with the data for **2** (Supporting Information) showed that the molecules were closely related. One of the main differences in the NMR data for the two molecules was the presence of a series of resonances in the spectrum of **3** that could be assigned by analysis of the COSY and HMBC data to an isovalerate fragment (δ_H 0.89, d, $J = 5.4$ Hz, 6H, Me-30/Me-29; 2.12, m, H-28; 2.12, m, H_2-27; δ_C 23.1, C-29/C-30; 25.6, C-28; 45.0, C-27; 172.4, C-26), which accounted for the five additional carbons in its molecular formula. The second difference in the NMR spectra of **2** and **3** was the replacement of the resonances assigned to the $\Delta^{11,23}$ olefinic methylene in **2** with a proton methyl singlet at δ 1.48 (Me-23) and a nonprotonated carbon resonance at δ 77.4 (C-11). These changes in the NMR data of **3** were consistent with the presence of a valerate ester at C-11 in **3** as shown. The near identity of the remainder of the 1D and 2D NMR data for **2** and **3** showed that, except for this difference at C-11, the two molecules were identical.

ROESY correlations observed between H-1 (δ 4.33) and both H-6 (δ 2.00) and H-13 (δ 5.15) confirmed that the relative configurations at C-1, C-6, C-9, and C-13 were the same in both **2** and **3**. The large scalar coupling constant observed for the H-13/H-12 coupling ($J = 10.8$ Hz) in **3** indicated that the C ring was in a chair conformation and that H-13 was axial. The absence of ROESY or 1D NOESY correlations between Me-23 (δ 1.48) and H-13 (δ 5.15) and the observation of a 1D NOESY correlation between Me-23 (δ 1.48) and H-12_{ax} (δ 1.24) suggested that Me-23 was in an equatorial orientation, *cis* to C-14 as shown. The CD spectra of **2** and **3** were nearly identical, and therefore, the configuration of **3** is 1*S*,6*S*,9*R*,11*S*,13*R*,14*E*.

Alotaketals A (**2**) and B (**3**) activate the cAMP signaling pathway in HEK293 cells transfected with the pHTS-CRE plasmid in the absence of hormone binding with EC_{50}'s of 18 and 240 nM, respectively (Figure 4). The decrease in alotaketal A's activity at higher concentrations is attributed to cytotoxicity. Alotaketal A is 170 times more potent than forskolin (**1**), which has an EC_{50} of 3 μM in the same assay (Supporting Information). However, forskolin elicits a much stronger response than the alotaketals. Experiments aimed at identifying the molecular target of the alotaketals are ongoing in our laboratories.

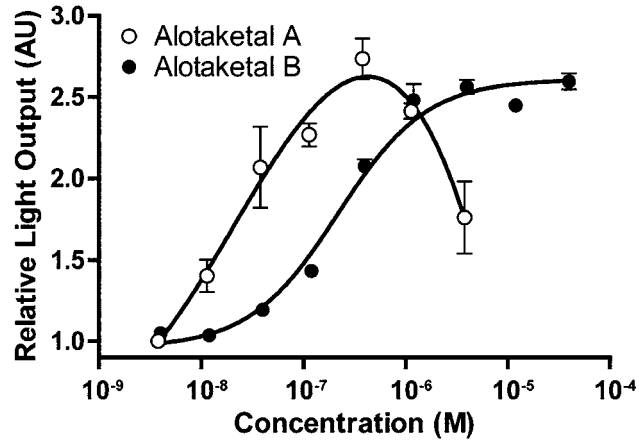


Figure 4. Dose response curves for activation of cAMP signaling by alotaketals A (**2**) and B (**3**).

The alotaketals are new sesterterpenoids containing an interesting spiroketal substructure. They have a monocyclic regular sesterterpenoid carbon skeleton that to the best of our knowledge has not been previously encountered in a natural product.[11] We propose the name "alotane" for this new terpenoid carbon skeleton **4**. Previous studies of sponges in the genus *Hamigera* have resulted in the isolation of the hamigeran terpenoids,[12] the hamigerols, which are sulfated sterol dimers,[13] and a small family of alkaloids.[14] Alotaketals A and B are the first sesterterpenoids reported from this sponge genus. The ability of the alotaketals to activate cAMP signaling at nanomolar concentrations suggests, that like forskolin, they might be useful chemical tools for cell biology research involving cAMP signaling.

Acknowledgment. The authors thank M. LeBlanc from the EOS Department at UBC for assistance with collecting the *Hamigera* sp. specimens. Financial support was provided by grants from the Natural Sciences and Engineering Research Council of Canada (RJA) and the Stem Cell Network (TJK). TJK is a grateful recipient of a Michael Smith Foundation for Health Research Senior Scholar award.

Supporting Information Available: Tables of NMR assignments for **2** and **3**, 1D and 2D NMR spectra for **2** and **3**, and experimental data including bioassay description. This material is available free of charge via the Internet at http://pubs.acs.org.

OL902066E

(11) A compound with this carbon skeleton has been prepared via synthesis. See: Calderon, J.; Quijano, L.; Guzman, M.; Rios, T. *Rev. Latinoam. Quim.* **1980**, *11*, 102–103.

(12) Wellington, K. D.; Cambie, R. C.; Rutledge, P. S.; Bergquist, P. R. *J. Nat. Prod.* **2000**, *63*, 79–85.

(13) Cheng, J.-F.; Lee, J.-S.; Sun, F.; Jares-Erijiman, E. A.; Cross, S.; Rinehart, K. L. *J. Nat. Prod.* **2007**, *70*, 1195–1199.

(14) Hasan, W.; Edrada, R.; Ebel, R.; Wray, V.; Proksch, P. *Mar. Drugs* **2004**, *2*, 88–100.