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Sesterterpenoids Isolated from a Northeastern Pacific *Phorbas* sp.

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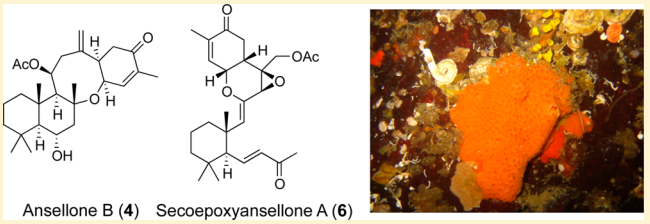
Supporting Information

ABSTRACT: Four new sesterterpenoids, ansellone B (**4**), phorbadione (**5**), secoepoxyansellone A (**6**), and alotaketal C (**7**), have been isolated from specimens of the sponge *Phorbas* sp. collected in British Columbia. Ansellone B (**4**) has an unprecedented heterocyclic skeleton featuring an oxocane ring, and secoepoxyansellone A (**6**) is the first example of the degraded "secoansellane" sesterterpenoid carbon skeleton. Alotaketal C (**7**) is an activator of cAMP signaling in HEK cells.



INTRODUCTION

Sesterterpenoids are one of the smallest groups of known isoprenoid natural products, despite the fact that they have been isolated from a wide variety of sources including terrestrial fungi, lichens, higher plants, insects, and marine invertebrates.[1,2] A broad range of pharmaceutically relevant biological activities have been reported for sesterterpenoids, making them attractive targets for chemical synthesis.[1−4] Sponges have been the richest source of marine sesterterpenoids.[5]

We recently reported the isolation of alotaketals A (**1**) and B (**2**), the first sesterterpenoids with the monocyclic "alotane" carbon skeleton, from the sponge *Hamigera* sp. collected in Papua New Guinea.[6] The alotaketals are potent activators of the cAMP signaling pathway in human embryonic kidney (HEK-293) cells. They are members of an emerging family of related sponge sesterterpenoids representing several new terpenoid carbon skeletons that can all be biogenetically derived from an "alotane" precursor. The first described members of this family were the "suberitane" sesterterpenoids suberitenones A and B isolated from the Antarctic sponge *Suberites* sp.[7] Additional "suberitanes"[8] and the rearranged "caminatane" sesterterpenoid caminatal were subsequently reported from the Antarctic sponge *Suberites caminatus*.[9] The "alotane" sesterterpenoids phorbaketals A−C have been reported from a Korean sponge in the genus *Phorbas*,[10] and we isolated the "ansellane" sesterterpenoid ansellone A (**3**) from the sponge *Phorbas* sp. and specimens of the nudibranch *Cadlina luteomarginata* collected in British Columbia.[11] Phorbasones A and B,[12] phorone A, and isophorbasone A[13] have all been reported from a Korean *Phorbas* sp., providing the first examples of the new rearranged "phorbasane", "phorane", and "isophorbasane" carbon skeletons.

Further chemical investigation of the minor terpenoid components in extracts of the B.C. *Phorbas* sp. that yielded ansellone A (**3**) has resulted in the isolation of ansellone B (**4**), phorbadione (**5**), secoepoxyansellone A (**6**), and alotaketal C (**7**). Ansellone B (**4**) contains an eight-membered cyclic ether embedded in an unprecedented heterocyclic skeleton, secoepoxyansellone A (**6**) represents the first example of the degraded "secoansellane" sesterterpenoid carbon skeleton, and alotaketal C (**7**), an activator of cAMP signaling in HEK293 cells, is the first "alotane" sesterterpenoid isolated from extracts of the B.C. *Phorbas* sp. Presented below are the details of the isolation, structure elucidation, and cAMP activating properties of the new sesterterpenoids **4**−**7**.

RESULTS AND DISCUSSION

Specimens of *Phorbas* sp. were collected at Ansell Point in Howe Sound, British Columbia. Freshly collected sponge tissue was extracted exhaustively with MeOH, and the extracts were combined and concentrated in vacuo to give an orange gum that was partitioned between EtOAc and H_2O. The EtOAc-soluble material was chromatographed on a Si gel flash column eluting with a hexanes to EtOAc gradient to give fractions enriched in sesterterpenoids **3** to **7**. The enriched fractions were subjected to normal- and reversed-phase HPLC to give pure samples of ansellone B (**4**), phorbadione (**5**), secoepoxyansellone A (**6**), and alotaketal C (**7**).

Ansellone B (**4**) gave an $[M + Na]^+$ ion at m/z 467.2783 in the HRESIMS consistent with a molecular formula of $C_{27}H_{40}O_5$ (calcd for $C_{27}H_{40}O_5Na$, 467.2773), requiring eight

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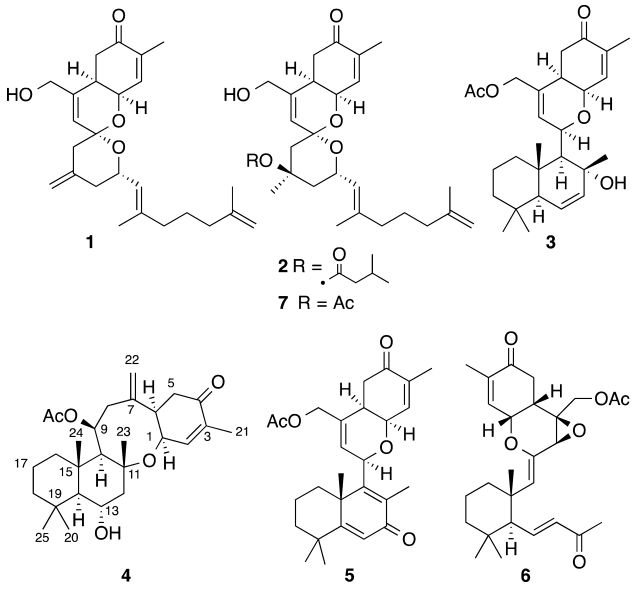

sites of unsaturation. The ^{13}C NMR spectrum obtained for **4** (C_6D_6) showed 26 well-resolved resonances and the HSQC and HMBC data showed that a resonance at δ 50.8 accounted for two carbon atoms (C-10 and C-12) (Table 1), thereby accounting for all 27 carbon atoms indicated by the HRESIMS. HSQC and COSY data identified 39 hydrogen atoms attached to carbon (6 × CH_3, 7 × CH_2, 7 × CH) and one hydrogen attached to an oxygen atom (δ 0.62, bs; OH-13). Downfield resonances in the ^{13}C NMR spectrum were assigned to four olefinic carbons [δ 116.4 (C-22), 146.6 (C-7), 136.0 (C-3), and 141.6 (C-2)], one α,β-unsaturated ketone carbonyl (δ 198.1, C-4), and an ester or carboxylic acid carbonyl (δ 169.2, C-26), accounting for four sites of unsaturation. The absence of ^{13}C NMR evidence for additional unsaturated functionality indicated that ansellone B (**4**) was tetracyclic.

A C-1 to C-6 cyclohexenone substructure (fragment **A**, Figure 1) typical of this family of terpenoids was readily identified in ansellone B (**4**) from its COSY, HSQC, and HMBC data. The COSY data revealed a second linear spin system labeled as fragment **B** in Figure 1, comprised of a pair of olefinic methylene hydrogens (δ 4.77, 4.89; H-22/H-22′), a pair of diastereotopic methylene hydrogens (δ 2.45, 2.82; H-8/H-8′), an oxymethine hydrogen (δ 5.52; H-9), and an aliphatic methine hydrogen (δ 1.39; H-10). A third spin system was identified from the COSY and the HMBC data (Figure 1, fragment **C**) starting at the H-12/H-12′ methylene hydrogens (δ 1.49/1.53), proceeding to an oxymethine hydrogen (δ 3.56; H-13), and ending with an aliphatic methine hydrogen (δ 0.81; H-14). A COSY correlation between H-13 (δ 3.56) and an exchangeable hydrogen resonance at δ 0.62 suggested the presence of a secondary alcohol at C-13. A fourth spin system identified in the COSY amd HSQC data (Figure 1**C**) contained three contiguous pairs of methylene hydrogens (δ ^1H 0.93, 1.86; H-16/H-16′: 1.33, 1.48; H-17/H-17′: 1.11, 1.30; H-18/H-18′).

The remaining structural elements not accounted for by the proton spin systems described above included an acetyl group (δ ^1H 1.67; ^{13}C 21.3, 169.2), four methyls (δ ^1H 0.97, s, Me-24; 1.03, s, Me-25; 1.34, s, Me-20; 1.55, s, Me-23), two quaternary carbons (δ 34.1, C-19; 39.1, C-15), and an oxygenated tertiary carbon (δ 79.4, C-11). A series of HMBC correlations provided evidence for the connectivity between the structural fragments (Figure 1, fragment **D**). HMBC correlations between both H-5 (δ 2.93) and H-6 (δ 2.37) and C-7 (δ 146.6), between H-6 and C-22 (δ 116.4), and between H-22/H-22′ (δ 4.77/4.89) and C-6 (δ 46.0) established a C-6/C-7 linkage between fragments A and B; correlations between Me-23 (δ 1.55) and C-11 (δ 79.4), between Me-24 (δ 0.97) and C-15 (δ 39.1), and between both Me-20 (δ 1.34) and Me-25 (δ 1.03) and C-19 (δ 34.1) showed that Me-23 was attached to C-11, Me-24 was attached to C-15, and both Me-20 and Me-25 were attached to C-19; correlations between both Me-23 and Me-24 and C-10 (δ 50.8) linked C-10 of fragment **B** to fragment **C** via bonds to both C-11 and C-15; a correlation between H-12 (δ 1.49) and Me-23 (δ 27.9) linked C-12 to C-11; correlations between Me-20, Me-24 and Me-25 and C-14 (δ 61.5) linked C-14 to both C-15 and C-19; correlations between Me-24 and C-16 (δ 41.3) and between Me-20 and Me-25 and C-18 (δ 43.6) established bonds between C-15 and C-16 and between C-18 and C-19; and a correlation between H-1 (δ 3.73) and C-11 (δ 79.4) identified an ether link between C-1 and C-11. Attaching the acetyl group to the oxygen atom at C-9 satisfied the remaining valences and was consistent with the downfield shift of H-9 (δ 5.52).

The tROESY correlations shown in Figure 1, fragment **E**, between Me-24 (δ 0.97) and both Me-23 (δ 1.55) and H-13 (δ 3.56); between Me-25 (δ 1.03) and H-13; and between H-14 (δ 0.81) and all of H-10 (δ 1.39), H-16α (δ 0.93), and H-18α (δ 1.11) demonstrated that the cyclohexane rings in the decalin substructure were in chair conformations, the rings were *trans* fused, OH-13 was equatorial, C-9 was equatorial, and the ether oxygen at C-11 was also equatorial as shown. Additional tROESY correlations between H-1 (δ 3.73) and H-6 (δ 2.37) showed that the cyclohexenone ring and the oxocane ring were *cis* fused, and correlations between H-9 (δ 5.52) and both H-10 (δ 1.39) and H-16α (δ 0.93) showed that H-9 was α, completing the relative configuration of **4**. We have assumed that the absolute configuration of **4** is the same as that of ansellone A (**3**) as shown.

Phorbadione (**5**) gave an $[M + Na]^+$ ion at *m*/*z* 461.2297 in the HRESIMS approriate for a molecular formula of $C_{27}H_{34}O_5$ (calcd for $C_{27}H_{34}O_5Na$, 461.2304), requiring 11 sites of unsaturation. Analysis of the 1D and 2D NMR data collected in C_6D_6 clearly identified a bicyclic fragment **A** in **5** containing cyclohexenone and dihydropyran rings that was identical to the corresponding fragment in anselone A (**3**) (Table 1). The same C_6D_6 1D ^1H NMR data set revealed that the remainder of the molecule (fragment **B** in Figure 2) had to account for a deshielded methyl resonance (δ 2.10, Me-23), an isolated deshielded olefinic methine resonance (δ 6.48, H-13), and two upfield methyl singlets (δ 0.93, Me-20; 0.99, Me-25). HMBC correlations observed between the methyl resonance at δ 2.10 (Me-23) and carbon resonances at δ 134.3 (C-11), 156.4 (C-10), and 186.3 (C-12), and between the olefinic methine resonance at δ 6.48 (H-13) and carbon resonances at δ 134.3 (C-11), 170.4 (C-14), and 186.3 (C-12) showed that the deshielded methyl and olefinic methine were part of a cross conjugated ketone substructure (C-10 to C-14). Further HMBC correlations between the methyl resonance at δ 0.93 (Me-20) and the carbon resonance at δ 28.5 (Me-25), and between the methyl resonance at 0.99 (Me-25) and the carbon resonance at 32.4 (Me-20), confirmed that the methyls were geminal. Both methyl resonances (δ 0.93, Me-20; 0.99, Me-25) showed HMBC correlations to the olefinic carbon resonance at δ 170.4 (C-14) and aliphatic carbon resonances at δ 37.1 (C-19) and 40.4 (C-18), and the olefinic resonance at δ 6.48 (H-

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Table 1. NMR Data for Ansellone B (4), Phorbadione (5), Secoepoxyansellone A (6), and Alotaketal C (7)

	4 (C_6D_6)		**5** (C_6D_6)		**5** (MeOH-d_4)		**6** (C_6D_6)		**7** (C_6D_6)	
C no.	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	64.9	3.73 (5.6, 3.6)	70.7	3.57 t (5.0)	71.8	4.34 bt (5.2)	65.9	4.37 bd (5.9)	62.9	4.27 dd (5.3, 3.3)
2	141.6	6.03 dq (5.6, 1.4)	138.2	6.17 dq (5.0,1.3)	140.9	6.74 dq (5.2,1.4)	138.4	6.50 bs	139.0	6.27 dq (5.3, 1.1)
3	136.0		138.8		139.6		138.6		138.5	
4	198.1		196.8		200.1		195.8		197.3	
5	39.4	2.22 dd (16.1, 3.5)	37.4	2.62 m	38.1	2.67 m	34.9	2.32[a]	38.2	2.38 d (8.8)
5′		2.93 (16.1, 13.5)		2.62 m		2.67 m		2.41 m		2.38 d (8.8)
6	46.0	2.37 dt (13.5, 3.5)	34.1	2.19 m	35.0	2.72 m	34.4	2.31[a]	33.7	2.00 td (8.8, 3.3)
7	146.6		133.8		135.3		59.5		142.2	
8	34.1	2.45 bdd (11.46, 6.9)	129.0	5.49 bs	129.8	5.91 bs	55.7	2.99[a]	124.7	5.43 bs
8′		2.82 td (12.9, 1.6)								
9	74.9	5.52 ddd (11.0, 6.9, 2.6)	75.5	4.73 m	76.4	5.30 bs	143.3		96.5	
10	50.8	1.39 d (2.6)	156.4		161.4 br		123.7	4.81 s	39.6	1.19 d (14.7)
10′										3.11 dd (14.7, 2.1)
11	79.4		134.3		134.6 br		198.1	/	77.1	
12	50.8	1.49[a]	186.3		189.5		129.2	5.89 d (11.7)	43.8	1.20 dd (13.8, 11.3)
12′		1.53[a]								1.81 dt (13.8, 2.1)
13	68.0	3.56 td (10.4, 4.4)	124.4	6.48 s	124.1	6.28 s	146.3	5.85 t (11.7)	63.7	5.13 ddd (11.3, 8.3, 2.1)
14	61.5	0.81 d (10.4)	170.4		176.3		49.3	3.89 d (11.7)	126.1	5.45 bd (8.3)
15	39.1		43.7		45.9		38.2		138.7	
16	41.3	0.93 td (12.5, 3.5)	34.3	1.40 td (12.7,3.5)	35.4	1.49 td (12.9,4.4)	37.3	1.48[a]	39.9	2.10 m 2.10 m
16′		1.86 bd (12.5)		1.92 m		2.36 bd (12.9)		1.69 m		
17	18.6	1.33[a]	18.5	1.27[a]	19.3	1.67 m	19.4	1.48[a]	26.9	2.20 m
17′		1.48[a]		1.50 m		1.99 dm (13.8)		1.48[a]		2.20 m
18	43.6	1.11 td (13.5, 4.1)	40.4	1.04[a]	41.6	1.39 td (13.3,4.0)	38.6	1.20 m	124.4	5.21 tm (7.0)
18′		1.30[a]		1.26[a]		1.73 dt (13.3,3.4)		1.30 m		
19	34.1		37.1		38.7		34.3		131.6	
20	37.2	1.34 s	32.4	0.93 s	32.9	1.23 s	31.8	1.08 s	17.7	1.54 bs
21	15.9	1.80 d (0.9)	16.0	1.75 bs	15.8	1.82 bs	15.9	1.72 bs	15.9	1.76 bs
22	116.4	4.77 bd (1.0)	65.1	4.21 d (13.0)	66.3	4.54 d (12.9)	63.8	3.60 d (12.6)	63.4	3.50 (14.2, 5.2)
22′		4.89 bs		4.35 d (13.0)		4.72 bd (12.9)		4.14 d (12.6)		3.54 (14.2, 5.2)
23	27.9	1.55 s	12.2	2.10 s	12.3	1.90 s	31.5	1.85 s	27.0	1.44 s
24	18.9	0.97 s	24.6	1.04[a]	24.7 br	1.41s	23.2	1.25 s	16.9	1.76 bs
25	22.5	1.03 s	28.5	0.99 s	29.0	1.33 s	26.4	0.94 s	25.8	1.66 bs
26	169.2		169.7		172.4		169.4		170.0	
27	21.3	1.67 s	20.3	1.63 s	20.7	2.05 s	19.9	1.53 s	22.1	1.76 bs
OH		0.62 bs								0.62 t (5.2)

[a]Multiplicity not determined due to overlapping signals/chemical shifts determined from 2D data.

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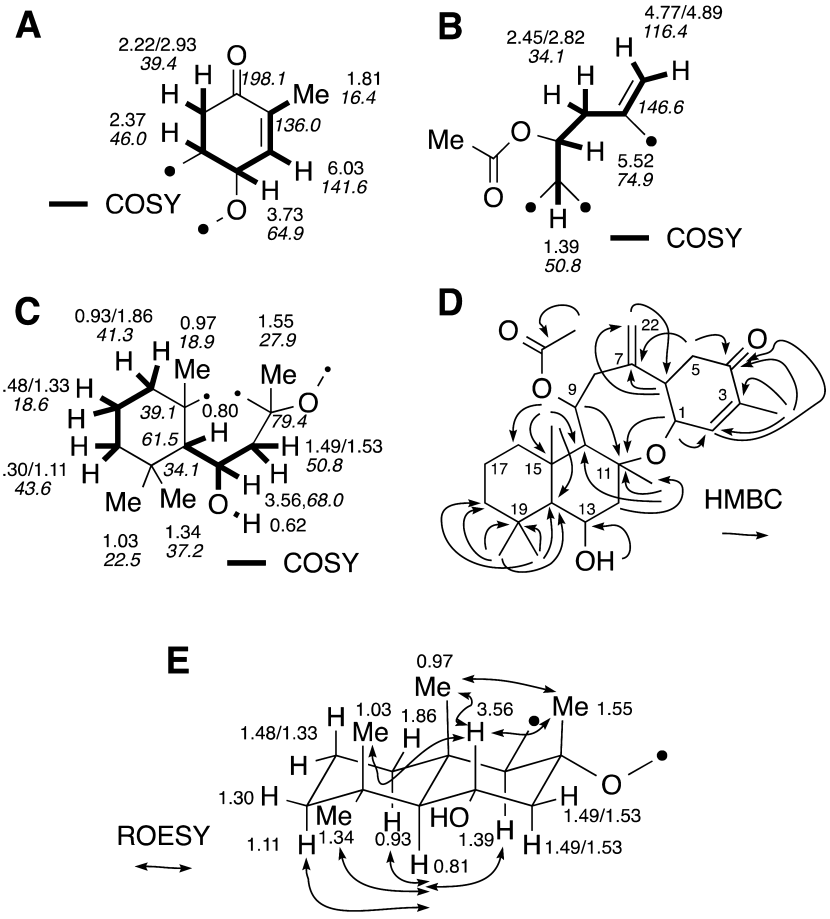

Figure 1. Fragments of ansellone B (**4**).

13) showed HMBC corrrelations into carbon resonances at δ 37.1 (C-19) and 43.7 (C-15). This set of correlations established a bond between C-14 and C-19 and between C-14 and another aliphatic carbon (C-15). NOESY correlations between both H-1 (δ 3.57) and H-8 (δ 5.49) and Me-23 (δ 2.10) supported the expected linkage between C-9 and C-10 as shown. Fragment **A** accounted for six sites of unsaturation and the cross conjugated ketone for another 3 sites. The lack of NMR evidence for additional sites of unsaturation required that fragment **B** was bicyclic and the absence of additional methyl resonances suggested that the remaining carbons were methine or methylene carbons.

The NMR data for **5** collected in C_6D_6 at 600 MHz contained a number of extremely broad carbon resonances, presumably caused by a slow conformational reorientation, that did not show correlations to proton resonances in the either the HSQC or HMBC spectra. This signal broadening made it impossible to conclusively assign a final structure to phorbadione (**5**). Fortunately, the NMR data for **5** recorded in CD_3OD showed sharp well-resolved resonances for all 27 carbon atoms in the molecule and gave HSQC and HMBC correlations that revealed the rest of the structure. The most significant difference in the ^{1}H NMR spectrum of **5** recorded in CD_3OD compared with the spectrum recorded in C_6D_6 (Table 1) was the presence of 6 upfield methyl singlets in the CD_3OD spectrum (δ 1.23, Me-20; 1.33, Me-25; 1.41, Me-24; 1.82, Me-21; 1.90, Me-23; 2.05, Me-27) compared with only 5 clear upfield methyl resonances in the spectrum recorded on C_6D_6 (δ 0.93, Me-20; 1.75, Me-21; 2.10, Me-23; 0.99, Me-25; 1.63, Me-27). In addition, from the CD_3OD data it was possible to identify a spin system comprised of three contiguous apliphatic methylene groups (δ ^{1}H 1.49/2.36 H-16/H-16′, 35.4, C-16; 1.67/1.99 H-17/H-17′, 19.3, C-17; 1.39/1.73 H-18/H-18′, 41.6, C-18) identical to that found in ansellone B (**4**) (Table 1). HMBC correlations in the CD_3OD data (Figure 2, fragment **C**) routinely assigned the methyl resonances at δ 1.23 (Me-20), 1.33 (Me-25), and 1.90 (Me-23) and confirmed the C-14/C-15, C-14/C-19 nd C-19/C-18 bonds identified from the C_6D_6 NMR data. The remaining methyl resonance at δ 1.41 (Me-24) showed HMBC correlations into resonances assigned to C-15 (δ 45.9), C-10 (δ 161.4), C-14 (δ 176.3), and C-16 (δ 35.4) consistent with the presence of a decalin ring system typical of ansellane sesterterpenoids (Figure 2, fragment **E**). HMBC correlations observed in the CD_3OD data between H-1 and C-9, and between H-9 and both C-1 and C-10 provided additional support for the C-1 to C-9 ether linkage and the C-9 to C-10 bond in **3** that was not present in the C_6D_6 data (Figure 2, fragment **E**). We have assumed that phorbadione (**5**) has the same absolute configurations at C-1 (*R*), C-6 (*R*), C-9 (*R*), and C-15 (*S*) as ansellones A (**3**) and B (**4**). This was supported by comparing the calculated electronic CD spectrum for **5** with the observed spectrum (Supporting Information).

Secoepoxyansellone A (**6**) gave a $[M + Na]^+$ ion at *m*/*z* 479.2414 in the HRESIMS appropriate for a molecular formula of $C_{27}H_{36}O_6$ (calcd for $C_{27}H_{36}O_6Na$, 479.2410), requiring 10 sites of unsaturation. Analysis of the COSY, HMBC, and HSQC data obtained for **6** readily identified the cyclohexenone substructure (Figure 3, fragment **A**) in **6** that is a hallmark of

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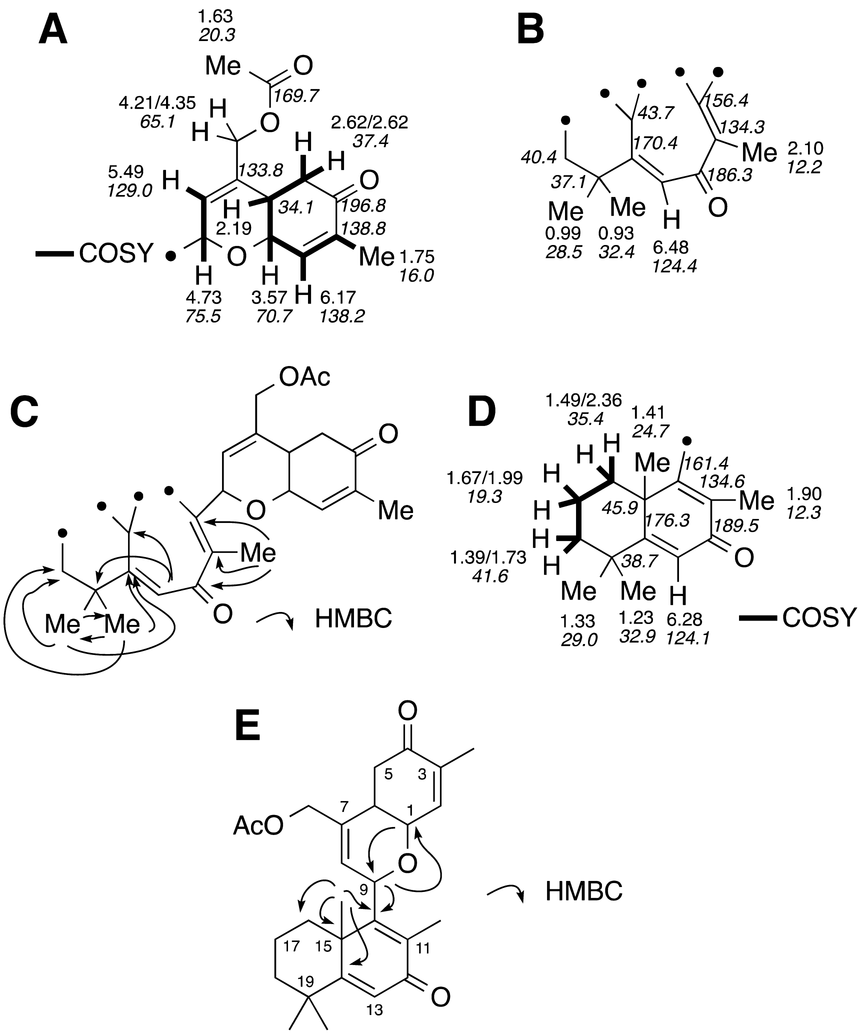

Figure 2. Fragments of phorbadione (**5**). Panels A–C are data recorded in C_6D_6. Panels D and E are data recorded in CD_3OD.

this family of sesterterpenoids. The acetoxymethyl fragment found in ansellone A (**3**) could also be identified in the 2D NMR data for **6** as shown in Figure 3**A** and 3**C**. HMBC correlations between the acetoxy methylene proton resonances (δ 3.60/4.14 H-22/H-22′) and carbon resonaces at δ 34.4 (C-6), 59.5 (C-7), and 55.7 (C-8) indicated that the $\Delta^{6,7}$ alkene in **3** had been replaced by an epoxide in **6**. The H-8 resonance at δ 2.99 in the ^{1}H NMR spectrum of **6** was a sharp singlet indicating that the H-9 proton in ansellone A (**3**) was missing in **6**. Correlations between H-8 and carbon resonances at δ 143.3 (C-9) and 123.7 (C-10), and between H-10 (δ 4.81) and carbon resonances at δ 143.3 (C-9) and 55.7 (C-8) in the HMBC spectrum of **6** were consistent with the presence of an enol ether at C-9 and C-10 in place of the aliphatic C-1 to C-9 ether linkage in **3**.

A second fragment identified in **6** shown in Figure 3**B** contained a conjugated methyl ketone (δ ^{1}H 1.85, s: δ ^{13}C 31.5, C-23; 198.1, C-11), a proton spin system consisting of a disubstituted alkene (δ ^{1}H 5.89, H-12; 5.85, H-13: ^{13}C 129.2, C-12; 146.3, C-13) and an aliphatic methine (δ ^{1}H 3.89, H-14; ^{13}C 49.3, C-14), a pair of geminal methyls (δ ^{1}H 0.94, Me-25; 1.08, Me-20: ^{13}C 26.4, C-25; 31.8, C-20) attached to a quaternary carbon (δ ^{13}C 34.3, C-19), a methyl (δ ^{1}H 1.25, Me-24; ^{13}C 23.2, C-24) attached to a quaternary carbon (δ 38.2, C-15), and a spin system consiting of three contiguous methylene groups (δ ^{1}H 1.48/1.69, H-16/H-16′; 1.48, H-17/H-17′; 1.20/1.30, H-18/H-18′: ^{13}C 37.3, C-16; 19.4, C-17, 38.6, C-18).

HMBC correlations between Me-24 (δ 1.25) and carbon resonances at δ 123.7 (C-10), 49.3 (C-14), 38.2 (C-15), and 37.3 (C-16) established linkages between the quaternary carbon C-15 and each of the C-10 alkene, the C-14 methine, and C-16 methylene carbons. Similarly, HMBC correlations observed between both Me-20 (δ 1.08) and Me-25 (δ 0.94) and carbon resonances at δ 34.3 (C-19), 49.3 (C-14), and 38.6 (C-18) established linkages between the quaternary carbon C-19 and both of the C-14 methine and C-18 methylene carbons completing a cyclohexane ring as shown in Figure 3, fragment **C**. Finally, HMBC correlations between all of Me-23 (δ 1.08), H-12 (δ 5.89), and H-13 (δ 5.85) and the ketone carbonyl resonance at δ 198.1 (C-11) confirmed that the methyl ketone was a substituent on the disubstituted $\Delta^{12,13}$ alkene.

tROESY data established the relative configuration of **6** as shown in Figure 3, fragment **D**. Correlations between H-1 (δ 4.37) and H-6 (δ 2.31) showed that they were *cis*; correlations between H-8 (δ 2.99) and H-10 (δ 4.81) established the $\Delta^{9,10}$ alkene configuration as *Z*; correlations between H-8 (δ 2.99) and H-22/22′ (δ 3.60/4.14), and between H22/H22′ and H-5/H-5′ (δ 2.32/2.41) established that H-8 and C-22 were *cis* substituents on the C-7/C-8 epoxide and they were both *trans* to H-6; and correlations between Me-24 (δ 1.25) and both Me-

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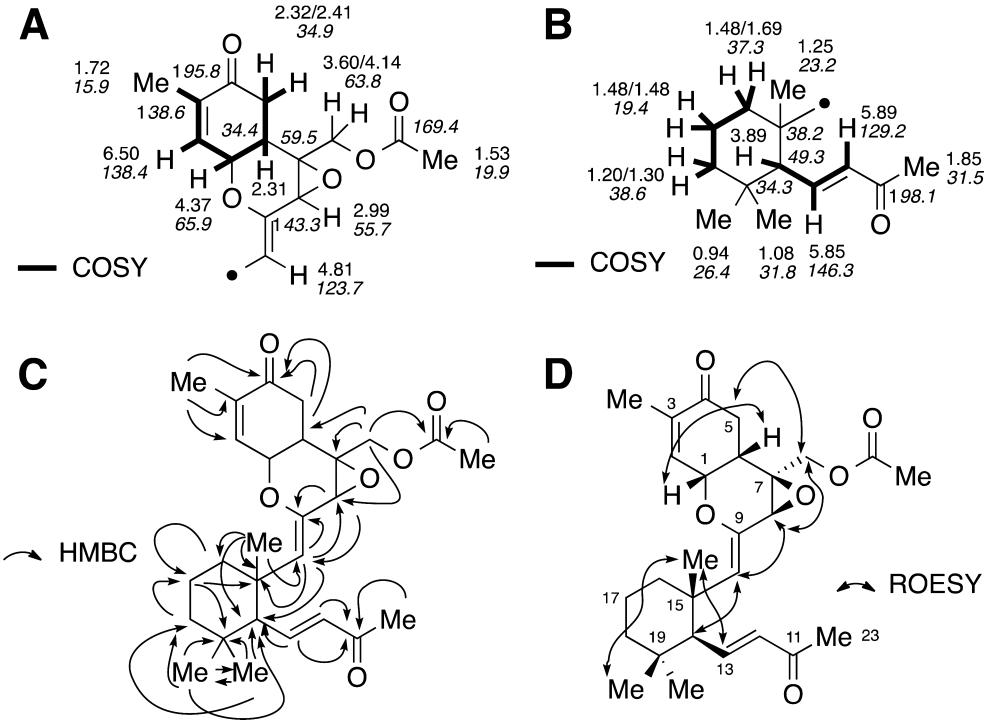

Figure 3. Fragments of secoepoxyansellone A (**6**).

25 (δ 0.94) and H-13 (δ 5.85), and between H-14 (δ 3.89) and H-10 (δ 4.81), were consistent with the C-14 to C-19 ring being in a chair conformation with H-14 and C-10 *cis* to each other as shown in Figure 3, fragment **D**. We have assumed that C-1, C-6, C-14, and C-15 have the same absolute configurations in **6** as in ansellone A (**3**).

Alotaketal C (**7**) was isolated as an oil that gave a $[M + Na]^+$ peak at *m*/*z* 481.2565 in the HRESIMS appropriate for a molecular formula of $C_{27}H_{38}O_6$ (calcd for $C_{27}H_{38}O_6Na$, 481.2566). Analysis of the 1D and 2D NMR data obtained for alotaketal C (**7**) (Table 1 and Supporting Information) showed that it was an analogue of alotaketal B in which the isovalerate ester at C-11 in alotaketal B (**2**) had simply been replaced by an acetate ester in **7**.

Alotaketal C (**7**) activated cAMP signaling in HEK293 cells with an EC_{50} of 6.5 μM, while ansellone B (**4**) was toxic at all concentrations tested. Interestingly, alotaketal B (**2**), which differs from alotaketal C (**7**) simply by having the acetate ester in **7** replaced by an isovalerate ester is active in the assay with an EC_{50} of 0.24 μM, and alotaketal A (**1**), which has an olefinic methylene at C-22 has an EC_{50} of 0.018 μM. These data further emphasize that the C-22 functionality is an important part of the cAMP activating pharmacophore of the alotaketals. The small quantities of phorbadione (**5**) and secoepoxyansellone A (**6**) that were available were not sufficient for biological testing. Forskolin, a plant diterpenoid that is widely used as a cell biology tool to activate cAMP signaling in cells, is active in the HEK293 cell-based assay with an EC_{50} of 3.0 μM, which is comparable to the potency of alotaketal C (**7**), but is much weaker than the potency of alotaketals A (**1**) and B (**2**).[6]

Ansellone B (**4**) has an unprecedented heterocyclic skeleton that contains an oxocane ring. Secoepoxyansellone A (**6**) represents the first example of the degraded "secoansellane" sesterterpenoid carbon skeleton. The discovery of alotaketal C (**7**) in the B.C. *Phorbas* species means that 'alotane' sesterterpenoids have now been isolated from sponges collected at three widely separated locations around the Pacific Rim [Papua New Guinea (South Western Pacific), Korea (North Western Pacific), and British Columbia (North Eastern Pacific)].

EXPERIMENTAL SECTION

Specimens of the sponge *Phorbas* sp. were collected by hand using SCUBA at a depth of 10 m off Ansell Place, in Howe Sound, British Columbia, Canada in February 2010 and twice in March 2012 to give 400 g, 222 g and 150 g sponge samples, respectively. In each case the freshly collected sponge material was immediately frozen and stored at −20 °C until extraction. A voucher sample (RMNH POR. 5227) has been deposited at the University of Amsterdam.

The frozen sponge samples (400 g, 222 and 150 g) were extracted exhaustively with MeOH (3 × 150 mL). The combined MeOH extracts were concentrated in vacuo to afford orange gums. The extracts were each partitioned between H_2O (100 mL) and EtOAc (3 × 50 mL) and the combined EtOAc extracts evaporated under reduced pressure to give three samples of dark orange oil.

The EtOAc soluble material obtained from the 400 g sponge sample was fractionated on Si gel flash chromatography (step gradient: hexanes to 4:6 hexanes/EtOAc, 2 g Sep pak). An orange oil (18 mg) eluting with 4:1 hexanes/EtOAc contained ansellone B (**4**) which was purified by normal-phase HPLC using a Alltech Apollo silica, 5 μm, 25 × 1.0 cm column, with 7:3 hexanes/EtOAc as eluent to give 3.4 mg of **4**. The fraction that eluted with 7:3 hexanes/EtOAc was fractionated by C_{18} reversed-phase HPLC using a CSC-Inertsil 150A/ODS2, 5 μm, 25 × 0.94 cm column, with 1:1 MeCN/H_2O as eluent to give phorbadione (**5**) (<0.1 mg). The fraction that eluted with 1:1 hexanes/EtOAc was first fractionated by C_8 reversed-phase HPLC using a Phenomenex, 5 μm, Luna 25 × 1.0 cm column, with 3:2 MeCN/H_2O as eluent, and gave a fraction that was then further purified on C_{18} reversed-phase HPLC using a InertSustain, 5 μm, 25 × 0.46 cm column, with 7:3 MeCN/H_2O as eluent, to give alotaketal C (**7**) (5.8 mg).

The EtOAc-soluble material obtained from the 222 g sponge sample was chromatographed on Sephadex LH20 with 4:1 MeOH/CH_2Cl_2 as eluent to give six fractions A-F. Fraction B (200 mg) was subjected to Si gel flash chromatography (step gradient: hexanes to EtOAc, 2 g Sep

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pak) to give 13 fractions BA-BM. A pure sample of secoepoxyansellone A (**6**) (0.4 mg) was isolated from fraction BH (87.7 mg) via C_{18} reversed-phase HPLC using a CSC-Inertsil 150A/ODS2, 5 μm, 25 × 0.94 cm column, with 13:7 MeCN/H_2O as eluent.

The EtOAc soluble material obtained from the 150 g sponge sample was chromatographed on Sephadex LH20 with MeOH as eluent to give five fractions. The major faction was subjected to Si gel flash chromatography (step gradient: hexanes to 1:1 hexanes/EtOAc, 2 g Sep pak). The fraction that eluted with 7:3 hexanes/EtOAc was fractionated by C_{18} reversed-phase HPLC using a InertSustain, 5 μm, 25 × 0.46 cm column, with 3:2 MeCN/H_2O as eluent, to give an additional and larger sample of phorbadione (**5**) (2.8 mg).

Ansellone B (4). Isolated as a white film: $[\alpha]^{25}_D$ −12.5 (*c* 0.05 MeOH); ^{1}H NMR and ^{13}C NMR, see Table 1; positive-ion HRESITOFMS $[M + Na]^+$ *m*/*z* 467.2783 (calcd for $C_{27}H_{40}O_5Na$, 467.2773).

Phorbadione (5). Isolated as a colorless oil: $[a]^{25}_D$ +34.6 (*c* 1.86 CH_2Cl_2); UV (1:1 MeCN/H_2O) λ_{max} 250 nm; CD spectrum (Supporting Information); ^{1}H NMR and ^{13}C NMR, see Table 1; positive-ion HRESITOFMS $[M + Na]^+$ *m*/*z* 461.2297 (calcd for $C_{27}H_{34}O_5Na$, 461.2304).

Secoepoxyansellone A (6). Isolated as a colorless oil: $[\alpha]^{25}_D$ +10.6 (*c* 0.08 MeOH); UV (MeOH) λ_{max} (log ε) 218.9 nm (4.95); ^{1}H NMR and ^{13}C NMR, see Table 1; positive-ion HRESITOFMS $[M + Na]^+$ *m*/*z* 479.2414 (calcd for $C_{27}H_{36}O_6Na$, 479.2410).

Alotaketal C (7). Isolated as a colorless oil: $[\alpha]^{25}_D$ −8.1 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ε) 230 nm (4.58); ^{1}H NMR and ^{13}C NMR, see Table 1; positive-ion HRESITOFMS $[M + Na]^+$ *m*/*z* 481.2556 (calcd for $C_{27}H_{38}O_6Na$, 481.2566).

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR Spectra for **4**−**7**; CD spectrum for **5**, details of CD calculation for **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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