Phorbasin B and C: Novel Diterpenes from a Southern Australian Marine Sponge, *Phorbas* Species

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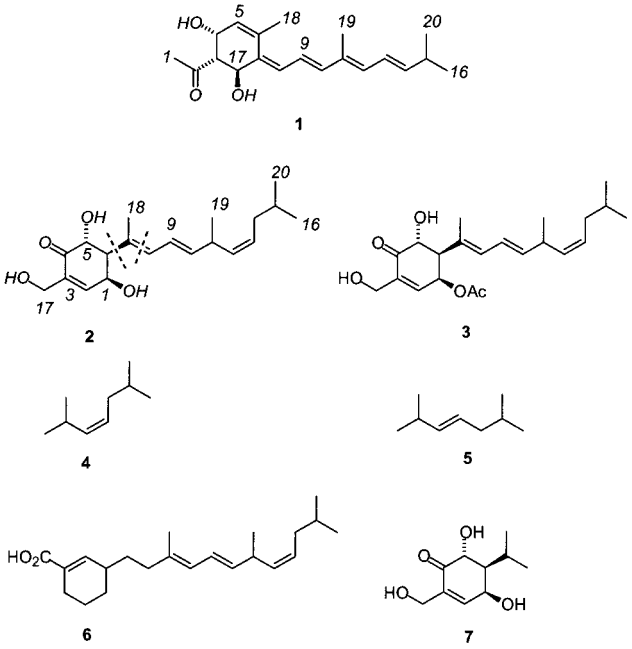
A southern Australian *Phorbas* sp. has yielded the novel diterpenes phorbasin B (**2**) and phorbasin C (**3**). Phorbasins B and C possess a hitherto unknown carbon skeleton, and their structures were assigned on the basis of detailed spectroscopic analyses.

Sponges of the *Phorbas* genus do not feature prominently in the marine natural products literature, with reports limited to accounts of heterocyclic phorboxazoles[1] and phorbazoles,[2] and more recently the novel rearranged diterpene phorbasin A (**1**).[3] In continuing our investigations into the chemistry of southern Australian marine sponges we report the isolation and structure elucidation of the two novel diterpenes phorbasin B (**2**) and phorbasin C (**3**). These compounds were isolated from a *Phorbas* sp. collected during scientific trawling operations in the Great Australian Bight.



The crude EtOH extract of the *Phorbas* sp. displayed growth inhibitory activity against the Gram positive bacteria *Staphylococcus aureus* and *Micrococcus luteus*. Bioassay-directed fractionation (silica SPE and HPLC) of the CH_2Cl_2-soluble portion of the crude *Phorbas* extract yielded as the antibacterial consitituents two structurally novel diterpenes, phorbasin B (**2**) and phorbasin C (**3**).

The high-resolution ESI(+)MS data for phorbasin B (**2**) revealed a pseudo molecular ion (M + Na) consistent with a formula ($C_{20}H_{30}O_4Na$, Δmmu = 0.9) requiring six double bond equivalents. NMR analysis of **2** revealed resonances for two magnetically equivalent isopropyl methyls (^1H δ 0.89; ^{13}C 22.4 ppm), a 2° methyl (^1H δ 1.14; ^{13}C 20.8 ppm), an olefinic methyl (^1H δ 1.83; ^{13}C 16.2 ppm), an isolated ABq spin system attributed to the diastereotopic methylene protons of a 1° allylic alcohol (^1H δ 4.15 and 4.06; ^{13}C 60.0 ppm), two oxymethines (^1H δ 3.75 and 4.56; ^{13}C 66.6 and 70.2 ppm), and a ketone (^{13}C 201.1 ppm) (see Table 1). The latter observation together with the presence of eight additional sp^2 hybridized carbons associated with two disubstituted and two trisubstituted double bonds accounted for five double bond equivalents. These observations required that phorbasin B (**2**) be monocyclic.

Analysis of the 2D NMR COSY and TOCSY data (Table 1) for **2** revealed two diagnostic correlation sequences; the first from H-8 to H_3-16, incorporating H_3-19 and H_3-20; and the second from H-5 to H-2, incorporating H-1, H-6, and H_2-17. The deshielded chemical shifts of C-1 (66.6 ppm), C-5 (70.2 ppm), and C-17 (60.0 ppm) confirmed that all were substituted by oxygen, which given the considerations outlined above was best accommodated by alcohol functionalities. Furthermore, gHMBC correlations from H-2 to C-4 and C-17, and from H-5 to C-4, permitted closure of the cyclohexenone ring system as shown and defined the three substructural units as indicated on the structure diagram for **2**. Connection of these substructural units as shown was achieved by observation of gHMBC correlations from (a) H-5 to C-6 and C-7, (b) from H-6 to C-7 and C-8, (c) from H-8 to C-6 and C-18, and (d) from H_3-18 to C-1, C-6, and C-7. Thus the gross structure for phorbasin B (**2**) is as shown.

The *Z* stereochemistry about $\Delta^{7,8}$ was determined from the shielded chemical shift for C-18 (16.2 ppm), while an *E* stereochemistry about $\Delta^{9,10}$ was apparent from the magnitude of $J_{9,10}$ (15.0 Hz). Overlap of the ^1H NMR (benzene-d_6) resonances for H-12 and H-13 prevented measurement of $J_{11,13}$ as a means to determine stereochemistry about Δ.[12,13] Instead a *Z* stereochemistry was assigned on the basis of comparison of ^{13}C NMR chemical shifts for olefinic carbons in the synthetic model compounds (*Z*)-2,6-dimethyl-3-heptene (**4**) and (*E*)-2,6-dimethyl-3-heptene (**5**).[4] This empirical comparison notes that ^{13}C NMR shift differences between the two olefinic carbons in *Z* stereoisomers (i.e., **4**) differ by ~12 ppm, while for *E* stereoisomers (i.e., **5**) this difference is ~1 ppm. We have recently employed this technique to determine double bond stereochemistry in the marine natural product clathrin A (**6**),[5] which featured a significant structural fragment in common with phorbasin B (**2**). In the case of phorbasin B (**2**) the ^{13}C NMR chemical shift difference between C-12 and C-13 (2.6 ppm) was consistent with a *Z* stereochemistry.

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Table 1. NMR (d_6-benzene, 400 MHz) Data for Phorbasin B (**2**)

no.	$^{13}C^a$ δ	^1H (δ, m, J Hz)	COSY	TOCSY	gHMBC ^1H to ^{13}C
1	66.6	3.75, br	H-2	H-2, H-6	
2	142.2	6.44, d, 9.4	H-1	H-1, H_2-17	C-17, C-6, C-4
3	136.8				
4	201.1				
5	70.2	4.56, d, 12.2	H-6	17-OH/5-OH, H-6, H_2-17	C-4, C-6, C-7
6	54.9	2.29, dd, 12.2, 3.2	H-5	H-1, H-5	C-5, C-7, C-8
7	133.4				
8	129.4	6.14, d, 10.8	H-9	H-9, H-18	C-6, C-9, C-10, C-18
9	125.0	6.44, dd, 10.8, 15.1	H-8, H-10	H-8, H-10	C-7, C-8, C-11
10	139.1	5.73, dd, 7.0, 15.1	H-11, H-9	H-9, H-11	C-8, C-11, C-12, C-19
11	40.3	2.92, m	H-10, H_3-19	H-10, H-12/H-13, H_3-19	C-10, C-12, C-13
12	135.7	5.46, m	H_2-14, H-13	H-11, H-13, H_2−14	C-11, C-14
13	128.3	5.46, m	H_2-14, H-12	H-11, H-12, H_2−14	C-11, C-12, C-14
14	42.4	1.92, dd, 6.6, 6.6	H-15, H-12/H-13	H-12/H-13, H-15, H_3-16/H_3-20	C-12, C-13, C-15, C-16, C-20
15	28.7	1.58, m	H_2-14, H_3-16/H_3-20	H_2-14, H_3-16/H_3-20	C-13, C-14, C-16, C-20
16, 20	22.4	0.89, d, 6.7	H-15	H-15, H_3-16/H_3-20	C-14, C-15, C-16, C-20
17	60.0	4.15/4.06, ABq, 14.5	H_2-17	H-2, H-5	
18	16.2	1.83, s		H-8	C-1, C-6, C-7, C-8, C-9
19	20.8	1.14, d, 6.9	H-11	H-11	C-10, C-11, C-12
17-OH,^{b} 5-OH		3.55, brs		H-5	C-6
1-OH		0.61, brs			

^{a} Assignments supported by gHMQC data. ^{b} Assignments can be interchanged.

The relative stereochemistry about C-1, C-5, and C-6 was successfully assigned based on $J_{1,6}$ and $J_{5,6}$. Molecular modeling of all relative stereoisomers about C-1, C-5, and C-6 provided theoretical measures of the H-1 to H-6, and H-5 to H-6, dihedral angles. These calculations were used to compare theoretical with experimentally measured values for $J_{1,6}$ and $J_{5,6}$ with the best fit being for the relative stereochemistry as shown ($J_{1,6}$ theory 2.8 Hz, expt 3.6 Hz, $J_{5,6}$ theory 9.8 Hz, expt 12.2 Hz). This stereochemical assignment was supported by spectroscopic comparison to the known terrestrial plant natural product 3β,5α,7-trihydroxycarvotacetone (**7**)[6] ($J_{1,6}$ 3.5 Hz, $J_{5,6}$ 12 Hz), which is an example of a large family of related metabolites[7,8] all possessing a cyclohexenone substructure in common with phorbasin B (**2**).

Phorbasin C (**3**) was spectroscopically very similar to, and readily identified as the C-1 acetate of, phorbasin B (**2**). The molecular formula for **3** differed from that for **2** by the requisite acetyl unit (42 mass units), and the ^1H NMR spectrum of **3** revealed both a characteristic acetate methyl resonance (^1H δ 2.03) and significant deshielding of H-1 (δ 5.58) compared to the corresponding ^1H NMR resonance for **2** (δ 4.45). Lack of material and the instability of phorbasin A and B prohibited a determination of absolute stereochemistry or quantification of their antibacterial properties.

To the best of our knowledge phorbasin B (**2**) and C (**3**) are the first examples of a new carbon skeleton. Although unique, the phorbasin B and C carbon skeleton embraces structural features in common with the marine natural product clathrin A (**6**) and a series of terrestrial monoterpene carvolactones exemplified by **7**. Although not possessing the same carbon skeleton, nor occurring as co-metabolites, it seems likely that phorbasin A (**1**) shares a common biosynthetic pathway with phorbasin B (**2**) and C (**3**).

Experimental Section

General Experimental. See ref 9. Computational studies were performed using MM2 energy minimization capabilities in Chem 3D Pro version 4.0.

Animal Material. A specimen of sponge identified as a *Phorbas* sp. (Museum of Victoria Registration No. F80008) was collected by epibenthic sled at a depth of 90 m in the Great Australian Bight in July 1997. Taxonomic description of this specimen is as follows: growth form macrobenthic, stalked, flabellate, biplanar, 5−10 mm thick; color in life sandy orange; color in EtOH beige-orange; texture firm, but compressible, cartilagenous, harsh; surface opaque, irregular, pockmarked with areoles; oscules discrete, conspicuous, marginal with membranous lip; spicules megascleres oxeas mammilliform (360−400 × 5−7.5 μm); acanthostyles curved (180−200 × 7.5−10 μm); microscleres arcuate isochelae (25 μm); ectosome a dense crust of chelae over an almost continuous palisade of paratangential oxeas, easily detachable; choanosome an almost halichondroid arrangement of heavy spongin fibers cored and occasionally echinated by acanthostyles with irregular tracts of oxeas throughout the collagen in the mesohyl. Chelae are scattered throughout and line pore areas.

Extraction and Isolation. The sample was transported frozen to the laboratory, where it was thawed, diced, and stored in EtOH at −20 °C. The EtOH extract was concentrated in vacuo and partitioned between CH_2Cl_2, BuOH, and H_2O. Silica SPE (10% stepwise gradient elution from hexane to CH_2Cl_2 to EtOAc) and HPLC (2 mL/min 50% EtOAc/hexane through a Phenomenex Spherex 5μ silica 250 × 10 mm column) fractionation of the CH_2Cl_2-soluble material yielded two novel antibiotic diterpenes, phorbasin B (**2**) (7.2 mg, 0.92%) and phorbasin C (**3**) (2.5 mg, 0.34%). Percentage yields are calculated from the mass of crude EtOH extract.

Phorbasin B (2): yellow oil; $[\alpha]_D$ −60.2° (*c* 0.064, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (9.33) 279 (7.74); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.00, d, J = 5.4 Hz, H-2; 6.44, dd, J = 10.8, 15.1 Hz, H-9; 6.14, d, J = 10.8 Hz, H-8; 5.73, dd, J = 7.0, 15.1 Hz, H-10; 5.46, m, H-12, H-13; 4.71, d, J = 12.3 Hz, H-5; 4.45, m, H-1; 4.45 and 4.34, ABq, J = 13.8 Hz, H_2-17; 2.92, m, H-11; 2.68, dd, J = 3.2, 12.3 Hz, H-6; 1.92, s, H_3-18; 1.83, dd, J = 6.1, 6.1 Hz, H_2-14; 1.58, m, H-15, partially obscured by H_2O; 1.14, d, J = 6.9 Hz, H_3-19; 0.89, d, J = 6.7 Hz, H_3-16, H_3-20; See Table 1 for NMR data in d_6-benzene; ESI(+)MS *m/z* $[M + H]^+$ 335, $[M + Na]^+$ 357; HR ESI(+)MS *m/z* $[M + Na]^+$ 357.2033 (calcd for $C_{20}H_{30}O_4Na$, 357.2042, Δmmu = 0.9). The sample decomposed before IR data could be acquired.

Phorbasin C (3): yellow oil; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.95, d, J = 6.8 Hz, H-2; 6.25, dd, J = 10.7, 15.1 Hz, H-9; 6.04, d, J = 10.7 Hz, H-8; 5.66, dd, J = 7.0, 15.1 Hz, H-10; 5.58, brs, H-1; 5.35, m, H-12 and H-13; 4.73, d, J = 12.2 Hz, H-5; 4.38 and 4.39, ABq, J = 4.4 Hz, H_2-17; 3.45, brs, 5-OH and 17-OH; 2.87, m, H-11; 2.78, dd, J = 12.2, 3.6 Hz, H-6; 2.03, s, OAc; 1.89, s, H_3-18; 1.87, dd, J = 6.1, 6.1 Hz, H_2-14; 1.58, m, H-15; 1.09, d, J = 6.9 Hz, H_3-19; 0.87, d, J = 6.7 Hz, H_3-16 and H_3-20; ESI(+)MS *m/z* $[M + H]^+$ 377. The sample decomposed before $[\alpha]_D$, UV, IR, and HR ESI(+)MS data could be acquired.

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