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Phorbasins D−F: Diterpenyl-taurines from a Southern Australian Marine Sponge, *Phorbas* sp.

Hua Zhang and Robert J. Capon*

Centre for Molecular Biodiversity, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Queensland 4072, Australia

r.capon@imb.uq.edu.au

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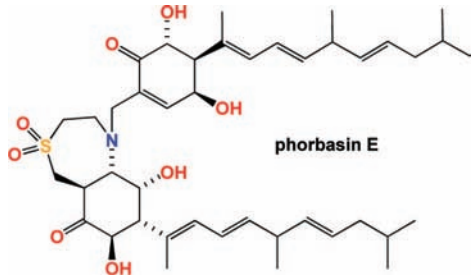
ABSTRACT



Fractionation of an Australian marine sponge, *Phorbas* sp., yielded the known phorbasins B (1) and C (2) together with three novel diterpenyl-taurines, phorbasins D−F (3−5). Partial absolute structures were assigned on the basis of detailed spectroscopic analysis. Phorbasins D−F (3−5) are the first reported examples of terpenyl-taurines linked via an amine moiety, and phorbasins E and F (4 and 5) incorporate an unprecedented heterocycle. A plausible biosynthetic route is proposed for the incorporation of taurine and heterocycle formation.

During an investigation into anticancer agents from marine organisms we examined a collection of invertebrates and algae acquired over a period of 20+ years from various southern Australian and Antarctic locations, ranging from intertidal to deep water, utilizing collection methods from hand (SCUBA) to commercial and scientific trawling. Solvent extracts prepared from these samples were screened for their growth inhibitory and cytotoxic activity against a panel of human cancer cell lines, leading to the identification of a number of noteworthy bioactive extracts. One extract that came to our attention was derived from a *Phorbas* sp. sponge collected in July 1995 during scientific trawling operations (epibenthic sled, depth 65 m) aboard the RV Franklin in the Great Australian Bight. The *Phorbas* genus is well-known in the scientific literature for its ability to yield novel metabolites, including in order of discovery the phorbazoles (1994),[1] phorboxazoles (1995),[2] phorbasins (2000 and 2001),[3,4] gagunins (2002),[5] phorbasterones (2004),[6] phorbasides,[7] and amaranzole[8] (2007). In our prior investigations into Great Australian Bight *Phorbas* species we reported phorbasin A (2000)[3] and phorbasins B and C (2001).[4] In this report we revisit the chemistry of Great Australian Bight *Phorbas* species and describe a bioassay-guided fractionation study leading to the reisolation and structure revision of phorbasins B and C (**1** and **2**), together with the discovery and structure elucidation of three new analogues, phorbasins D−F (**3**−**5**). Phorbasins D−F (**3**−**5**) are noteworthy in that they incorporate a terpenyl-taurine

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residue unprecedented in the scientific literature. Adding to their structural novelty, phorbasins E (**4**) and F (**5**) are dimers fused by an unprecedented seven-membered heterocycle. This report describes the isolation, characterization, and structure elucidation of phorbasins B−F (**1**−**5**), together with discussion on a plausible biosynthetic route to terpenyltaurines and the novel dimeric heterocycles.

Chemical fractionation of the EtOH extract of a Great Australian Bight *Phorbas* sp. using solvent partitioning, reverse phase solid phase extraction (SPE), and HPLC, yielded the cytotoxic agents as the known *Phorbas* diterpenes, phorbasins B (**1**) and C (**2**), together with three novel analogues, phorbasins D−F (**3**−**5**).

In a 2001 study Capon et al. reported[4] phorbasins B (**1**) and C (**2**) as the first examples of a new carbon skeleton and as the antibacterial principles from a Great Australian Bight *Phorbas* sp. The identity of **1** and **2** reisolated in this current study was confirmed by spectroscopic analysis and comparison to authentic spectroscopic data. When first reported,[4] assignment of the $\Delta^{12,13}$ configuration for phorbasins B and C (**1** and **2**) proved problematic as a result of poor dispersion of ^{1}H NMR (both $CDCl_3$ and C_6D_6) resonances for H-12 and H-13 (phorbasins B and C were unstable in $CDCl_3$). At that time a *tentative* $\Delta^{12,13}$ *Z* configuration was assigned on the basis of empirical ^{13}C NMR chemical shift comparisons for C-12 and C-13 with model compounds.[4] The current reisolation of **1** and **2** provided an opportunity to revisit that earlier tentative assignment. Acquisition of the NMR ($CDCl_3$) data for reisolated samples of **1** and **2** facilitated comparison to the literature data and confirmed the structure assignment. Acquisition of the NMR (CD_3OD) data for **1** and **2** facilitated comparison to the new cometabolites phorbasins D−F (**3**−**5**), while also providing a means to unambiguously resolve assignment of the $\Delta^{12,13}$ configuration. Unlike the earlier ^{1}H NMR data sets (both $CDCl_3$ and C_6D_6), the ^{1}H NMR (CD_3OD) data for **1** and **2** (see Supporting Information, Table S1) resolved resonances for H-12 and H-13 and revealed values for $J_{12,13}$ of 15.3 and 15.5 Hz, respectively, indicative of a $\Delta^{12,13}$ *E* configuration. In light of this observation we propose a stereochemical revision to phorbasins B and C (**1** and **2**) as indicated.

High resolution ESI(+)MS analysis of phorbasin D (**3**) revealed a pseudo molecular ion (M + Na) consistent with a molecular formula ($C_{22}H_{35}NO_6S$, Δmmu −0.5) incorporating six double bond equivalents (DBE) (assuming an S^{II} oxidation state). Analysis of the ^{13}C NMR (CD_3OD) data for **3** (see Supporting Information, Table S2) revealed eight sp^2 carbons (δ_C 126.1−151.4) attributed to four carbon−carbon double bonds, together with a deshielded sp^2 resonance (δ_C 202.1) consistent with a ketone, accounting for five DBE and requiring that **3** is monocyclic. Comparison of the ^{1}H and ^{13}C NMR (CD_3OD) data for phorbasins B (**1**) and C (**2**) with phorbasin D (**3**) confirmed the structure fragment extending from C-7 to C-16 and incorporating three methyls (C-18, C-19, and C-20).

This same structure fragment was also confirmed by a sequence of diagnostic 2D NMR (CD_3OD) gCOSY and HMBC correlations (see Supporting Information, Table S2), while a large value for $J_{9,10}$ (15.1 Hz) and $J_{12,13}$ (15.4 Hz) established the $\Delta^{9,10}$ and $\Delta^{12,13}$ *E* configurations. A shielded ^{13}C NMR (CD_3OD) chemical shift for C-18 (δ_C 16.0) was consistent with a $\Delta^{7,8}$ *E* configuration, as previously attributed to phorbasins B and C (**1** and **2**).[4] This $\Delta^{7,8}$ *E* configurational assignment was further supported by ROESY (CD_3OD) correlations noted during discussion on phorbasin E (**4**), shown below.

Further analysis of the 1D and 2D NMR (CD_3OD) data for phorbasin D (**3**) revealed resonances and correlations consistent with a substituted cyclohexenone structure fragment C-1 to C-6, incorporating C-17, similar to that found in phorbasins B and C (**1** and **2**). Attachment of the C-6 side chain as shown was evident from the ^{1}H NMR (CD_3OD) chemical shift for H-6 (δ_H 2.71) and HMBC correlations from the H-5 oxymethine (δ_H 4.71) and the H-6 (δ_H 2.71) methine to the C-7 quaternary sp^2 olefinic carbon (δ_C 135.1). The relative configuration about the cyclohexenone ring was confirmed by $J_{5,6}$ (12.3 Hz) and $J_{1,6}$ (3.7 Hz) measurements diagnostic for pseudo diaxial and pseudo equatorial-axial configurations as shown, again consistent with those previously observed for phorbasins B and C (**1** and **2**).[4] A key difference in the NMR data for phorbasins B and C (**1** and **2**) compared to phorbasin D (**3**) was conversion of the isolated C-17 hydroxymethylene moiety in **1** and **2** to a similarly deshielded diastereotopic methylene in **3**, but with 2D NMR correlations to an X-CH_2CH_2-Y spin system (δ_H 3.36 and 3.12; δ_C 45.2 and 48.2). The structural arguments presented above account for all six DBE and all but the elements of NH (X-) and SO_3H (-Y), suggestive of the incorporation of a C-17 taurinyl substituent. Key HMBC correlations from H_2-17 to C-1′ and from H_2-1′ to C-17, together with the deshielded ^{1}H NMR (CD_3OD) resonances for H_2-17, unambiguously established a C-17 *N*-taurinyl residue. Thus, the structure for phorbasin D (**3**) with relative stereochemistry (excluding C-11) is as shown. Taurinyl amides are common conjugates to bile acids, with some marine metabolites featuring taurine bound to quinones,[9–11] imidazoles,[12,13] and fatty acids,[14,15] as well as polyketide,[16] amino acid,[17] purine,[18] styrene,[19] and pyridinium[20,21] resi-

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dues. This prior literature not withstanding, to the best of our knowledge phorbasin D (**3**) represents the first reported example of a terpenyl-taurine conjugated via an amine functionality.

High resolution ESI(+)MS analysis of phorbasin E (**4**) revealed a pseudo molecular ion (M + H) consistent with a molecular formula ($C_{42}H_{63}NO_8S$, Δmmu 0.8) incorporating 12 DBE (assuming an S^{II} oxidation state) suggestive of a dimeric phorbasin incorporating a single taurinyl residue. Analysis of the 1D and 2D NMR (CD_3OD) data for **4** (see Supporting Information, Table S3) confirmed the dimeric/taurinyl hypothesis and revealed two structure fragments. The NMR (CD_3OD) data for fragment A was very similar to that for phorbasin D (**3**), with a common carbon skeleton, functionality and stereochemistry. Principle differences in the NMR (CD_3OD) data between phorbasin E fragment A and phorbasin D (**3**) were focused around C-2, C-3, and C-17 and the taurinyl moiety (C-1′ and C-2′), suggestive of substitution between the taurinyl moiety and fragment B (see below). The 1D and 2D NMR (CD_3OD) data for phorbasin E fragment B displayed excellent comparisons with phorbasin D (**3**) across the full extent of the C-6″ side chain, including functionality and stereochemistry, with principle differences being focused around the carbocyclic moiety (C-1″ to C-6″). Whereas phorbasin D (**3**) incorporated a substituted cyclohexenone carbocycle, phorbasin E fragment B incorporated a substituted cyclohexanone carbocycle. Loss of the $\Delta^{2'',3''}$ residue in phorbasin E fragment B was accompanied by the appearance of two sp^3 methines (CH-2″ δ_H 3.00, δ_C 68.7 and CH-3″ δ_H 3.77, δ_C 44.4). Analysis of the gCOSY NMR (CD_3OD) data for the phorbasin E fragment B carbocycle revealed a correlation sequence across five contiguous sp^3 methine protons (H-5″ to H-3″, as indicated) to a deshielded sp^3 methylene (H-17″). The relative configuration about the chiral centers embedded within the phorbasin E fragment B carbocycle were determined by analysis of the ^1H NMR (CD_3OD) coupling constants $J_{5'',6''}$ (12.2 Hz, pseudo diaxial), $J_{6'',1''}$ (1.7 Hz, pseudo axial-equatorial), $J_{3'',2''}$ (13.1 Hz, pseudo diaxial), and $J_{2'',1''}$ (<2 Hz, pseudo axial-equatorial), with the 1,3 diaxial relationship between H-5″ and H-3″ further supported by a ROESY correlation. These observations confirmed that the substituents at C-5″, C-6″, C-2″, and C-3″ were all pseudo equatorial, while the substituent at C-1″ was pseudo axial. They also confirmed that C-2″ and C-17″ were substituted by heteroatom moieties, which on the basis of considerations from phorbasin E fragment A (as presented above) were attributed to the taurinyl heteroatom moieties N and SO_2.

The HMBC NMR (CD_3OD) data for phorbasin E (**4**) revealed a correlation from H_2-17 (δ_H 3.74, 3.60) to C-1′ (δ_C 44.2) of the taurinyl moiety, as well as from H_2-17 to a deshielded methine carbon(s) attributed to either C-1 (δ_C 68.8) of fragment A or C-2″ (δ_C 68.7) of fragment B. If this latter correlation to C-2″ were correct, it would reveal the nature of the connection between fragments A and B; however, the resolution of the ^{13}C and HMBC NMR (CD_3OD) data was insufficient to unambiguously differentiate correlations from H_2-17 to C-1 and/or C-2. Fortunately, the 1D and 2D NMR (d_5-pyridine) (see Supporting Information, Table S4) data for phorbasin E (**4**) did provide adequate resolution of the critical ^{13}C NMR resonances (C-1, δ_C 68.7; C-2″, δ_C 69.3) and revealed a key HMBC correlation (H-17b to C-2″) supportive of the amine connection as indicated. Given this latter connection, C-17″ was required to form a novel seven-membered heterocycle through the taurinyl SO_2 moiety, as indicated. Given the dimeric nature of phorbasin E (**4**) we propose that fragments A and B share a common biosynthetic origin and therefore a common absolute stereochemistry. The structure diagram for phorbasin E (**4**) is presented with the proposed relative configuration (excluding C-11 and C-11′) but an arbitrary absolute stereochemistry.

High resolution ESI(+)MS analysis of phorbasin F (**5**) revealed a pseudo molecular ion (M + H) consistent with a molecular formula ($C_{44}H_{65}NO_9S$, Δmmu 0.2) incorporating one additional DBE (assuming an S^{II} oxidation state) and being 42 amu heavier than phorbasin E (**4**), suggestive that phorbasin F (**5**) was a monoacetate analogue of phorbasin E (**4**). This hypothesis was confirmed by analysis of the NMR (CD_3OD) data for phorbasin F (**5**) (see Supporting Information, Table S5), which revealed an excellent comparison to phorbasin E (**4**) with the only significant differences being associated with deshielding of the H-1″ methine resonance in **5** compared to **4** ($\Delta\delta_H$ 1.41), together with the appearance of resonances for an *O*-acetate (δ_H 2.07; δ_C 21.3, 171.7). On the basis of these observations, we propose that phorbasin F (**5**) is the C-1″ acetate of phorbasin E (**4**).

In an attempt to assign absolute stereochemistry to the phorbasins, we acquired CD spectra for phorbasins B−E (**1**−**4**). CD data for phorbasin F was not acquired because of a scarcity of material. On the basis of the empirical rule by Snatzke[22] that acknowledges that the sign of the enone $n \rightarrow \pi^*$ Cotton effect is determined by the cyclohexenone ring conformation, the positive Cotton effects observed for phorbasin B (**1**) ($\Delta\varepsilon$ +0.7 at 330 nm), C (**2**) ($\Delta\varepsilon$ +0.8 at 334 nm), D (**3**) ($\Delta\varepsilon$ +0.5 at 337 nm), and E (**4**) ($\Delta\varepsilon$ +0.7 at 334 nm) are indicative of a 1*S*,5*R*,6*S* configuration. This conclusion was further supported by the comparison with CD data for a model compound, 5α-acetoxy-3β,7-ditigloyloxycarvotacetone ($\Delta\varepsilon$ +0.12 at 332 nm)[23] possessing the same stereochemical unit as found in phorbasins B−F (**1**−**5**). From these empirical observations and on biosynthetic grounds, we assign a common absolute stereochemistry to **1**−**5** as shown.

Phorbasins B−F (**1**−**5**) are likely derived from a common biosynthetic pathway that involves the formation of a monocarbocyclic diterpene such as **1**. Selective acetylation of the phorbasin B (**1**) C-1 hydroxy would yield phorbasin C (**2**), and nucleophilic displacement of the phorbasin B 17-hydroxy by hypotaurine/taurine could yield the terpenyl-taurine conjugate phorbasin D (**3**) (see Scheme 1). A

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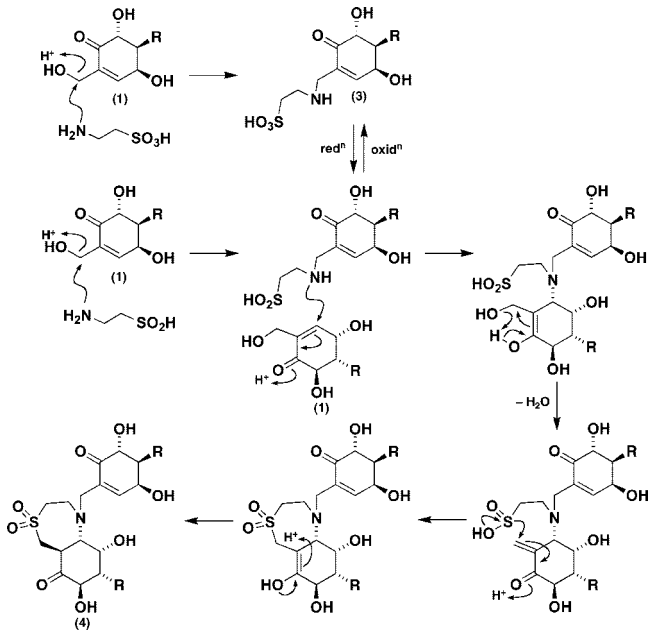
Scheme 1. Plausible Biosynthetic Pathway Linking Phorbasin B (**1**), Phorbasin D (**3**), and Phorbasin E (**4**)



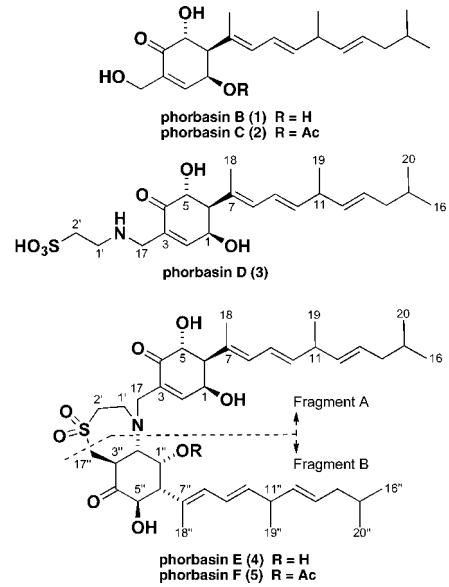


Figure 1. *Phorbas* sp. metabolites, phorbasins B−F (**1**−**5**).

plausible biosynthesis for phorbasin E (**4**) could proceed by way of Michael addition of a phorbasin B (**1**) hypotaurinyl intermediate to phorbasin B (**1**) with a subsequent intramolecular dehydration facilitating enone formation. Quenching of the enone by an intramolecular Michael addition would return the sulfone heterocycle as shown. The stereochemical preference about C-2″ and C-3″ in the dimeric product could be indicative of enzymatic control or, alternatively, could reflect a thermodynamic preference for pseudo diequatorial substitution at these two new sp^3 centers. A very similar process could be invoked to transform phorbasin C (**2**) to phorbasin F (**5**) or, alternatively, selective acetylation of phorbasin E (**4**) would yield phorbasin F (**5**). Preliminary analyses indicated that phorbasins B and C (**1** and **2**) and E (**4**) displayed GI_{50} values against lung (A549), colorectal (HT29), and breast (MDA-MB-231) cancer cell lines in the range 5−15 μM, whereas phorbasin D (**3**) was inactive at the highest tested concentration of 30 μM. Lack of material precluded testing of phorbasin F (**5**). The unprecedented taurine-substituted phorbasins D−F (**3**−**5**) described above are accompanied in the *Phorbas* extract by a number of minor biosynthetically related cometabolites that do not incorporate a taurinyl residue. The isolation and structure elucidation of these minor phorbasins, along with a comparative assessment of the in vitro cytotoxicity of all phorbasin cometabolites, remains a work in progress.

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Supporting Information Available: General experimental procedures together with NMR data and spectra for phorbasins B−F (**1**−**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

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