Phorbasin A: A Novel Diterpene from a Southern Australian Marine Sponge, *Phorbas* Species

Dat Vuong and Robert J. Capon*

School of Chemistry, University of Melbourne, Parkville, Victoria 3010, Australia

Received May 10, 2000

A southern Australian *Phorbas* species has yielded a novel diterpene, phorbasin A (**1**), possessing an unprecedented carbon skeleton. The structure for phorbasin A was determined by detailed spectroscopic analysis.

Reports of novel metabolites from the *Phorbas* genus of marine sponge [Duchassaing and Michelotti (1864), order Poecilosclerida, family Anchinoidae] are limited to only two accounts, which describe the heterocylic phorboxazoles[1] and phorbazoles.[2] Having secured a collection of several thousand unique southern Australian marine sponges, we were curious to explore the chemistry of other *Phorbas* specimens. To this end we examined a *Phorbas* species collected during scientific trawling operations in the Great Australian Bight, Australia, which was found to contain a novel unstable polyene diterpene to which we have attributed the structure and given the trivial name phorbasin A (**1**).

The EtOH extract of the *Phorbas* sp. was decanted and concentrated in vacuo, after which it was triturated with CH_2Cl_2, and the residue partitioned between *n*-BuOH and H_2O. The *n*-BuOH solubles were quickly fractionated by C_{18} solid-phase extraction (H_2O to MeOH) to yield phorbasin A (**1**) as an unstable pale yellow solid. Initial ^{1}H NMR analysis revealed a conjugated polyene, and care was taken to quickly acquire spectroscopic data while avoiding unnecessary exposure to chromatographic media, intense light, heat, air, and pH variations. When not being used to acquire spectroscopic data, samples of phorbasin A (**1**) were stored in sealed vials in the dark at −20 °C. Despite precautions, phorbasin A (**1**) eventually decomposed during the course of investigations. Decomposition was accelerated in CH_2Cl_2 and retarded in MeOH.

Positive ESIMS analysis of **1** revealed a pseudo-molecular ion (M + Na, Δmmu −0.1) consistent with a molecular formula ($C_{20}H_{28}O_3$) requiring seven double-bond equivalents. Analysis of the NMR data for phorbasin A (**1**) revealed resonances consistent with two isopropyl methyls, two olefinic methyls, a methyl ketone, two hydroxymethines, and a total of five substituted olefins (see Table 1). These observations required that **1** be monocyclic. Supportive of these conclusions, the IR spectrum for **1** displayed characteristic absorbances for carbonyl and hydroxyl moieties (1688 and 3500 cm^{-1}), while the UV spectrum displayed absorbances consistent with a highly conjugated polyene (326, 312, and 232 nm).

The homonuclear 2D NMR COSY and TOCSY data for **1** (Table 1) identified connectivity sequences indicative of subunit A: H-17 through H-3 to H-5; subunit B: H-8 to H-10; and subunit C: H_3-19 through H-12 to H_3-16 and H_3-20. Also identified were isolated methyl units attributed to subunit D (H_3-1) and subunit E (H_3-18) (Figure 1). Heteronuclear 2D NMR correlations (see gHMBC data in

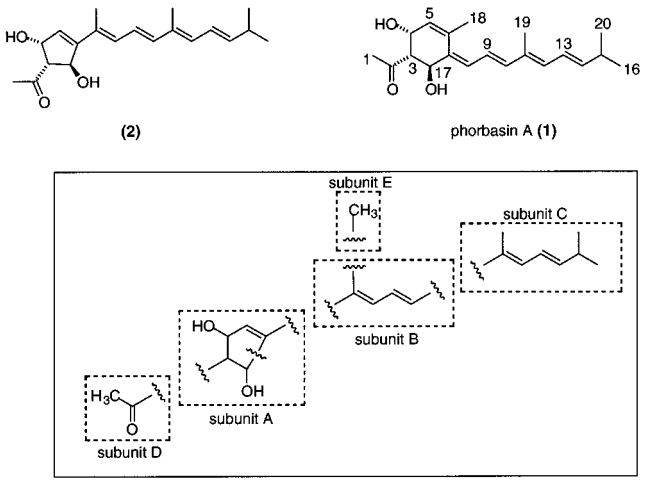

Figure 1. Structure subunits for phorbasin A (**1**).

Table 1) permitted connection of subunits as follows; subunits A−D, H-3 to C-2 and C-1; subunits A−E, H-5 to C-18 and H-17 to C-6 and C-18; subunits E−B, H_3-18 to C-8; and subunits B−C, H-10 to C-11 and H-12 to C-10. In this way two plausible gross structures could be proposed for phorbasin A (**1** or **2**), differing in the nature of the cyclic portion of the molecule. Whereas the cyclopentenyl alternative **2** required a five bond COSY correlation from H-5 to H_3-18, no such long-range correlations were required for the cyclohexenyl alternative **1**. Consequently, we are inclined to support structure **1** for phorbasin A. The proposed structure can be rationalized biosynthetically as a rearranged diterpenoid, and the numbering scheme we have applied reflects this hypothesis.

Stereochemical assignments about $\Delta^{9,10}$ and $\Delta^{13,14}$ double bonds were determined as *E* on the basis of $J_{9,10} = 14.8$ Hz and $J_{13,14} = 15.0$ Hz. These assignments were supported by NOE difference measurements from H-10 to H-8 (6%) and from H-14 to H-12 (8%). Similarly, irradiation of H-9 resulted in a 4% NOE to H_3-18, while irradiation of H-13 returned a 9% enhancement to H_3-19, requiring the stereochemistry about $\Delta^{7,8}$ and $\Delta^{11,12}$ double bonds as indicated. The magnitude of $J_{3,4}$ (4.4 Hz) was consistent with a small dihedral angle and, hence, a cis relationship between H-3 and H-4. Conversely, $J_{3,17}$ (12.0 Hz) was consistent with a large dihedral angle and, hence, a trans relationship between H-3 and H-17. An NOE difference measurement of 11% to H-17 on irradiation of H_3-1 confirmed this relative stereochemistry.

Attempts to secure the absolute stereochemistry about the three chiral centers in phorbasin A (**1**) were precluded

* To whom correspondence should be addressed. Tel.: 61 3 8344 6468. Fax: 61 3 9347 5180. E-mail: r.capon@chemistry.unimelb.edu.au.

10.1021/np000239t CCC: $19.00
Published on Web 11/01/2000

Table 1. NMR (CD_3OD, 400 MHz) Data for Phorbasin A (**1**)

	$^{13}C^a$ δ (m)	^1H δ (m, J Hz)	COSY	gHMBC ($^1H-^{13}C$)
1	22.8 (q)	2.08 (s)		C-3, C-4
2	202.7 (s)			
3	48.0 (d)	3.33 (dd, obscured)	H-4, H-17	C-2, C-6, C-4, C-17, C-1
4	69.3 (d)	4.32 (t, 5.1)	H-5, H-3,	C-5, C-7, C-17, C-3
5	144.7 (d)	6.74 (dd, 1.6, 5.1)	H-4, H-18	C-2, C-4, C-3, C-18
6	138.3 (s)			
7	135.3 (s)			
8	130.6 (d)	6.11 (d, 10.8)	H-9	C-10, C-9,
9	123.5 (d)	6.43 (dd, 10.8, 14.8)	H-8, H-10	C-10, C-11, C-8
10	138.1 (d)	6.21 (d, 14.8)	H-9	C-11
11	134.7 (s)			
12	132.6 (d)	6.02 (d, 11.0)	H-13, H-19	C-14, C-10, C-13, C-19
13	125.3 (d)	6.35 (ddd, 0.8, 11.0, 15.0)	H-12, H-14	C-11, C-15
14	143.6 (d)	5.68 (dd, 7.2, 15.0)	H-13, H-15	C-12, C-15, C-16, C-20
15	32.9 (d)	2.37 (m)	H-14, H-16, H-20	C-14, C-16, C-20
16	22.9 (q)	1.02 (d, 6.8)	H-15	C-14, C-15, C-20
17	71.3 (d)	4.62 (d, 12.0)	H-3	C-2, C-6, C-3, C-4, C-18
18	15.6 (q)	1.84(s)	H-5	C-5, C-7, C-8
19	12.7 (q)	1.82 (s)	H-12	C-10, C-11, C-12, C-13
20	22.9 (q)	1.02 (d, 6.8)	H-15	C-14, C-15, C-16

[a] Assignments supported by DEPT, gHMQC, and TOCSY experiments.

by instability, with all available material decomposing during characterization. The enantiomer portrayed in the structure diagram is arbitrary.

Experimental Section

General Experimental Procedures. For procedures see Ovenden and Capon.[3]

Animal Material. A *Phorbas* sp. (Museum of Victoria registry number F79989) of marine sponge was collected during a scientific expedition to the Great Australian Bight aboard the RV *Franklin* in July 1995. The specimen was collected by epibenthic sled at a depth of 100 m at position 34° 02′ S: 114° 44′ E. A description of the specimen is as follows: growth form macrobenthic, stalked, flabelliform, biplanar, branching−lobate; color in life bright orange-red; color in EtOH beige; texture firm but compressible, cartilagenous; surface opaque, glossy, evenly tuberculose; oscules conspicous, discrete, raised on tubercles with a marked membrane lip; spicules megascleres anisostrongyles (300−450 × 2−8 μm); microscleres arcuate isochelae (20−30 μm); ectosome a thin, outer layer of chelae on a thick collagenous layer filled with multispicular, plumose tracts of megascleres and lightly scattered microsclere; choansome lightly collagenous with plumose, multispicular tracts of megascleres coring thick spongin fibers that become less evident in the subectosomal region. Auxiliary megascleres and miroscleres are scattered throughout. The sponge was frozen shortly after collection and transported to the laboratory, where it was documented, diced, and steeped in EtOH at −20 °C, prior to chemical analysis.

Extraction and Isolation. The EtOH extract of the *Phorbas* sp. was decanted and concentrated in vacuo to yield a bright orange solid (280 mg) that was partitioned between *n*-BuOH (59 mg, 21%) and H_2O (220 mg). The *n*-BuOH solubles were concentrated in vacuo and the residue subjected to C_{18} solid-phase extraction (10% stepwise gradient elution from 100% H_2O to 100% MeOH) to yield phorbasin A (**1**) (13 mg, 4.6% by wt of the EtOH extract).

Phorbasin A (1): an unstable pale yellow solid; $[\alpha]^{22}_D$ +2° (*c* 0.38, MeOH); IR (CCl_4) ν_{max} 3500 (br), 1688 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 326 (20 300), 312 (25 500), 232 nm (11 000); ^1H and ^{13}C NMR data, see Table 1; positive HRESIMS *m*/*z* 339.1935 (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

Acknowledgment. We acknowledge the CSIRO Division of Oceanography, and the crew and scientific personnel aboard the O. R. V. *Franklin* for collection of the *Phorbas* specimen. We also acknowledge taxonomic classification by L. Goudie. This research was supported by the Australian Research Council.

References and Notes

(1) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126−8131.
(2) Rudi, A.; Stein, Z.; Green, S.; Goldberg, I.; Kashman, Y. *Tetrahedron Lett.* **1994**, *35*, 2589−2592.
(3) Ovenden, S. P. B.; Capon, R. J. *Aust. J. Chem.* **1998**, *51*, 573−579.

NP000239T