Pergamon

Tetrahedron Letters 43 (2002) 3079–3081

TETRAHEDRON LETTERS

A new pentacyclic sulfated hydroquinone from the marine sponge *Haliclona* sp.

Heidi R. Bokesch,[b] Amy C. Stull,[a] Lewis K. Pannell,[c] Tawnya C. McKee[a] and Michael R. Boyd[a,*]

[a]*Molecular Targets Drug Discovery Program, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702-1201 USA*

[b]*Intramural Research Support Program, SAIC Frederick, Frederick, MD 21702-1201, USA*

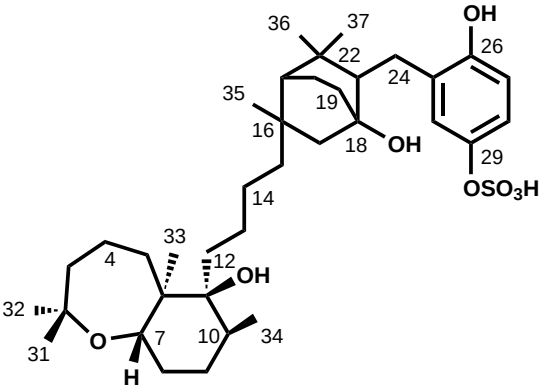
[c]*Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892, USA*

Received 20 November 2001; revised 11 February 2002; accepted 12 February 2002

Abstract—A new pentacyclic sulfated hydroquinone, phuklona sulfate, has been isolated from the marine sponge *Haliclona* sp. collected near Phuket Island, Thailand. The structure was elucidated by a variety of spectroscopic methods including 1D and 2D NMR experiments. Phuklona sulfate contains a novel triterpenoid carbon skeleton linked to a sulfated hydroquinone moeity. Published by Elsevier Science Ltd.

Haliclona sponges have produced metabolites encompassing many biosynthetic classes including steroids,[1] polyketides,[2] macrolides,[3] alkaloids,[4] and terpenes.[5] A group of bioactive sulfated hexaprenoid hydroquinones have also been isolated from sponges, including HIV-1 RT inhibitors from *Toxiclona toxius*,[6] a phosphatidylinositol-specific phospholipase C inhibitor from *Callyspongia* sp.,[7] and the kinesin motor protein inhibitors, adociasulfates, from *Adocia*[8] and *Haliclona*[9] sp. Although phuklona sulfate contains a sulfated hydroquinone and appears to be triterpene-derived, it possesses a novel pentacyclic carbon skeleton similar to the polyepoxysqualene-derived sodwanone triterpenes.[10]

The organic extract (2.1 g) of *Haliclona* sp. collected in the South Andaman Sea near Phuket Island, Thailand, was subjected to a solvent–solvent partitioning scheme[11] that concentrated the anti-HIV activity in the EtOAc (274 mg) and H_2O (882 mg) soluble fractions. EtOH precipitation of the combined fractions resulted in a filtrate (288 mg) which was chromatographed on Sephadex LH-20 (MeOH–H_2O 7:3). Final purification was achieved by reversed-phase HPLC (Rainin Dynamax, C_{18}, 1×25 cm) eluting with a 20–100% CH_3CN/H_2O gradient to give 10.0 mg of phuklona sulfate (**1**).[†]



The molecular formula of $C_{36}H_{58}O_8S$ was determined by HRFABMS of the $[M-H+2Cs]^+$ ion at m/z 915.1891 and suggested the presence of a sulfate group. The ^{13}C NMR spectrum (Table 1) contained resonances for 36 carbon atoms, including six aromatic signals and four oxygen bearing sp^3 carbons. The ^1H NMR spectrum of **1** contained overlapping resonances in the upfield region for most of the protons, therefore complete spectral data was obtained in CD_3OD, DMSO-d_6, and pyridine in order to com-

Keywords: pentacyclic sulfated hydroquinone; marine natural product; *Haliclona*.

* Corresponding author. Tel.: (301) 846-5391; fax: (301) 846-6919; e-mail: boyd@dtpax2.ncifcrf.gov

0040-4039/02/$ - see front matter. Published by Elsevier Science Ltd.
PII: S0040-4039(02)00355-6

[†] Compound **1**: white glass; $[\alpha]_D = -17.4°$ (*c* 0.91, MeOH); UV (MeOH): λ_{max} (log ε) 280 (3.25), 209 (4.04), 208 (4.22) nm; HRFABMS: $[M-H+2Cs]^+$ m/z 915.1891, $[M-H+2Cs]^+$ calcd for $C_{36}H_{57}Cs_2O_8S$, 915.1876; for ^1H and ^{13}C NMR data, see Table 1.

Table 1. NMR spectral data for phuklona sulfate (**1**) recorded in CD_3OD (500 MHz)

C#	δ ^{13}C	δ ^{1}H mult., J Hz	HMBC (H to C#)	TOCSY
2	75.7			
3	41.8	a 1.54, m	4, 32	5b
		b 1.60, m	4, 5, 32	
4	20.4	a 1.47, m		
		b 1.58, m	3, 6	3a, 5a, 5b
5	38.8	a 1.49, m	4, 7, 11, 33	
		b 1.88, m	3, 4, 6, 33	4b
6	47.4			
7	72.7	3.76, dd, J=6.0, 9.0	2, 6, 8, 33	8a, 8b, 9a, 9b, 10, 34
8	31.4	a 1.41, m		
		b 1.44, m	9	
9	29.8	a 1.46, m	8, 10	
		b 1.36, m	7, 8, 10, 11	34
10	33.9	1.81, m	9, 34	34
11	79.9			
12	31.8	a 1.29, m	6, 11	12b, 13a, 14a, 14b, 15a, 15b
		b 1.57, m	10, 13	
13	42.0	a 0.77, m	14	13b, 14a, 14b, 15a, 15b
		b 1.80, m	14, 15	
14	19.3	a 1.33, m	12, 13, 15, 16	12b, 13a, 13b, 14b, 15b
		b 1.50, m	15, 16	
15	37.5	a 0.81, m	13, 14, 35	
		b 1.66, m	13, 14, 21	
16	37.4			
17	28.1	a 1.26, d, J=13.5	16, 18, 19	
		b 1.49, d, J=13.5	18	
18	75.1			
19	44.8	a 1.51, m	20, 21	
		b 1.88, m	18, 20, 21, 23	19a, 20a, 20b, 21
20	21.4	a 1.37, m	19, 21, 22	
		b 1.65, m	18, 19, 22	
21	59.8	0.96, t, J=11.5	16, 17, 19, 20, 22, 35	19a, 19b, 20a, 20b
22	40.9			
23	63.7	1.56, d, J=5.0	17, 18, 22, 24, 25, 36, 37	24a, 24b
24	27.1	a 2.52, dd, J=6.0, 15.0	22, 23, 25, 26, 30	
		b 2.79, d, J=15.0	18, 22, 23, 25, 26, 30	
25	132.0			
26	153.3			
27	116.8	6.64, d, J=9.0	25, 26, 28, 29	
28	121.0	6.94, dd, J=3.0, 9.0	26, 29, 30	
29	146.2			
30	125.6	7.09, d, J=3.0	24, 26, 28, 29	
31	27.0	1.14, 3H, s	2, 3, 32	
32	29.0	1.09, 3H, s	2, 3, 31	
33	16.8	1.00, 3H, s	5, 6, 7, 11	
34	16.2	0.86, 3H, d, J=6.5	9, 10, 11	
35	29.4	0.83, 3H, s	15, 17	
36	24.2	1.24, 3H, s	36	
37	16.9	0.99, 3H, s	21, 22, 23, 36	

plete the NMR assignments. In particular, use of 1D-TOCSY experiments acquired using a series of different mixing times allowed for sequential assignment of overlapping protons. The NMR spectral data used to illustrate the structure elucidation was run in CD_3OD unless otherwise indicated.

The ^{1}H NMR spectrum in DMSO-d_6 indicated one aromatic and two non-aromatic hydroxyl groups, which along with the sulfate, left one oxygen as an ether linkage. The ^{1}H and ^{13}C NMR data indicated the presence of a 1,2,4-trisubstituted aromatic ring, which was supported by HMBC correlations, leaving four of the eight degrees of unsaturation attributed to additional rings. Methylene proton signals at δ 2.79 and 2.52 showed HMBC correlations to C25, C26 and C30 of the aromatic ring as well as C23 and quaternary carbons at C18 and C22. This attached the methylene at C25 and an additional HMBC correlation from H23 (δ 1.56) to C25 placed the C23 methine adjacent to the C24 methylene. A hydroxyl group was placed on C26 based on NOE correlations in DMSO-d_6 to H24b and the C18 hydroxyl group. The remaining position on the aromatic ring was substituted with a sulfate group

based on its ^{13}C chemical shift (δ 146.2), which was in agreement with the literature values reported for the adociasulfates.[8]

HMBC and 1D-TOCSY correlations were used to elucidate the remaining structure. HMBC correlations from Me-37 (δ 0.99) to C21, C22 and C23 as well as a correlation from H23 (δ 1.56) to C36 allowed the positioning of the *gem*-dimethyls at C22. 1D-TOCSY correlations from H21 to all four protons of the C19 and C20 methylenes [(δ 0.96) to H20a (δ 1.37), H20b (δ 1.65), H19a (δ 1.51) and H19b (δ 1.88)], along with HMBC correlations from H21 to C16, C17, and C35 formed an additional fragment. HMBC correlations from H19b (δ 1.88) to C18, and H17a (δ 1.26) to C18 and C19 allowed the two pieces to be joined, forming a bicyclo[2.2.2]octane ring fused at C18 and C21, which accounted for two of the remaining four rings and completed the 'upper' half of the structure.

1D-TOCSY correlations from H7 (δ 3.76) to H8a (δ 1.41), H8b (δ 1.44), H9a (δ 1.46), H9b (δ 1.36), H10 (δ 1.81) and Me-34 (δ 0.86), along with HMBC correlations from Me-34 (δ 0.86) to C9, C10, and C11, and Me-33 (δ 1.00) to C5, C6, C7, and C11 allowed establishment of an additional six-membered ring. HMBC correlations from Me-32 (δ 1.09) and Me-31 (δ 1.14) to C2 and C3, H7 (δ 3.76) to C2, and H3b (δ 1.60) to C4, along with a 1D-TOCSY correlation from H4b (δ 1.58) to H5b (δ 1.88) allowed the closing of the last seven-membered ring to form a cyclohexane–oxepane system.

A 1D-TOCSY experiment indicated that the remaining four carbons were joined in a chain which linked the two halves of the molecule. HMBC correlations from H12a (δ 1.29) to C6 and C11 and from H15a (δ 0.81) to C35 supported this link. A hydroxyl group was placed on C11 based on HMBC correlations in DMSO-d_6 from the hydroxyl proton to C10 and C12, and the remaining hydroxyl was placed on C18 based on the chemical shift of the carbon (δ 75.1).

The relative stereochemistry of phuklona sulfate was elucidated on the basis of difference NOE experiments obtained in DMSO-d_6. Irradiation of the H7 signal caused an enhancement of H9a and the C11 hydroxyl group, indicating they were in a *cis* 1,3 diaxial position relative to one another, and defining a chair conformation for the cyclohexane ring. Irradiation of H7 also enhanced the signal of the H31 methyl group indicating it was on the same face of the molecule as H7. An enhancement of H10 on irradiation of the H6 methyl signal indicated they were *cis* to each other and placed the methyl on C34, and the adjoining ring at C6, equatorial to the C11 hydroxyl group. The presence of a large vicinal coupling of H7 to H8 supported a *trans*-fused ring system. On comparison of the NMR data of the shaagrockols,[12] which have a *cis*-fused cyclohexane–oxepane system, we found significant differences in the chemical shifts and coupling constants of C6, C7, and C24, which support our assignment of a *trans*-fused cyclohexane–oxepane system. We were unable to assign the relative stereochemistry of the bicyclo[2.2.2]octane ring due to overlapping proton resonances which would not allow unambiguous assignments of the observed enhancements by difference NOE or NOESY experiments in multiple solvents.

Phuklona sulfate showed modest cytoprotection against HIV in the NCI assay with an EC_{50} of 30 μg/mL and an IC_{50} of 117 μg/mL.

Acknowledgements

The organism was collected under contract for the National Cancer Institute by P. Murphy. Taxonomic identification was provided by Jane Fromont; a voucher specimen (Q66C4110) has been deposited at the Smithsonian Institution, Washington, DC. We thank K. Snader and D. Newman (NPB) for collections, T. McCloud for extractions, and R. Gardella and D. Rosser for anti-HIV evaluations. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract no. NO1-CO-12400. The content of this article does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade name, commercial products, or organization imply endorsement by the US Government. This report constitutes number 77 in the NCI Laboratory of Drug Discovery Research and Development series 'HIV-inhibitory Natural Products'. For part 76 see Ref. 13.

References

1. Findlay, J. A.; Patil, A. D. *Can. J. Chem.* **1985**, *63*, 2406–2410.
2. Williams, D. H.; Faulkner, D. J. *J. Nat. Prod.* **1996**, *59*, 1099–1101.
3. Erickson, K. L.; Beutler, J. A.; Cardellina, J. H., II; Boyd, M. R. *J. Org. Chem.* **1997**, *62*, 8188–8192.
4. Koren-Goldshlager, G.; Kashman, Y.; Schleyer, M. *J. Nat. Prod.* **1998**, *61*, 282–284.
5. Crews, P.; Harrison, B. *Tetrahedron* **2000**, *56*, 9039–9046.
6. Isaacs, S.; Hizi, A.; Kashman, Y. *Tetrahedron* **1993**, *49*, 4275–4282.
7. Fukami, A.; Ikeda, Y.; Kondo, S.; Naganawa, H.; Takeuchi, T.; Furuya, S.; Hirabayashi, Y.; Shimoike, K.; Hosaka, S.; Watanabe, Y.; Umezawa, K. *Tetrahedron Lett.* **1997**, *38*, 1201–1202.
8. Blackburn, C. L.; Hopmann, C.; Sakowicz, R.; Berdelis, M. S.; Goldstein, L. S. B.; Faulkner, D. J. *J. Org. Chem.* **1999**, *64*, 5565–5570.
9. Blackburn, C. L.; Faulkner, D. J. *Tetrahedron* **2000**, *56*, 8429–8432.
10. Rudi, A.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *J. Nat. Prod.* **1997**, *60*, 700–703.
11. McKee, T. C.; Bokesch, H. R.; McCormick, J. L.; Rashid, M. A.; Spielvogel, D.; Gustafson, K. R.; Alavanja, M. M.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 431–438.
12. Isaacs, S.; Kashman, Y. *Tetrahedron Lett.* **1992**, *33*, 2227–2230.
13. Barrientos, L. G.; Mori, T.; Botos, I.; Louis, J. M.; O'Keefe, B. R.; Boyd, M. R.; Wlodawer, A.; Gronenborn, A. M. *Structure*, submitted.