Vasculyne, a New Cytotoxic Acetylenic Alcohol from the Marine Sponge *Cribrochalina vasculum*

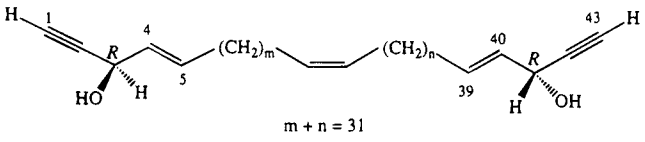
Jin-Rui Dai, Yali F. Hallock, John H. Cardellina, II, and Michael R. Boyd*

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis, and Centers, National Cancer Institute, Building 1052, Room 121, Frederick, Maryland 21702-1201

Received July 17, 1995[⊗]

A new C_{43} acetylenic alcohol, vasculyne (**1**), was isolated by cytotoxicity-guided fractionation of the Caribbean sponge *Cribrochalina vasculum*. The structure of **1** was determined by spectroscopic methods.

Previous studies of marine sponges from the genus *Cribrochalina* have led to the discovery of long-chain acetylene and polyacetylene metabolites.[1–4] During our continuing search for antitumor compounds from natural sources, we noted a collection of the Caribbean sponge *Cribrochalina vasculum* van Soest (Niphatidae) from deep water (174 m) that demonstrated a cytotoxicity profile in the NCI 60-cell antitumor screen[5] different from that of another collection of *C. vasculum* made in the same general location at 79 m depth. Further examination of the extracts of this sponge sample resulted in the isolation of a new high-molecular-weight acetylenic alcohol, vasculyne (**1**). This note describes the isolation and structure determination of **1**.



1

The organic extract was first fractionated via a solvent-solvent partition protocol.[6] The cytotoxic activity was concentrated in the nonpolar hexane and CCl_4 fractions, which were combined and further separated by gel permeation through Sephadex LH-20, followed by reversed-phase HPLC (C_{18}) to give vasculyne (**1**) as a colorless powder.

The molecular formula of **1** was established by HR-FABMS as $C_{43}H_{74}O_2$. The ^{1}H- and ^{13}C-NMR spectral data indicated the presence of two terminal acetylenes, three disubstituted double bonds, two oxymethines, and a long methylene chain with no methyl groups. In the ^{1}H–^{1}H COSY spectra, the oxymethine (δ 4.82) was coupled to an olefinic proton at δ 5.59, suggesting an allylic alcohol functional group. HMBC correlation from the terminal acetylene proton (δ 2.54) to the oxymethine carbon (δ 62.8) linked the acetylene to the allylic alcohol, thus providing a 1-yn-3-ol-4-ene partial structure. The observation of a mass fragment at m/z 95, corresponding to $HC{\equiv}CCHOHCH{=}CHCH_2^+$, further substantiated this assignment. The configuration of this double bond was assigned as *E*, based on the vicinal coupling constant (15 Hz). The proton signal at δ 5.33 (HMQC: δ_C 129.9), which showed an HMBC correlation to the signal at δ 27.2 (δ_H 1.99), was assigned to an isolated double bond with *Z*-configuration on the basis of the ^{13}C-NMR chemical shift of the allylic carbon. Because no methyl groups were observed in the ^{1}H- and ^{13}C-NMR spectra, the partial structures were connected in a linear fashion to give **1**. The exact location of the isolated double could not be ascertained from mass spectral fragmentation patterns before or after addition of dimethyldisulfide.

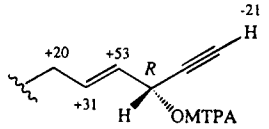


Figure 1. ^{1}H NMR $\Delta\delta$ ($\delta_S - \delta_R$) values obtained for the Mosher's esters of **1**.

We applied the modified Mosher's method to determine the absolute configurations of the carbinol centers.[7] The oxymethine protons at C-3 and C-41 appeared as overlapped signals in both the natural product and the Mosher's ester derivatives, indicating the same stereochemistry at each site. Based on the $\Delta\delta$ ($\delta_S - \delta_R$) values (Figure 1), the absolute configuration of **1** was assigned as 3*R*,43*R*.

Vasculyne (**1**) yielded mean average GI_{50}, TGI, and LC_{50} values[5] of 0.2, 0.7, and 6.7 μg/mL, respectively, and modest differential cytotoxicity toward the melanoma and colon tumor cell-line subpanels when tested in the NCI's 60-cell antitumor screening panel.[5,8] Structurally, **1** is closely related to the C_{30} compound duryne, previously isolated from the Caribbean sponge *C. dura*.[3]

Experimental Section

General Experimental Procedures. Instrumentation and general procedures have been descibed elsewhere.[9]

Animal Material. Samples of *Cribrochalina vasculum* were collected at a depth of 174 m off Egg Island, Bahamas, in March 1987. Sponge samples were kept frozen prior to extraction. A voucher specimen is on deposit at the Smithsonian Institution, Washington, D.C.

Extraction and Isolation. The frozen sponge (26.3 g) was ground with dry ice and extracted with H_2O at 4 °C; the aqueous extract was removed by centrifugation and was then lyophilized. The sponge residue was lyophilized and extracted overnight at room temperature with MeOH–CH_2Cl_2 (1:1), followed by MeOH. Solvents from the combined organic extracts were removed *in vacuo* to give a residue (1.09 g). A portion of this extract (300 mg) was fractionated by solvent-solvent partition to give hexane (67.8 mg), CCl_4 (34.5

[⊗] Abstract published in *Advance ACS Abstracts*, December 1, 1995.

mg), $CHCl_3$ (10.9 mg), and H_2O (119.3 mg) fractions. The cytotoxic hexane and CCl_4 fractions were pooled and chromatographed on a Sephadex LH-20 column eluted with hexane–CH_2Cl_2–MeOH (2:5:1). The cytotoxic fraction (20.3 mg) was further separated by reversed-phase HPLC (C_{18}, Rainin Microsorb, 5 μm, 1 × 25 cm), using MeOH–H_2O (19:1) as mobile phase, yielding vasculyne (**1**) (7.9 mg, 0.07% wet wt).

Vasculyne (**1**): white powder; $[\alpha]_D$ −24° (*c* 0.25, $CDCl_3$); HRFABMS $[MH]^+$ *m/z* 623.5780 (calcd for $C_{43}H_{75}O_2$, 623.5767); LRCIMS (CH_4) *m/z* 623 $[MH]^+$ (1), 605 $[623 - H_2O]^+$ (1), 524 (1), 486 (22), 430 (21), 410 (12), 348 (18), 308 (23), 266 (28), 199 (23), 179 (22), 165 (22), 151 (21), 137 (34), 123 (36), 109 (35), 95 (39), 81 (26) 69 (100); UV (MeOH) λ_{max} (log ϵ) 244 (2.1), 239 (2.1), 206 (3.2) nm; IR (film) ν_{max} 3400–3200, 3283, 3003, 2917, 2849, 2135, 1468, 1275, 1086, 1008, 964, 719, 658 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (2H, *ddt*, *J* = 15, 1, 7 Hz; H-5 and H-39), 5.59 (2H, *ddt*, *J* = 15, 6, 1.5 Hz; H-4 and H-40), 5.33 (2H, *m*, protons of the isolated olefin), 4.82 (2H, *ddd*, *J* = 6, 2, 1 Hz; H-3 and H-41), 2.54 (2H, *d*, *J* = 2 Hz; H-1 and H-43), 2.04 (4H, *q*, *J* = 7 Hz; H-6 and H-38), 1.99 (*dt*, *J* = 4.5, 7 Hz), 1.83 (*br*), 1.55 (*br*), 1.37 (*m*), 1.25 (*m*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.7 (C-5 and C-39), 129.9 (two carbons of the isolated olefin), 128.3 (C-4 and C-40), 83.3 (C-2 and C-42), 73.9 (C-1 and C-43), 62.8 (C-3 and C-41), 31.9 (C-6 and C-38), 29.7–27.2 (29C).

MTPA Esters of Vasculyne. Vasculyne (2 mg) was dissolved in 1 mL freshly distilled pyridine. To this solution were added a 4× molar excess of MTPA-Cl and a granule of DMAP. The mixture was stirred under nitrogen overnight, then dried in *vacuo*. Reaction products were purified by VLC[10,11] on cyano-bonded phase (hexane). Both the *S*-ester (from *R*-MTPA-Cl) and *R*-ester (from *S*-MTPA-Cl) were prepared.

Acknowledgment. We thank K. Snader for coordinating collections, Harbor Branch Oceanographic Institution for the sponge collection and identification, T. McCloud for extractions, G. Gray for mass spectral data, and A. Monks and D. Scudiero for primary antitumor screening data.

References and Notes

(1) Gunasekera, S. P.; Faircloth, G. T. *J. Org. Chem.* **1990**, *55*, 6223–6225.
(2) Aiello, A.; Fattorusso, E., Menna, M. *J. Nat. Prod.* **1992**, *55*, 1275–1280.
(3) Wright, A. E.; McConnell, O. J.; Kohmoto, S.; Lui, M. S.; Thompson, W.; Snader, K. M. *Tetrahedron Lett.* **1987**, *28*, 1377–1380.
(4) Hallock, Y. F.; Cardellina, J. H., II; Balaschak, M. S.; Alexander, M. R.; Prather, T. R.; Shoemaker, R. H.; Boyd, M. R. *J. Nat. Prod.*, in press.
(5) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91–109.
(6) Van Wagenen, B. C.; Larsen, R.; Cardellina, J. H. II; Randazzo, D.; Lidert, Z. C.; Swithenbank, C. *J. Org. Chem.* **1993**, *58*, 335–337.
(7) Ohtani, I.; Kusukmi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296–1297.
(8) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
(9) Rashid, M. A.; Gustafson, K. R.; Cardellina, J. H. II; Boyd, M. R. *J. Nat. Prod.* **1995**, *58*, 594–597.
(10) Pelletier, S. W.; Chokshi, H. P.; Desai, H. K. *J. Nat. Prod.* **1986**, *49*, 892–900.
(11) Coll, J. C.; Bowden, B. F. *J. Nat. Prod.* **1986**, *49*, 934–936.

NP9600199