Symplostatin 1: A Dolastatin 10 Analogue from the Marine Cyanobacterium Symploca h ydnoides

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · OCTOBER 1998

Impact Factor: 3.8 · DOI: 10.1021/np980321c · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

Volume 61, Number 9 September 1998

Rapid Communications

Symplostatin 1: A Dolastatin 10 Analogue from the Marine Cyanobacterium *Symploca hydnoides*

George G. Harrigan,[†] Hendrik Luesch,[†] Wesley Y. Yoshida,[†] Richard E. Moore,*[,†] Dale G. Nagle,[‡,§] Valerie J. Paul,*[,‡] Susan L. Mooberry,[⊥] Thomas H. Corbett,[∥] and Fred A. Valeriote[▽]

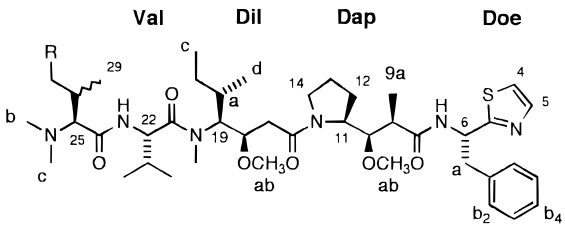
Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96923, Cancer Research Center of Hawaii, 1236 Lauhala Street, Honolulu, Hawaii 96813, Karmanos Cancer Center, Wayne State University, Detroit, Michigan 48201, and Department of Internal Medicine, Wayne State University, Detroit, Michigan 48201

Received July 23, 1998

Abstract: A new solid tumor selective cytotoxic analogue of dolastatin 10 (**1**) has been isolated from the marine cyanobacterium *Symploca hydnoides*, collected near Guam. This metabolite has been assigned the trivial name symplostatin 1 (**2**). This discovery supports the proposal that many compounds isolated from the seahare *Dolabella auricularia*, the original source of the dolastatins, are of dietary origin.

The dolastatins are a series of remarkable cytotoxic compounds isolated from the Indian Ocean seahare *Dolabella auricularia*.[1a−c] The most important of these is dolastatin 10 (**1**),[2] which is in phase I trials as an anticancer agent.[3a−c] The exceedingly low yields of dolastatins and other metabolites obtained from *D. auricularia*, however, imply that this mollusk is not the true producer of these compounds. *D. auricularia* is a known generalist herbivore. Moreover, many metabolites that were originally isolated from seahares have been shown to be of dietary origin.[4a,b] The cytotoxic peptolide dolastatin 12 and the closely related analogue lyngbyastatin 1 were recently isolated from collections of the cyanobacterium (blue-green alga) *Lyngbya majuscula* and assemblages of *L. majuscula* and *Schizothrix calcicola*.[5] This finding demonstrates that some metabolites isolated from *D. auricularia* are of cyanobacterial origin. Our ongoing investigations of cyanobacteria as sources of novel anticancer drugs has now afforded symplostatin 1 (**2**),[6,7] an analogue of dolastatin 10 (**1**), from a Guamanian variety of *Symploca hydnoides* Kutzing and Gomout (UOG strain VP377).[8]

Liquid−liquid partition of an organic extract of VP377, which exhibited solid tumor selective cytotoxicity (460 differential for $Z_{C38}-Z_{L1210}$) and equal cytotoxicity against a drug-sensitive and a MDR solid tumor cell line (M17Adr) in the Corbett assay,[9,10] followed by normal-phase, gel filtration, and reversed-phase chromatographic steps, afforded **2** as a white amorphous powder.[6,7] The UV and ^{1}H and ^{13}C NMR spectral data of **2** (see Table 1) corresponded closely with the same data for dolastatin 10 (**1**). The HRFABMS of **2** established the molecular formula as $C_{43}H_{70}N_6O_6S$, one methylene unit greater than the one for **1**. Spectral analysis, however, was complicated by extensive broadening and considerable overlap of several signals in the ^{1}H NMR spectrum and also by the presence of a minor conformer,[11] which doubled the number of signals. Despite the analytical difficulties, direct comparison of NMR spectra (see the Supporting Information) indicated that **2** differed from **1** at only one site in the molecule.[12]



1 R = H
2 R = CH_3

* To whom correspondence should be addressed. (R.E.M.) Tel.: (808) 956-7232. Fax: (808) 956-5908. E-mail: moore@gold.chem.hawaii.edu. (V.J.P.) Tel.: (671) 735-2186. Fax: (671) 734-6767. E-mail: vpaul@uog9.uog.edu.
[†] Department of Chemistry, University of Hawaii at Manoa.
[‡] University of Guam Marine Laboratory.
[§] Current address: Department of Pharmacognosy, University of Mississippi, University, MS 38677.
[⊥] Cancer Research Center of Hawaii.
[∥] Karmanos Cancer Center, Wayne State University.
[▽] Department of Internal Medicine, Wayne State University.

S0163-3864(98)00321-8 CCC: $15.00
Published on Web 09/11/1998

Table 1. NMR Spectral Data for the Major Conformer of Compound **2** in CD_2Cl_2 at 500 MHz

C/H no.[a]	δ_H (*J*, Hz)	δ_C	HMBC[b]
2		172.4	H-5, H-6
4	7.72, d (3.4)	142.7	
5	7.26, d (3.3)	119.2	
6	5.50, ddd (5.9, 7.6, 9.2)	52.9	H-6a
6a	3.22, dd (9.2, −13.9) 3.39	41.3	H-6
6b1		137.6	H-6, H-6a
6b2, b6	7.24	128.7 × 2	H-6b6, b2
6b3, b5	7.22	129.7 × 2	H-6a, H-6b4, H−b3, b5
6b4	7.21	127.0	
7	7.31 br d (5.9)		
8		174.0	H-6, H-7, H-9, H-9a, H-10
9	2.28	44.7	H-9a, H-10
9a	1.07, d (7.0)	14.5	H-9, H-10
10	3.83, dd (1.5, 8.2)	81.9	H-9, H-9a, H-10ab, H-12
10ab	3.30, s	60.9	H-10
11	3.96, m	59.7	H-9, H-10
12	1.60, 1.78	24.9	H-10
13	1.72, 1.94	25.4	H-12
14	3.40	48.0	H-12
16		170.4	H-17
17	2.33,br d (14.3)[c] 2.40, dd (9.7, 14.3)	37.9	
18	4.11, m	78.7	H-17, H-18ab
18ab	3.31, s	58.1	
19	4.77, dd (5.4, 10.1)[c,d]	57.2[f]	H-19d
19a	1.75	33.4	H-19c, H-19d
19b	1.03, 1.35	26.1	H-19c, H-19d
19c	0.82, t (7.4)	10.8	
19d	0.97, d (6.7)	15.9	
20a	3.01, s	32.3	
21		173.4	H-20a, H-22
22	4.76, br t (7.7)	54.5	H-22b, H-22c
22a	2.01, m	31.2	H-22, H-22b, H-22c
22b	0.96	18.3	H-22, H-22c
22c	1.00, d (6.7)	19.6	H-22b
23			
24		174.0	
25	*e*	74.5[g]	
25bc	2.40, br s	42.6	
26	1.85, m	34.5	H-28, H-29
27	1.25, 1.62	26.8	H-28, H-29
28	0.91	15.0	
29	0.93	11.8	

[a] In order to allow direct comparison with the data presented by Pettit et al. in ref 2, their numbering system has been adopted. [b] Proton showing long-range correlation with indicated carbon. [c] Value determined at − 20 °C. [d] At 25 °C, this signal is present only as a broad, nearly indistinguishable overlap with the signal for H-22. [e] Not observed, presumably due to signal broadening. [f] Broad signal at 25 °C, showed HMQC correlation with H-19 only at −20 °C. [g] Broad signal; no HMQC correlations observed at +25 or −20 °C.

Additional CH_2 signals at δ_C 26.8 and δ_H 1.25/1.62 and chemical shift differences for the signals of two methyl groups in the NMR spectra of **2** clearly showed that one of the isopropyl groups in **1** had been replaced by a *sec*-butyl group in **2**. HMBC correlations between H-28/H-29 and C-26/C-27 indicated that **2** differed from **1** in the presence of a terminal *N*,*N*-dimethylisoleucine or *N*,*N*-dimethylalloisoleucine residue instead of a terminal *N*,*N*-dimethylvaline residue.[13]

Symplostatin 1 (**2**) exhibited a cytotoxicity IC_{50} value of 0.3 ng/mL against KB cells (an epidermoid carcinoma line), as opposed to <0.1 ng/mL for **1**. Since **2** induced 80% microtubule loss at 1 ng/mL when tested on A-10 cells,[14] its mechanism of action must be similar, if not identical, to that of dolastatin 10 (**1**).

Dolastatin 10 appears to be one of the most potent antineoplastic compounds known to date.[1−3] The isolation of a closely related analogue from a cultivable source is significant, as this potentially allows the study of its biosynthesis. We are currently isolating further quantities of **2** to complete a more rigorous biological evaluation.

Acknowledgment. Funding was provided by NCNPDDG Grant No. CA53001 from the National Cancer Institute and by NIH Grant No. GM38624 to V.J.P. The cyanobacterium was identified by Dr. G. M. L. Patterson, and cytotoxicity assays were performed by Dr. M. Liebermann, Department of Chemistry (UH). Microtubule-depolymerizing assays were performed by Mrs. A. Hernandez, Cancer Research Center of Hawaii. Mass spectral analysis was performed at the UCR Mass Spectrometry Facility, Department of Chemistry, University of California at Riverside. We are extremely grateful to Professor G. R. Pettit and Dr. C. L. Herald, Department of Chemistry, Arizona State University, for generously providing us with a sample of dolastatin 10.

Supporting Information Available: 500 MHz ^1H and 125 MHz ^{13}C NMR spectra of **1** and **2** in CD_2Cl_2; HMQC spectrum of **2** in CD_2Cl_2 (15 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151−9170. (b) Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812−821. (c) Pettit, G. R. In *Progress In the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer-Verlag: Vienna, New York, 1997; Vol. 70, pp 1−79.

(2) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883−6835.

(3) (a) Pettit, G. R.; Herald, C. L.; Kamano, Y. US Patent 4,816,444, Mar 28, 1989, 26 pages. (b) Pettit, G. R.; Srirangam, J. K.; Kantoci, D. US Patent 5,663,149, Sept 2, 1997, 50 pages. (c) Sakakibara, K.; Gondo, M.; Miyazaki, K. US Patent 5,654,399, Aug 5, 1997, 48 pages.

(4) (a) Carefoot, T. H. *Oceanogr. Mar. Biol. Annu. Rev.* **1987**, *25*, 167−284. (b) Faulkner, D. J. In *Ecological Roles of Marine Secondary Metabolites*; Paul, V. J., Ed; Cornell University Press: Ithaca, NY, 1992; pp 119−163.

(5) Harrigan, G. G.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Park, P. U.; Biggs, J.; Paul, V. J., Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, in press.

(6) Organism VP377 is a *Symploca hydnoides* that was collected at the reef flat of Pago Bay, Guam, on April 8, 1996. The freeze-dried cyanobacterium was extracted with CH_2Cl_2−MeOH (1:1) to afford a lipophilic extract (4.34 g). This extract was partitioned between hexanes and 80% aqueous MeOH. The aqueous MeOH fraction was dried and further partitioned between *n*-BuOH and H_2O. The *n*-BuOH fraction was subjected to silica gel chromatography. Elution was initiated with hexanes−CH_2Cl_2 (1:4) followed by CH_2Cl_2, CH_2Cl_2 mixtures containing progressively increasing amounts of *i*-PrOH, and finally MeOH. The MeOH fraction was chromatographed on Sephadex LH-20 (Sigma), eluting with $CHCl_3$−MeOH (3:7). The earliest eluting material was subjected to reversed-phase C_{18} chromatography on YMC-ODS-A. Elution was initiated with 10% aqueous MeCN followed by H_2O mixtures containing progressively increasing amounts of MeCN. The 80−100% MeCN fraction was chromatographed on a Bond-Elut C_{18} column, eluting with 30% aqueous MeCN followed by mixtures containing progressively increasing amounts of MeCN. The Me_2CO-soluble portion of the 60% MeCN fraction was absorbed on a Bond-Elut phenyl column and washed with CH_2Cl_2 followed by MeOH. The MeOH fraction was chromatographed on Bond-Elut Si gel, eluting with EtOAc followed by EtOAc−MeOH mixtures containing progressively increasing amounts of MeOH. The EtOAc fraction afforded 3.2 mg of symplostatin 1 (**1**).

(7) Symplostatin 1 (**2**): $[\alpha]_D$ −45° (*c* 1.6, CH_3OH); UV λ_{max} (ϵ) 209 (20 420), 245 (5430) nm; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 1; HRFABMS *m/z* $[M + H]^+$ 799.5221 (calcd for $C_{43}H_{71}N_6O_6S$, 799.5156).

(8) A distinctive feature of **1** and **2** is the presence of the unique dolaphenine unit at one of the terminuses. This novel unit is also found in the cyanobacterial metabolite, barbamide, from a Caribbean variety of *Lyngbya majuscula* (Orjala, J.; Gerwick, W. H. *J. Nat. Prod.* **1996**, *59*, 427−430).

(9) Corbett, T. H.; Valeriote, F. A.; Polin, L.; Panchapor, C.; Pugh, S.; White, K., Lowichik, N.; Knight, J.; Bissery, M.-C.; Wozniak, A.;

LoRusso, P.; Biernat, L.; Polin, D.; Knight, L.; Biggar, S.; Looney, D.; Demchik, L.; Jones, J.; Jones, L.; Blair, S.; Palmer, K.; Essenmacher, S.; Lisow, L.; Mattes, K. C.; Cavanaugh, P. F.; Rake, J. B.; Baker, L. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development;* Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic Publishers: Norwell, MA, 1993; pp 35−87.

(10) Corbett, T.; Vaeriote, F.; LoRusso, P.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Knight, J.; Demchik L.; Jones, J.; Jones, L.; Lisow, L. In *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*; Teicher, B., Ed.; Humana Press Inc.: Totowa, NJ, 1997; pp 75−99.

(11) Two conformers were observed in a ratio of about 3:1 in CD_2Cl_2. This is the same as reported for dolastatin 10.[2,]

(12) HMBC data (Table 1) support the attachments of dolaphenine (Doe) to dolaproine (Dap) and dolaisoleuine (Dil) to Val in **2**. Although HMBC cross-peak signals are missing between Dap and Dil nuclei and between Val and *N*,*N*-dimethylisoleucine (or *N*,*N*-dimethylalloisoleucine) nuclei, the sequence of the five units as shown in structure **2** is the only one that can be concluded.

(13) The striking similarities of the NMR spectra and biological activities for **1** and **2** strongly suggest that the two compounds have the same relative and absolute stereochemistries.

(14) Smith, C. D.; Mooberry, S. L.; Zhang, X.; Helt, A. *Cancer Lett*. **1994**, *79*, 213−119.

NP980321C