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

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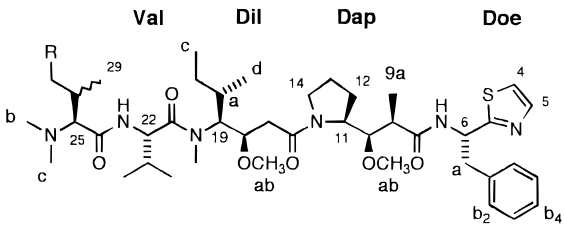
Abstract: A new solid tumor selective cytotoxic analogue of dolastatin 10 (**1**) has been isolated from the marine cyanobacterium *Symploca hydnoides*, collected near Guam. This metabolite has been assigned the trivial name symplostatin 1 (**2**). This discovery supports the proposal that many compounds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

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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.

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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.

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(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).

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(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.

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