

## Supporting Information

### **Psymberin, A Potent Sponge-Derived Cytotoxin from *Psammocinia* Distantly Related to the Pederin Family**

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#### **General Experimental Procedures.**

##### **Animal Material.**

##### **Extraction and Isolation.**

**Figure S1.** Fractionation scheme used for the isolation of psymberin.

**Figure S2.** <sup>1</sup>H NMR spectrum of psymberin.

**Figure S3.** <sup>13</sup>C NMR spectrum of psymberin.

**Figure S3.** <sup>1</sup>H-<sup>1</sup>H COSY spectrum of psymberin.

**Figure S4.** HETCOR spectrum of psymberin.

**Figure S5.** HMBC spectrum of psymberin.

**Figure S6.** Circular dichroism (CD) spectrum of psymberin in EtOH.

**Figure S7.** Source locations for all marine and terrestrial members of the pederin family.

**Figure S8.** *Psammocinia* sp. used in this study.

**Table S1.** Structures, optical rotations, and source organisms of all members of the pederin family of secondary metabolites.

**Table S2.** Summary of source organisms of all members of the pederin family of secondary metabolites.

**General Experimental Procedures.** All 1D- and 2D-NMR spectra were recorded in CD<sub>3</sub>OD (Cambridge Isotope Laboratories, Inc.) except where noted on Varian UNITY INOVA 500 instruments. Electrospray and FAB mass spectral data were obtained on Mariner Biospectrometry Workstation and TYPE instruments. The optical rotation was determined on a Jasco DIP 370 polarimeter.

Silica gel (230-400 mesh) was obtained from Fisher. Analytical and preparative thin layer chromatography was performed on Macherey-Nagel Polygram G/UV<sub>254</sub> and Analtech Uniplates (1000 µm) plates, respectively. Resin (XAD-4) and Sephadex LH-20 was obtained from Sigma-Aldrich. Preparative HPLC was carried out on a Waters 600E system controller and pumps with a Prep LC 25 mm radial compression column using 25×100 mm C<sub>18</sub> Nova-Pak HR18 (6 µm) cartridges. Peak detection utilized a Sedex 55 light scattering and Pharmacia LKB UV-1 (254 nm) detectors. Semi-preparative HPLC was performed on a C<sub>18</sub> Phenomenex 10×250 mm Synergi Hydro-RP (4µm) column with Waters 510 HPLC pumps and gradient controller and with a Waters 484 tunable absorbance detector (210 nm). All HPLC solvents were of HPLC quality while all other solvents were of ACS grade (EMD Chemicals).

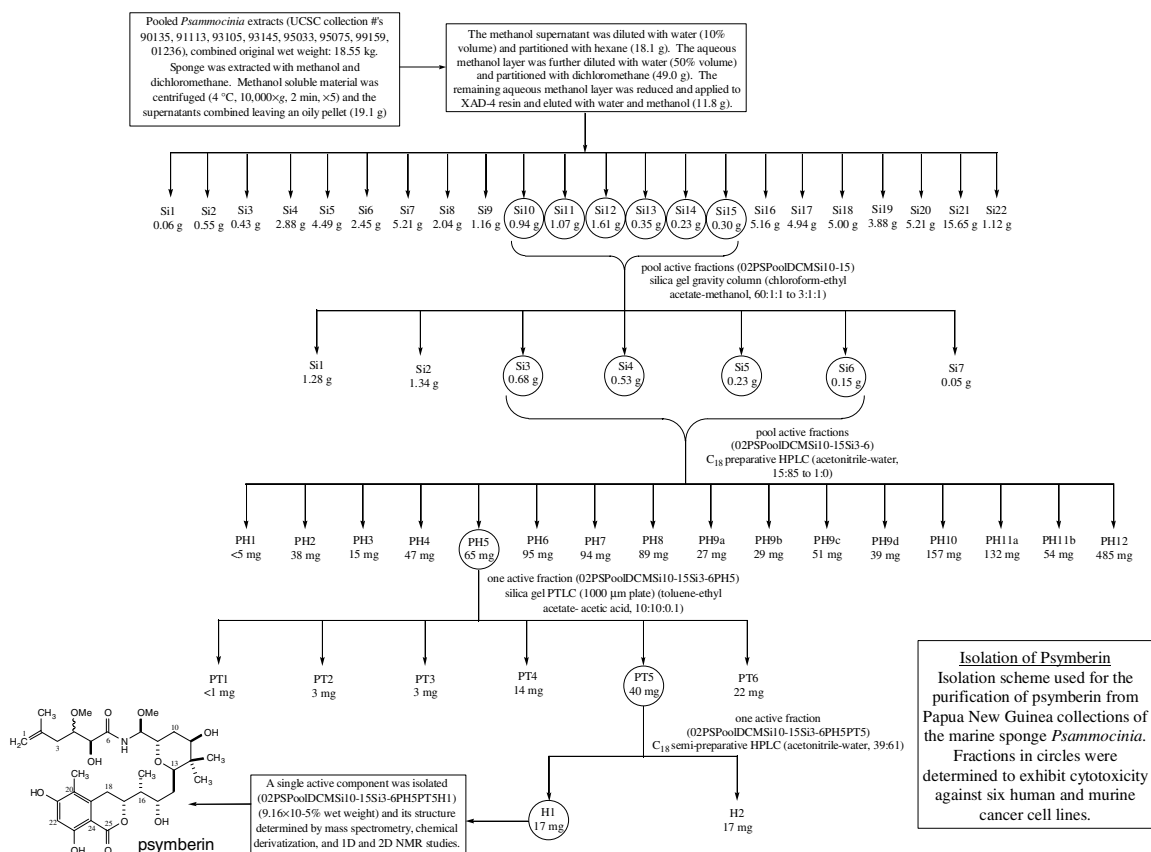
**Animal Material.** Extracts from several small collections of *Psammocinia* sp. (see Figure S8) obtained from multiple proveniences in the waters of Papua New Guinea during the period of 1990 to 2001 exhibited potent and selective activity against human solid tumors. In general, these *Psammocinia* sp. collections (18.6 kg wet weight total) were collected by SCUBA at depths ranging from 25-70 m along the coast of Papua New Guinea. The brown to grayish purple sponge material is characterized as possessing a globular shape with a compressible conulose surface and tan interior. Immediately following collection, the sponges were soaked in ethanol-sea H<sub>2</sub>O (1:1) for 24 hr after which the liquid was decanted and the sponge transported to UCSC. Upon arrival, the sponges were immediately immersed in MeOH and placed in cold storage at 4 °C until extracted. Voucher specimens have been retained at UCSC for reference.

**Extraction and Isolation.** The sponge material was combined and thoroughly extracted with MeOH (×3) and CH<sub>2</sub>Cl<sub>2</sub> (×3) yielding a viscous dark brown extract (>100g). The total organic extract was dissolved in MeOH and centrifuged (10,000×g, 20 min, 4 °C) (×5) and supernatants pooled leaving an insoluble oily pellet (19.1 g). The combined supernatants were diluted with deionized H<sub>2</sub>O (10% vol.) and partitioned with hexane (18.1 g). The aqueous MeOH was further diluted with deionized H<sub>2</sub>O (50% vol.) and partitioned with CH<sub>2</sub>Cl<sub>2</sub> (49.0 g). The aqueous MeOH reduced and applied to XAD-4 resin and flushed with 20% MeOH and 100% MeOH. The 100% MeOH fraction was possessed modest cytotoxicity and was determined to contain cyclocinamide A (5 mg).

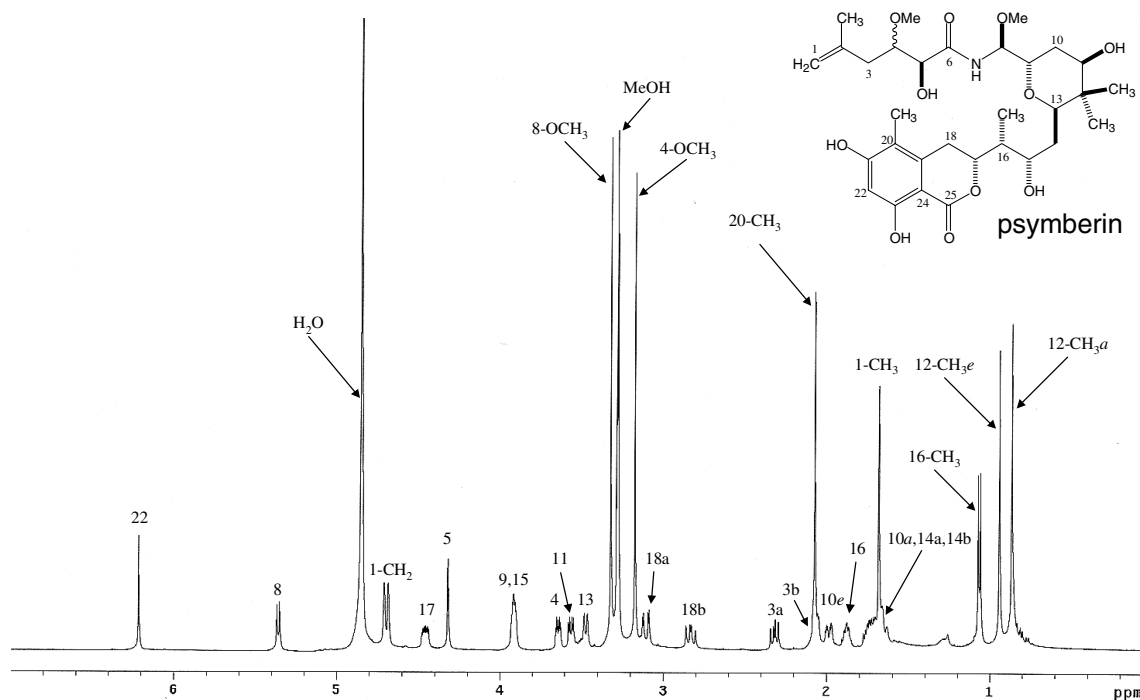
The hexane and CH<sub>2</sub>Cl<sub>2</sub> fractions were each separately passed over silica gel (100% toluene to 100% ethyl acetate to 100% MeOH) providing 8 and 22 fractions, respectively that were tested for cancer cell cytotoxicity. The CH<sub>2</sub>Cl<sub>2</sub>-derived silica fractions 10-15 were pooled (4.5 g) based on their remarkable cancer cell cytotoxicity. No activity was observed in the hexane fractions. The combined active fractions were subjected to additional silica gel column chromatography with CH<sub>3</sub>Cl and increasing proportions of ethyl acetate-MeOH (1:1) yielding seven fractions. Fractions 3-6

possessed strong activity and were pooled (1.7 g). This combined material was further separated by reverse phase-preparative HPLC (15 to 100% acetonitrile) providing 16 fractions. Only fraction 5 (65 mg) exhibited potent cytotoxic activity at nanogram/milliliter concentrations. A single active component was subsequently isolated following preparative-TLC with toluene-ethyl acetate-acetic acid (10:10:0.1) (40 mg) and semi-preparative reverse phase HPLC (isocratic, 39% acetonitrile) yielding 17 mg of psymberin (UCSC PC#833) ( $9.1 \times 10^{-5}\%$  yield).

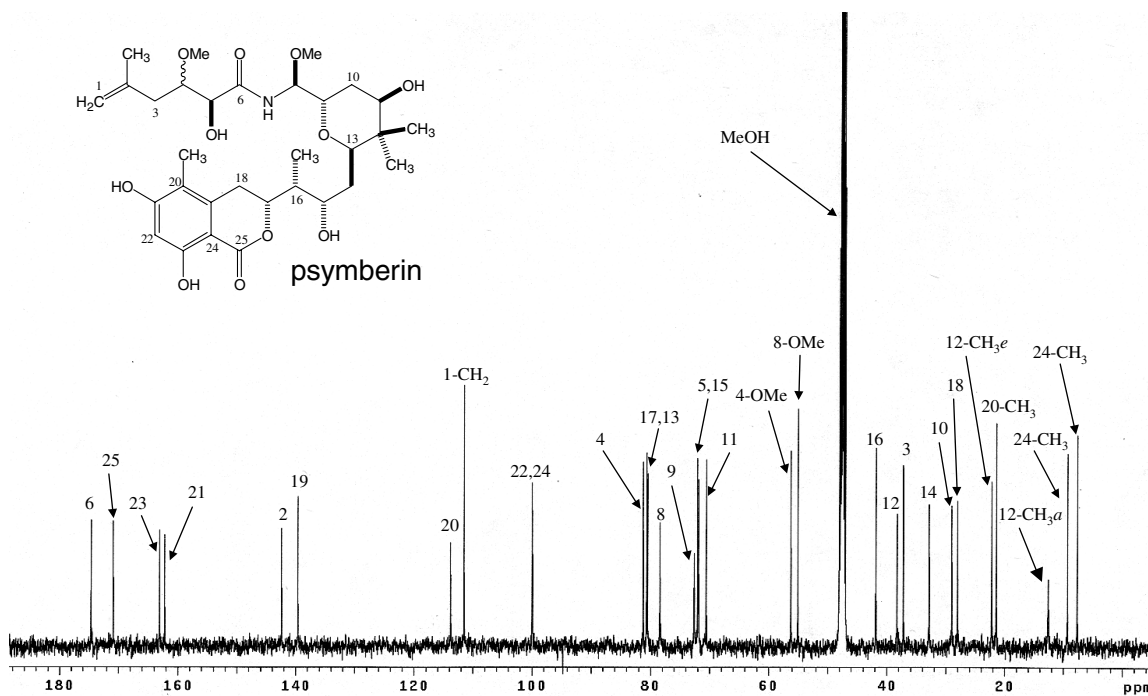
**Figure S1.** Fractionation scheme used for the isolation of psymberin.



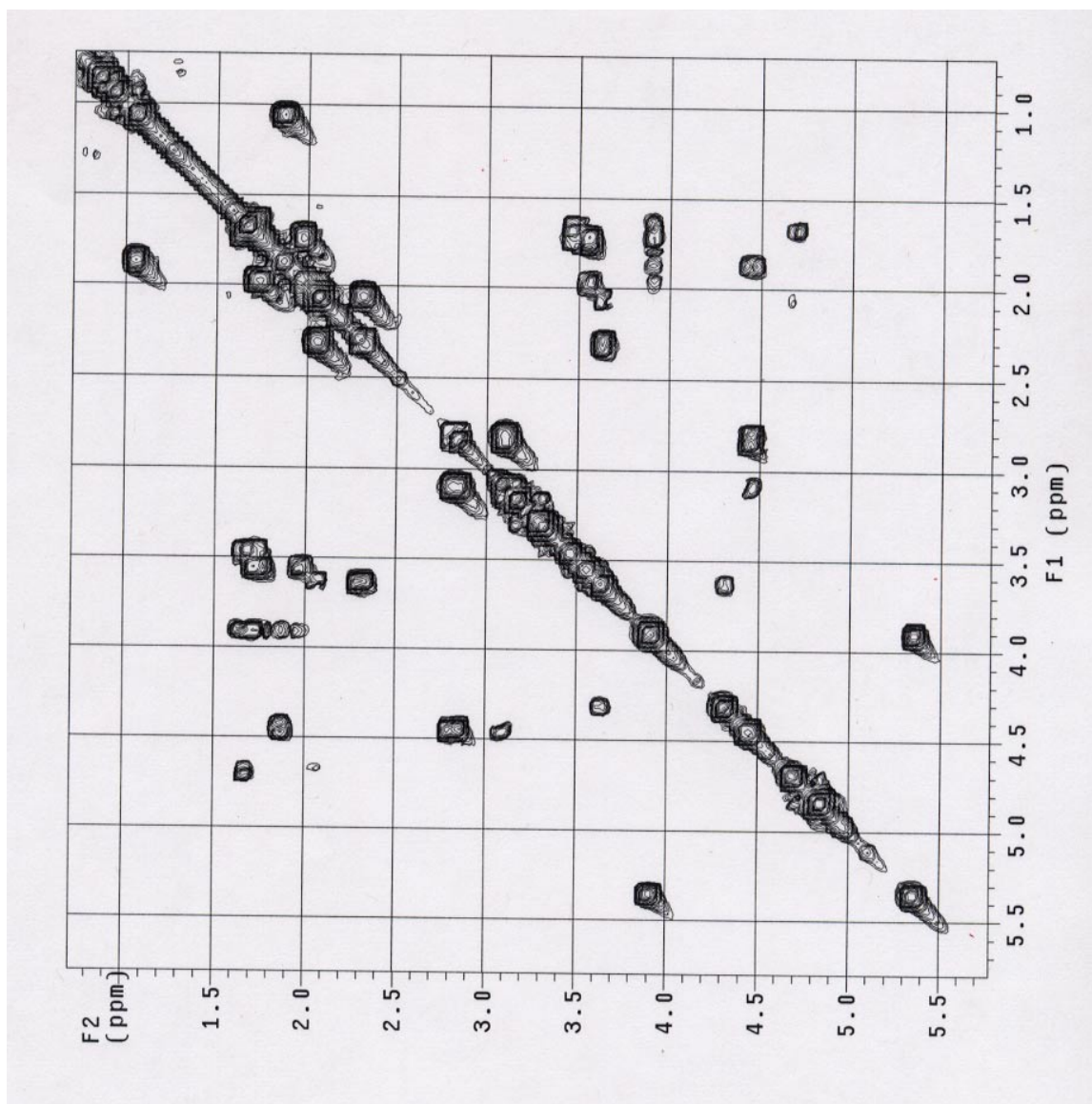
**Figure S2.**  $^1\text{H}$  NMR spectrum of psymbenin (500 MHz,  $\text{CD}_3\text{OD}$ ).



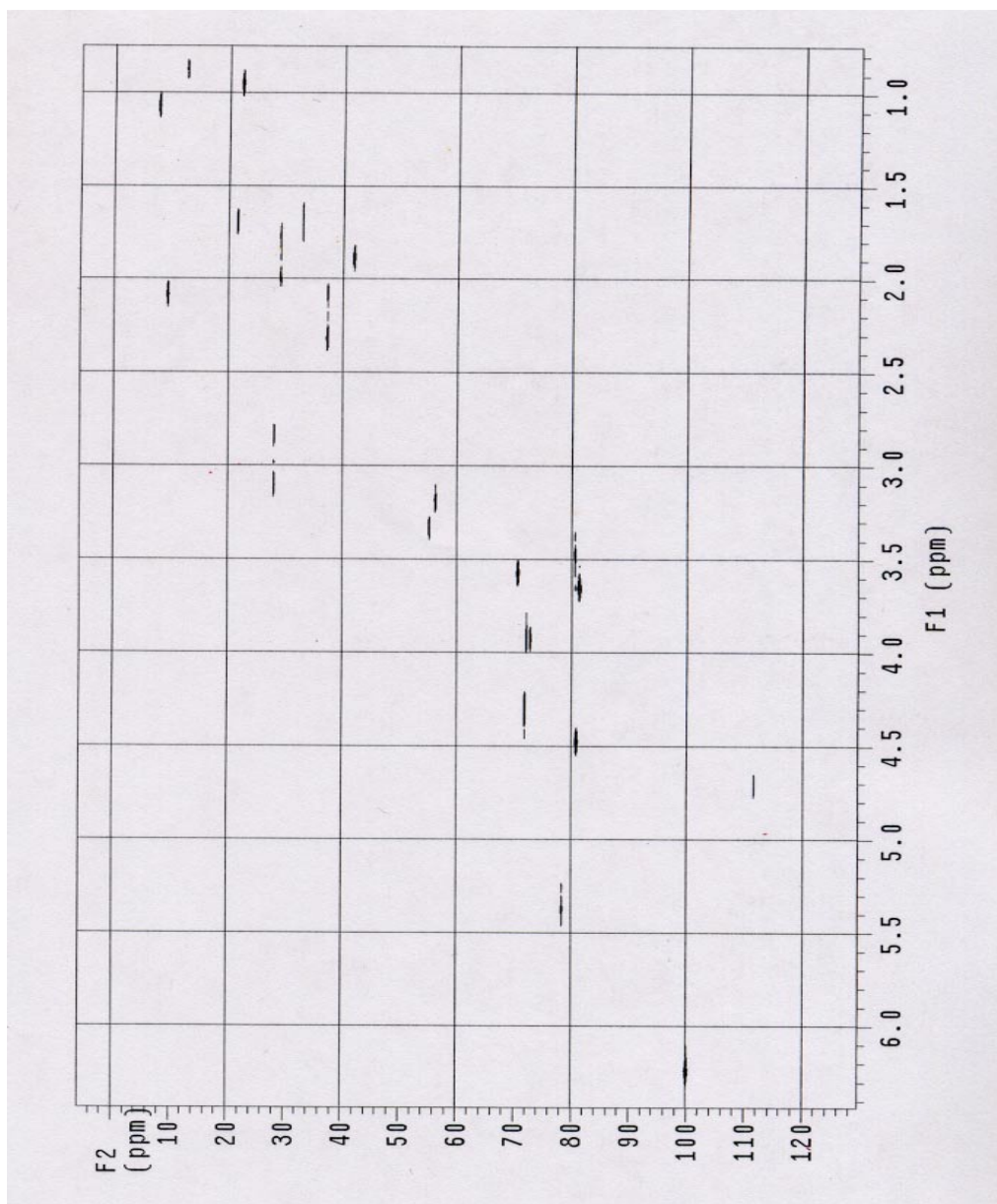
**Figure S3.**  $^{13}\text{C}$  NMR spectrum of psymbenin (125 MHz,  $\text{CD}_3\text{OD}$ ).



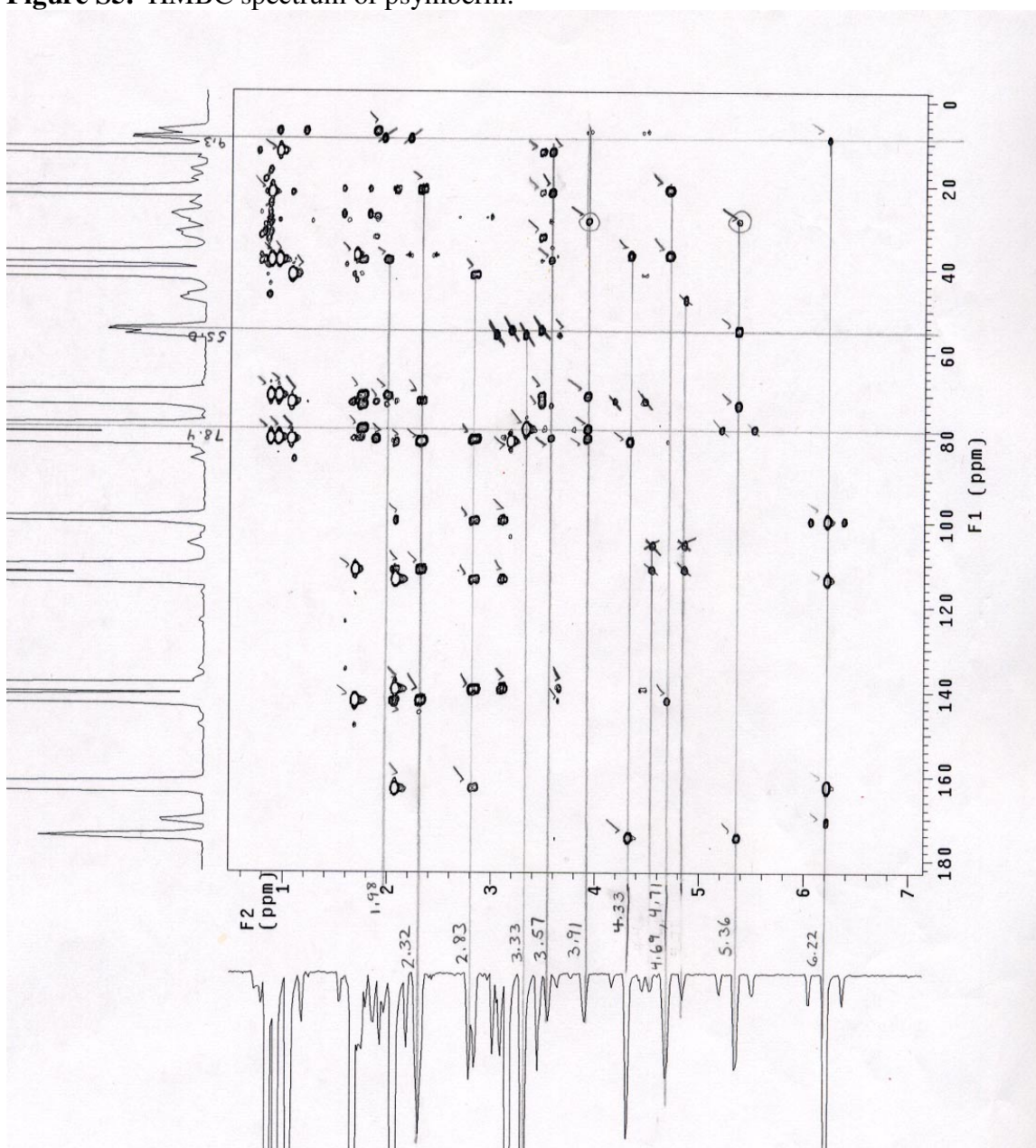
**Figure S3.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of psymbenin.



**Figure S4.** HETCOR spectrum of psymberin.

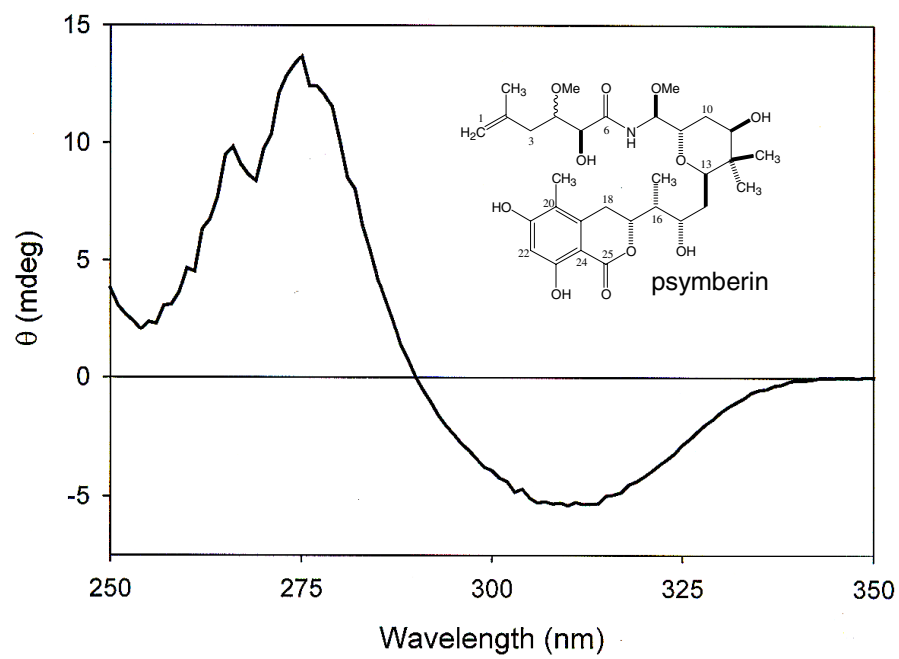


**Figure S5.** HMBC spectrum of psymblerin.

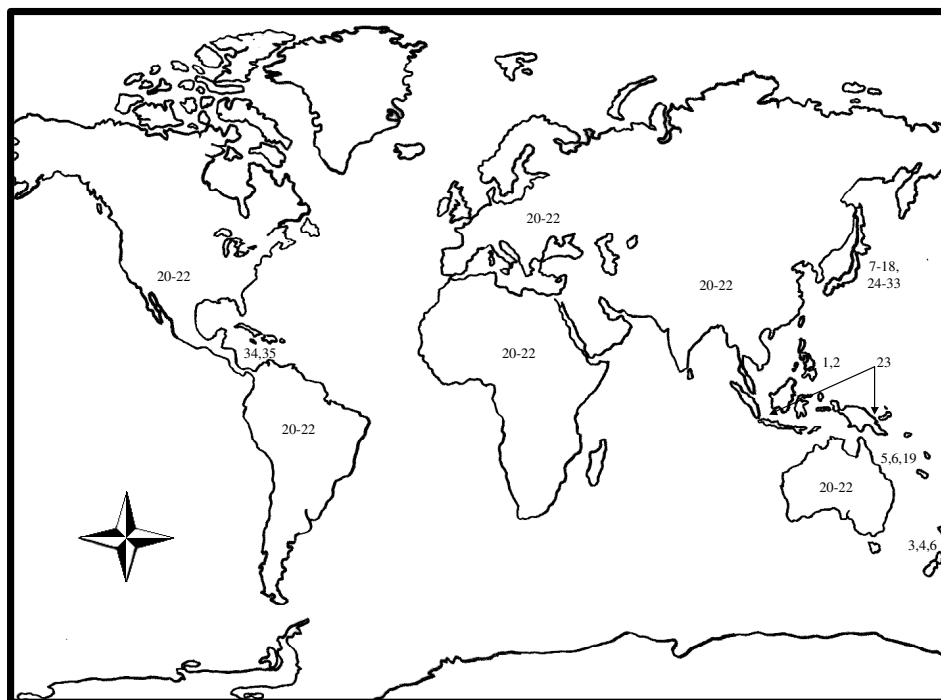




**Figure S6.** Circular dichroism (CD) spectrum of psymberin in EtOH.



**Figure S7.** Source locations for all marine (1-19, 23-35) and terrestrial (20-22) members of the pederin family of secondary natural products (refer to Table S1 for compound name, structure, and source organism).



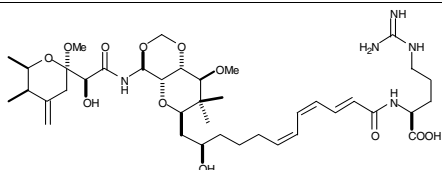
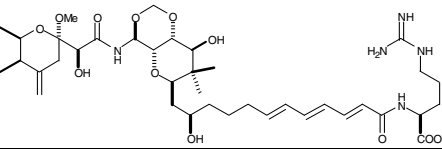
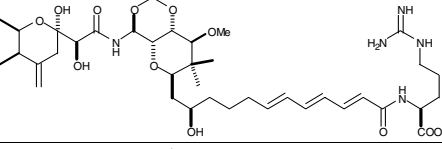
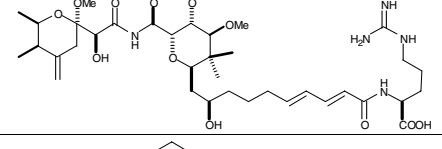
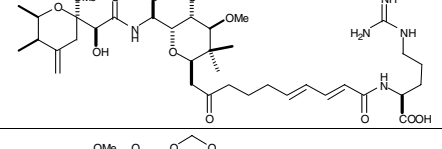
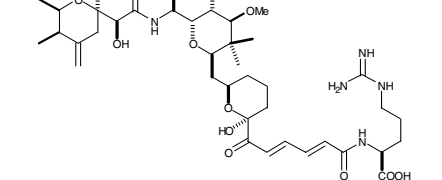
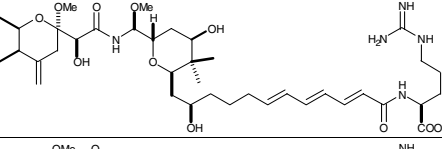
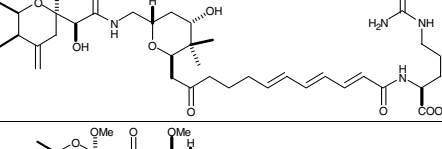
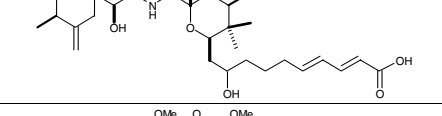
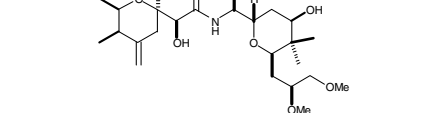
**Figure S8.** *Psammocinia* sp. used in this study.



**Table S1.** Structures, optical rotations, and source organisms of all members of the pederin family of secondary metabolites.

compound	optical rotation	source organism <sup>a</sup> (location)	reference <sup>b</sup>	structure
icadamide A (1)	$[\alpha]_{\text{D}}^{25} = +81^\circ$ (c 0.12, MeOH)	<i>Leiosella</i> sp. (Philippines)	Clardy and He, 1995	
icadamide B (2)	$[\alpha]_{\text{D}}^{25} = +96^\circ$ (c 0.9, MeOH)	<i>Leiosella</i> sp. (Philippines)	Clardy and He, 1995	
mycalamide A (3)	$[\alpha]_{\text{D}}^{25} = +110^\circ$ (c 0.2, CHCl <sub>3</sub> )	<i>Mycale</i> sp. (New Zealand)	Perry et al., 1988	
mycalamide B (4)	$[\alpha]_{\text{D}}^{25} = +39^\circ$ (c 0.2, CHCl <sub>3</sub> )	<i>Mycale</i> sp. (New Zealand)	Perry et al., 1990	
mycalamide C (5)	ND <sup>c</sup>	<i>Stylinos</i> sp. (Australia)	Simpson et al., 2000	
mycalamide D (6)	$[\alpha]_{\text{D}}^{25} = +41^\circ$ (c 0.3, CHCl <sub>3</sub> )	<i>Mycale</i> sp. (New Zealand) & <i>Stylinos</i> sp. (Australia)	West et al., 2000; Simpson et al. 2000	
onnamide A (7)	$[\alpha]_{\text{D}}^{25} = +99.1^\circ$ (c 5.5, MeOH)	<i>Theonella</i> sp. (Japan)	Sakemi et al., 1988	
dihydroonnamide A (8)	$[\alpha]_{\text{D}}^{25} = +74.0^\circ$ (c 0.2, MeOH)	<i>Theonella</i> sp. (Japan)	Matsunaga et al., 1992	
6,7-dihydro-11-oxo-onnamide A (9)	$[\alpha]_{\text{D}}^{25} = +39^\circ$ (c 0.42, MeOH)	<i>Theonella</i> sp. (Japan)	Kobayashi et al., 1993	
11-oxo-onnamide A (10)	$[\alpha]_{\text{D}}^{25} = +90^\circ$ (c 0.24, MeOH)	<i>Theonella</i> sp. (Japan)	Kobayashi et al., 1993	

**Table S2.** Continued

4Z-onnamide A (11)	$[\alpha]_D^{25} = +81^\circ$ (c 0.59, MeOH)	<i>Theonella</i> sp. (Japan)	Kobayashi et al., 1993	
13-des-O-methyl-onnamide A (12)	$[\alpha]_D^{25} = +70.0^\circ$ (c 0.1, MeOH)	<i>Theonella</i> sp. (Japan)	Matsunaga et al., 1992	
pseudoonnamide A (13)	$[\alpha]_D^{25} = +64^\circ$ (c 0.05, MeOH)	<i>Theonella</i> sp. (Japan)	Kobayashi et al., 1993	
onnamide B (14)	$[\alpha]_D^{25} = +61.8^\circ$ (c 0.5, MeOH)	<i>Theonella</i> sp. (Japan)	Matsunaga et al., 1992	
17-oxoonnamide B (15)	$[\alpha]_D^{25} = +59.7^\circ$ (c 0.2, MeOH)	<i>Theonella</i> sp. (Japan)	Matsunaga et al., 1992	
onnamide C (16)	$[\alpha]_D^{25} = +45.4^\circ$ (c 0.2, MeOH)	<i>Theonella</i> sp. (Japan)	Matsunaga et al., 1992	
onnamide D (17)	$[\alpha]_D^{25} = +51.4^\circ$ (c 0.1, MeOH)	<i>Theonella</i> sp. (Japan)	Matsunaga et al., 1992	
onnamide E (18)	$[\alpha]_D^{25} = +64.0^\circ$ (c 0.05, MeOH)	<i>Theonella</i> sp. (Japan)	Matsunaga et al., 1992	
onnamide F (19)	$[\alpha]_D^{25} = +22^\circ$ (c 0.16, MeOH)	<i>Trachycladus</i> <i>laevispiralifer</i> (Australia)	Vuong et al., 2001	
pederin (20)	ND	<i>Paederus</i> sp. (terrestrial, pandemic)	Narquizian and Kocienski, 2000	

**Table S2.** Continued

pseudopederin ( <b>21</b> )	ND	<i>Paederus</i> sp. (terrestrial, pandemic)	Narquizian and Kocienski, 2000	
pederone ( <b>22</b> )	$[\alpha]_D^{25} = +107.7^\circ$ (c 0.2, EtOH)	<i>Paederus</i> sp. (terrestrial, pandemic)	Cardani et al., 1967	
psymberin (irciniastatin A) ( <b>23</b> )	$[\alpha]_D^{25} = +29^\circ$ (c, MeOH)	<i>Psammocinia</i> sp. (Papua New Guinea and Indonesia)	new, see also Pettit et al., 2004	
theopederin A ( <b>24</b> )	$[\alpha]_D^{25} = +88.1^\circ$ (c 0.14, CHCl <sub>3</sub> )	<i>Theonella</i> sp. (Japan)	Fusetani et al., 1992	
theopederin B ( <b>25</b> )	$[\alpha]_D^{25} = +49.1^\circ$ (c 0.06, CHCl <sub>3</sub> )	<i>Theonella</i> sp. (Japan)	Fusetani et al., 1992	
theopederin C ( <b>26</b> )	$[\alpha]_D^{25} = +172.0^\circ$ (c 0.03, CHCl <sub>3</sub> )	<i>Theonella</i> sp. (Japan)	Fusetani et al., 1992	
theopederin D ( <b>27</b> )	$[\alpha]_D^{25} = +80.0^\circ$ (c 0.04, CHCl <sub>3</sub> )	<i>Theonella</i> sp. (Japan)	Fusetani et al., 1992	
theopederin E ( <b>28</b> )	$[\alpha]_D^{25} = +136.7^\circ$ (c 0.03, CHCl <sub>3</sub> )	<i>Theonella</i> sp. (Japan)	Fusetani et al., 1992	
theopederin F ( <b>29</b> )	$[\alpha]_D^{25} = +32^\circ$ (c 0.30, MeOH)	<i>Theonella</i> <i>swinhoei</i> (Japan)	Tsukamoto et al., 1999	

**Table S2.** Continued

theopederin G (30)	$[\alpha]_D^{25} = +45^\circ$ (c 0.12, MeOH)	<i>Theonella</i> <i>swinhoei</i> (Japan)	Tsukamoto et al., 1999	
theopederin H (31)	$[\alpha]_D^{25} = +60^\circ$ (c 0.023, MeOH)	<i>Theonella</i> <i>swinhoei</i> (Japan)	Tsukamoto et al., 1999	
theopederin I (32)	$[\alpha]_D^{25} = +54^\circ$ (c 0.077, MeOH)	<i>Theonella</i> <i>swinhoei</i> (Japan)	Tsukamoto et al., 1999	
theopederin J (33)	$[\alpha]_D^{25} = +48^\circ$ (c 0.038, MeOH)	<i>Theonella</i> <i>swinhoei</i> (Japan)	Tsukamoto et al., 1999	
theopederin K (discalamide A) (34)	$[\alpha]_D^{25} = +90.3^\circ$ (c 0.43, MeOH)	<i>Discodermia</i> sp. (Honduras)	Paul et al., 2002; Gunasekera et al., 2001	
theopederin L (discalamide B) (35)	$[\alpha]_D^{25} = +34^\circ$ (c 0.05, MeOH)	<i>Discodermia</i> sp. (Honduras)	Paul et al., 2002; Gunasekera et al., 2001	

a: The taxonomy of each source organisms is given here as reported in its original publication. A listing of these species with their revised taxonomy is provided in **Table S2**.

b: Cardani, C.; Ghiringhelli, D.; Quilico, A.; Selva, A. *Tetrahedron Lett.* **1967**, 41, 4023-4025.

Clardy, J.; He, H. U.S. Patent 5,476,953, 1995.

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Kobayashi, J.; Itagaki, F.; Shigemori, H.; Sasaki, T. *J. Nat. Prod.* **1993**, 56, 976-981.

Matsunaga, S.; Fusetani, N.; Nakao, Y. *Tetrahedron* **1992**, 48, 8369-8376.

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Vuong, D.; Capon, R.J.; Lacey, E.; Gill, J.H.; Heiland, K.; Friedel, T. *J. Nat. Prod.* **2001**, 64, 640-642.

West, L.M.; Northcote, P.T.; Hood, K.A.; Miller, J.H.; Page, M.J. *J. Nat. Prod.* **2000**, 63, 707-709.

c: Not determined.

**Table S2.** Summary of source organisms of all members of the pederin family of secondary metabolites.<sup>a</sup>

Phylum	Class	Order	Family	Genus	Compounds <sup>b</sup>
Arthropoda	Insecta	Coleoptera	Staphylinidae	<i>Paederus</i>	20-22
Porifera	Demospongiae	Axinellida (Hadromerida)	Spirastrellidae (Trachycladidae)	<i>Trachycladus</i>	19
Porifera	Demospongiae	Dictyoceratida	Irciniidae	<i>Ircinia</i> <i>Psammocinia</i>	23
Porifera	Demospongiae	‘Lithistids’	Theonellidae	<i>Theonella</i>	7-18, 24-33
Porifera	Demospongiae	Poecilosclerida	Mycalidae	<i>Mycale</i>	3, 4, 6
Porifera	Demospongiae	Poecilosclerida (Halichondrida)	Mycalidae (Halichondriidae)	<i>Stylinos</i> ( <i>Hymeniacidon</i> )	5, 6
Porifera	Demospongiae	Dictyoceratida	Spongiidae	<i>Leiosella</i>	1, 2
Porifera	Demospongiae	‘Lithistids’	Theonellidae	<i>Discodermia</i>	34, 35

<sup>a</sup>Revised taxonomic nomenclature was taken from *Systema Porifera: A Guide to the Classification of Sponges*; Hooper, J.N.A.; VanSoest, R.W.M., Eds.; Kluwer Academic/Plenum Publishers: New York, 2002. <sup>b</sup>Compound numbers refer to those used in Table S2.