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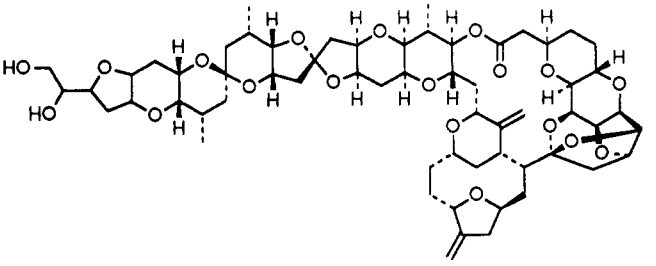
Communications to the Editor

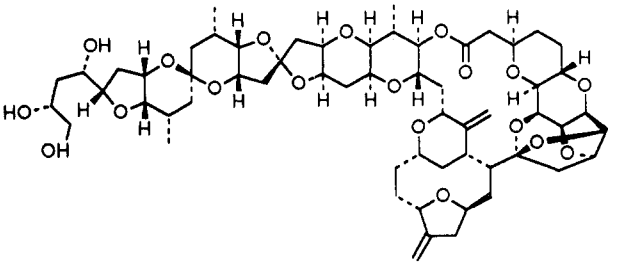
Isolation and Structure of the Cell Growth Inhibitory Constituents from the Western Pacific Marine Sponge *Axinella* sp.[1a]

While isolation of alkaloids from marine Porifera has been accelerating,[2] only a small number of antineoplastic[3] or peptide[3,4] constituents have been recovered from these invertebrates. Our isolation and structural determination of the P388 lymphocytic leukemia (PS system)[5] cell growth inhibitory cyclooctapeptide hymenistatin 1[4] from a Palau sponge in the genus *Hymeniacidon* represented the first such combination of source, structural type, and biological activity. We have also found an *Axinella* sp. (Demospongiae class) collected (in 1979) in Palau (at −40 m) to yield a methylene chloride–2-propanol extract that provided a 101% increase in life span (at 100 mg/kg) against the PS leukemia[5] with ED_{50} 2.5 μg/mL in the corresponding cell line.

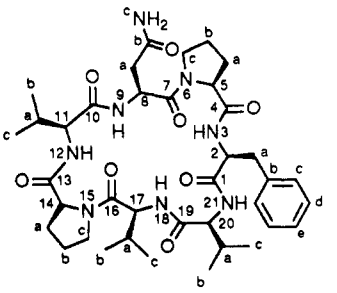
In 1985 the sponge was recollected (Palau) and preserved in 2-propanol. A 220-kg (wet weight) portion was extracted with methylene chloride–methanol. By means of PS guided bioassay and a series of detailed[3,4] solvent partition, gel permeation (and gel partition, Sephadex LH-20), partition (silica gel including reversed phase), and adsorption column chromatographic techniques, a series of structurally diverse antineoplastic constituents were detected in this very productive sponge. We now report that the most potent in vivo components were established[6] as the polyether macrolides, homohalichondrin B (1, 900 μg, 4.1×10^{-8}% yield, PS T/C 285 at 150 μg/kg) and halichondrin B (2, 400 μg, 1.8×10^{-8}% yield, PS T/C 238 at 25 μg/kg), heretofore found in trace amounts in one difficultly accessible Japanese sponge.[7,8] A new PS inhibitory (ED_{50} 0.21 μg/mL) peptide (3, 100 mg, 4.54×10^{-5}% yield) designated axinastatin 1 was also isolated, accompanied by axinohydantoin[3] (30 mg) and hymenialdisine[3] (0.53 mg).



1
(homohalichondrin B)



2
(halichondrin B)



3
(axinastatin 1)

Axinastatin 1 (3) crystallized from methylene chloride: mp 283–7 °C dec; $[\alpha]^{25}_D$ −161.6° (c 0.099, CH_3OH); TLC (R_f 0.18 in 95:5 CH_2Cl_2/MeOH); UV (CH_3OH) λ_{max} 208 nm (ε 18 000); IR (NaCl plate), ν_{max} 3320, 2960, 1640, 1520, 1465, 1430 cm^{-1}; high-resolution FAB MS 753.4293 $[M + H]^+$, theoretical mass for $[M + H]^+$ of $C_{38}H_{56}N_8O_8$ requires

(1) (a) Antineoplastic Agents series contribution 219. For part 218, refer to Bradshaw, T. D.; Gescher, A.; Pettit, G. R. *Int. J. Cancer* **1991**, *47*, 929. (b) Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701. (c) Midwest Center for Mass Spectrometry, The University of Nebraska, Lincoln, NE 68588-0362. (d) Northern Territory Museum of Arts and Sciences, Bullock Point, Fannie Bay, Darwin, N.T. 0801, Australia. (e) National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560.

(2) (a) Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925. (b) Cimino, G.; Mattia, C. A.; Mazzarella, L.; Puliti, R.; Scognamiglio, G.; Spinella, A.; Trivellone, E. *Tetrahedron* **1989**, *45*, 3863. (c) Kobayashi, M.; Kawazoe, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1989**, *37* (6), 1676. (d) Kubo, A.; Kitahara, Y.; Nakahara, S. *Chem. Pharm. Bull.* **1989**, *37* (5), 1384. (e) Burres, N. S.; Sazesh, S.; Gunawardana, G. P.; Clement, J. J. *Cancer Res.* **1989**, *49*, 5267. (f) Kobayashi, J.; Murayama, T.; Ohizumi, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1989**, *30*, 4833. (g) Kobayashi, M.; Kawazoe, K.; Kitagawa, I. *Tetrahedron Lett.* **1989**, *30*, 4149. (h) Fedoreyev, S. A.; Ilyin, S. G.; Utkina, N. K.; Maximov, O. B.; Reshetnyak, M. V.; Antipin, M. Y.; Struchkov, T. P. *Tetrahedron* **1989**, *45*, 3487. (i) Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1989**, *30*, 2809. (j) Sakemi, S.; Sun, H. H.; Jefford, C. W.; Bernardinelli, G. *Tetrahedron Lett.* **1989**, *30*, 2517.

(3) For leading references, see: Pettit, G. R.; Herald, C. L.; Leet, J. E.; Gupta, R.; Schaufelberger, D. E.; Bates, R. B.; Clewlow, P. J.; Doubek, D. L.; Manfredi, K. P.; Rützler, K.; Schmidt, J. M.; Tackett, L. P.; Ward, F. B.; Bruck, M.; Camou, F. *Can. J. Chem.* **1990**, *68*, 1621.

(4) Pettit, G. R.; Clewlow, P. J.; Dufresne, C.; Doubek, D. L.; Cerny, R. L.; Rützler, K. *Can. J. Chem.* **1990**, *68*, 708.

(5) U.S. National Cancer Institute's murine P388 lymphocytic leukemia cell line.

(6) By high-resolution FAB, NMR (2D, 400 MH_z) and comparison with an authentic sample of polyether 2 provided by Prof. D. Uemura.

(7) Uemura, D.; Takahashi, K.; Yamamoto, T.; Katayama, C.; Tanaka, J.; Okumura, Y.; Hirata, Y. *J. Am. Chem. Soc.* **1985**, *107*, 4796.

(8) (a) Hirata, Y.; Uemura, D. *Pure Appl. Chem.* **1988**, *58*, 701. (b) Tasumasa, T.; Toshitaka, M.; Keizo, Y.; Hiroyuki, K. Fujisawa Pharmaceutical Co., Ltd., Jpn Kokai Tokkyo Kohe JP 61,191,687 [86,191,687] (C1. C07D493/22) 1986, Appl. 85/32,253 1985; *Chem. Abstr.* **1987**, *106*, 23261g.

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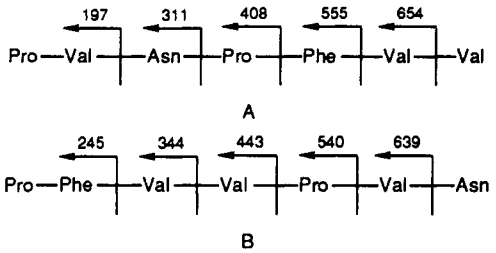
753.4299; amino acid analyses Asp, (or Asn), Phe, Pro, and Val in the ratio 1:1:2:3. The molecular formula for axinastatin 1 (3) was deduced from high-field (400 MHz) ^1H and ^{13}C NMR studies (see Table I of the supplementary material) in conjunction with the high-resolution FAB MS peak matching experiments just noted. Combined ^1H, ^1H COSY, ^1H, ^{13}C COSY, ^1H, ^1H relayed COSY,[9a] HMBC,[9b] and NOESY experiments confirmed the amino acid sequence and cyclic structure 3. The amino acid components and sequence of axinastatin 1 were confirmed as *cyclo*-(Asn-Pro-Phe-Val-Val-Pro-Val) by tandem (MS/MS) mass spectrometry.[10]

Protonation upon FAB results in ring opening of the cyclic peptide at an N-acyl bond to give a linear acylium ion.[10] The major fragmentation processes observed by tandem mass spectrometry involve losses of amino acid residues from the C terminus. Protonation is favored at proline, and with axinastatin 1 there are two possibilities. The FAB MS/MS spectrum of the [M + H] species contains two series (A and B) of ions resulting from protonation at the two proline units. All of the ions in both series



were observed. Additional supporting information for the sequence was obtained by MS/MS experiments on source-produced fragment ions to confirm the interrelationship of the fragment ions and by exact mass measurements on the fragment ions to verify elemental composition and correct assignment.

The absolute configuration of cycloheptapeptide 3 was ascertained by analyzing the acid hydrolysate *N*-pentafluoropropionyl-isopropyl ester[4] derivatives using chiral GC (Chirasil-Val III column). Each amino acid was found to have the L configuration. The disproportionatly high representation of L-Pro and L-Val in axinastatin 1 (3) and other strongly antineoplastic peptides[4] we have discovered in marine animals suggests that the presence of these amino acids may be an important structural requirement for controlling cell growth in peptide mediated systems.

The halichondrins proved to be remarkably potent against all of the 60 cell lines in the U.S. NCI's human tumor cell line in vitro screen,[11] yet with sufficient differences in relative sensitivity among the lines to yield a distinctive mean graph[12] profile. For halichondrin B and homohalichondrin B, the log molar GI_{50}'s for each line ranged from -8.10 to -9.70 and -8.05 to -10.08, respectively. The mean log molar GI_{50}'s were -8.95 and -8.99 for halichondrin B and homohalichondrin B, respectively. The characteristic mean graph "fingerprints" of the halichondrins were very similar; an analysis by the COMPARE pattern-recognition algorithm[13] showed their mean graph profiles to be most highly correlated to those produced by structurally unrelated, tubulin-binding standard agents[11] such as vincristine and taxol.

Discovery of the halichondrins in an *Axinella* sp., a sponge unrelated to their original source,[7] suggests that these exceptionally active[14] Porifera constituents may have a microorganism source. Either by exogenous and/or endogenous biosynthetic processes the marine porifera continue to be an especially fruitful source of potentially useful antineoplastic substances of novel structure.

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Supplementary Material Available: NMR spectra of axinastatin 1 and interpretation of tandem MS-MS spectra (28 pages). Ordering information is given on any current masthead page.

(9) (a) Bax, A.; Drobny, G. *J. Magn. Res.* **1985**, *61*, 306. (b) Bax, A.; Summers, M. A. *J. Am. Chem. Soc.* **1986**, *108*, 2094.
(10) Cerny, R. L.; Gross, M. L. Tandem Mass Spectrometry for Determining the Amino Acid Sequences of Cyclic Peptides and for Assessing Interactions of Peptides and Metal Ions. In *Mass Spectrometry of Peptides*; Desiderio, D. M., Ed.; CRC Press: Boca Raton, FL, 1990; pp 289-314.
(11) Boyd, M. R. Status of the NCI preclinical antitumor drug discovery screen. In *Principles and Practices of Oncology*; DeVita, V. T., Jr., Hellman, S.; Rosenberg, S. A., Eds.; Lippincott: Philadelphia, 1989; pp 1-12.
(12) Boyd, M. R.; Paull, K. D.; Rubinstein, L. R. Data display and analysis strategies from the NCI disease-oriented *in vitro* antitumor drug screen. In *Antitumor Drug Discovery and Development*; Valeriote, F. A., Corbett, T., Baker, L., Eds.; Kluwer Academic Press: Amsterdam, 1990, in press.
(13) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R.: Display and analysis of patterns of differential activity of drugs against human tumor cell lines: Development mean graph and COMPARE algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088-1092.
(14) Cooper, A. J.; Salomon, R. G. *Tetrahedron Lett.* **1990**, *31*, 3813.

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Novel Binding Mode of Highly Potent HIV-Proteinase Inhibitors Incorporating the (*R*)-Hydroxyethylamine Isostere

The recent communication from Professor Rich and his colleagues[1] describing their model of the binding of a hydroxyethylamine containing inhibitor (Ro 31-8959, compound 6, Table I) to the active site of HIV-1 proteinase

(1) Rich, D. H.; Sun, Q-C.; Prasad, J. V. N. V.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M.; Mueller, R. A.; Houseman, K. Effect of Hydroxyl Group Configuration in Hydroxyethylamine Dipeptide Isosteres on HIV Protease Inhibition. Evidence for Multiple Binding Modes. *J. Med. Chem.* **1991**, *34*, 1222-1225.

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