Antineoplastic Agents 300. Isolation and Structure of the Rare Human Cancer Inhibitory Macrocyclic Lactones Spongistatins 8 and 9†

George R. Pettit,*[a] Zbigniew A. Cichacz,[a] Cherry L. Herald,[a] Feng Gao,[a] Michael R. Boyd,[b] Jean M. Schmidt,[a] Ernest Hamel[c] and Ruoli Bai[c]

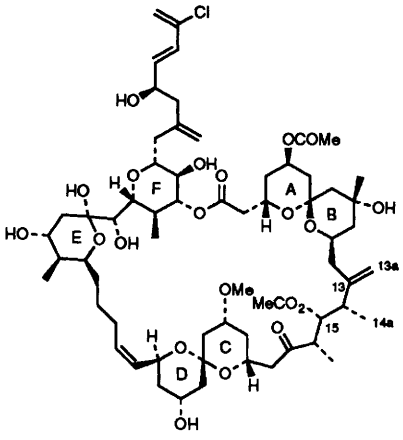
[a] *Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287-1604, USA*

[b] *Laboratory of Drug Discovery Research and Development, Developmental Therapeutic Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, USA*

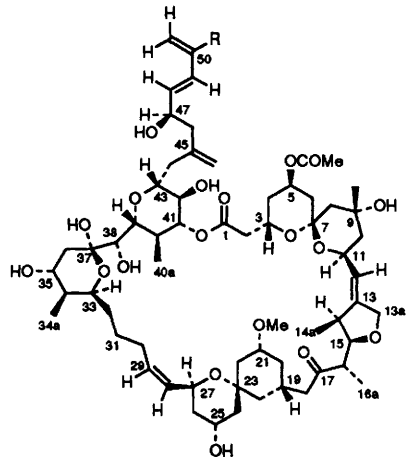
[c] *Laboratory of Molecular Pharmacology, Developmental Therapeutics Program Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*

Two trace (0.7 and 2.2 × 10^{-7}% yields) potent antineoplastic macrocyclic lactones termed spongistatins 8 (**2a**) and 9 (**2b**) have been isolated from the African marine sponge *Spirastrella spinispirulifera* and found to be very potent inhibitors of glutamate-induced tubulin polymerization.

Since our early investigations,[2] the discovery of cytotoxic and/or antineoplastic marine animal constituents has continued to accelerate. Current examples include potentially useful substances from marine sponges,[3–8] tunicates,[9] soft coral,[10] and starfish.[11] To date the astonishingly potent spongistatin 1 (**1**) discovered[12] in a Republic of Maldives, *Spongia sp.* has proved to be one of the most potent cancer cell growth inhibitory substances known. We later discovered spongistatins **2–7**[13–15] bearing variations in substituents and in the spongipyran ring system.† We now report the isolation and structural elucidation of two new and prodigiously cytotoxic macrocyclic lactones designated spongistatins 8 (**2a**) and 9 (**2b**) from the Southwest African marine sponge, *Spirastrella spinispirulifera.*



1, Spongistatin 1



2a, R = H Spongistatin 8
b, R = Cl Spongistatin 9

Further detailed study of P388 lymphocytic leukemia (PS cell line) active fractions prepared[13] from *Spirastrella spinispirulifera* (2409 kg wet wt.) employing an extensive series of gel permeation and partition chromatographic separations on Sephadex LH-20 followed by HPLC (*e.g.* using reversed phase Prepex RP8, 5-20 μ, columns and water–acetonitrile gradients) afforded 1.8 mg (7.5 × 10^{-8}% yield, PS ED_{50} 8 × 10^{-4} μg ml^{-1}) of colourless spongistatin 8 (**2a**): mp 158–159 °C; $[\alpha]_D^{22}$ −32° (*c* 0.18, MeOH); IR (film) cm^{-1} 3439, 2936, 1736, 1653, 1602, 1383, 1252, 1178, 1090; high resolution FABMS, *m/z* 1183.5821 $[M + K]^+$, $C_{61}H_{32}KO_{20}$ (calc. 1183.5819) and 5.4 mg (2.2 × 10^{-7}% yield, PS ED_{50} 2.7 × 10^{-5} μg ml^{-1}) of spongistatin 9 (**2b**): mp 164–165 °C; $[\alpha]_D^{22}$ −33.3° (*c* 0.14, MeOH); IR (film) cm^{-1} 3435, 2940, 1736, 1647, 1591, 1385, 1254, 1178, 1090; high resolution FABMS, *m/z* 1217.5425 $[M + K]^+$ corresponding to $C_{61}H_{91}ClKO_{20}$ (calc. 1217.5429).

Due to the paucity of spongistatin 8 (**2a**) structural elucidation was simplified by first deducing the structure of spongistatin 9 (**2b**). Once the high resolution FABMS and high field 2D NMR interpretations were in hand for lactone **2b**, the structure of spongistatin 8 (**2a**) was completed. The THF ring of spongistatin 8 was recognized by chemical shifts at δ 4.45 (br d, *J* = 13 Hz), 4.10 (br d, *J* = 13 Hz)/70.70 and 1.95 (acetyl, s, 3H)/21.31 and 172.56 (acetyl). A signal at δ 6.33 (ddd, *J* = 10, 10, 17 Hz) indicated that C-50 was devoid of the usual spongistatin chlorine atom at that position. A series of ^1H-^1H NMR COSY experiments allowed assignment of the remaining ^1H and ^{13}C NMR signals by comparison with the analogous NMR carbon data from spongistatin 9.

The structure of spongistatin 9 was determined mainly by high field NMR spectroscopy utilizing results of ^1H–^1H COSY, ^1H–^{13}C COSY, APT, and HMBC NMR experiments. Both the ^1H- and the ^{13}C-NMR spectra of spongistatin 9 indicated it was a member of the spongistatins by signals at δ 1.13 (3H, s)/30.17, 1.04 (3H, d)/14.59, 1.14 (3H, d)/15.10, 0.89 (3H, d)/11.52, 0.84 (3H, d)/13.00, a lactone carbonyl signal at δ 173.66 and a ketone carbonyl signal at δ 213.42. Spongistatin 9 was found to possess a THF ring by signals at δ 4.45 (br d, *J* = Hz) and 4.10 (br d, *J* = 13 Hz) corresponding to two H-13a. An acetyl group was evident by signals at δ 1.95 (3H)/21.35 and 172.61. That the acetyl group was attached to the C-5 oxygen atom was evidenced by the chemical shift of H-5 at δ 4.96. The two broad singlets at δ 5.42 and 5.33 and the lack of an ^1H signal for C-50 were indicative of a chlorine atom at that position.

Comparative testing of spongistatins **2a** and **2b** in the NCI 60 cell line *in vitro* screening panel,[18,20] revealed an overall potency comparable to or exceeding the potency of the most active members of the series heretofore reported. The mean panel $GI_{50's}$ determined from quadruplicate testing of spongistatins **2a** and **2b** over three different concentration ranges (10^{-7}, 10^{-8}, 10^{-9} mol dm^{-3} upper limits; five, log_{10}-spaced

concentrations in each range) were 2.3×10^{-10} and 0.4×10^{-10} mol dm^{-3}, respectively (standard errors averaged less than 10% of the respective means). The distinctive mean-graph profiles (pattern of relative cellular sensitivity) produced by spongistatins **2a** and **2b** were strongly correlated (*e.g.* compare correlation coefficients[19,20] >0.8) with the profiles obtained with other members of the series, as well as with structurally unrelated but mechanistically similar members of the important general class of tubulin-interactive antimitotics.[20]

Spongistatins 1 (**1**), 8 (**2a**) and 9 (**2b**) were found to potently inhibit the glutamate-induced polymerization of tubulin[21] with IC_{50} values respectively of 3.6, 5.5 and 4.2 μmol dm^{-3}. By comparison the novel peptide dolastatin 10[22] presumed to be the most potent inhibitor of tubulin assembly known, gave in the same series of experiments, an IC_{50} of 2.1 μmol dm^{-3}. While these spongistatins did not prevent the binding of colchicine to tubulin, they strongly inhibited the classic binding of vinblastine and nucleotide (GTP) to tubulin. Thus the spongistatins represent a completely new class of structurally unique biosynthetic products that inhibit mitosis by binding to tubulin in the Vinca alkaloid domain.[23] Very importantly, spongistatins 1 and 9 appear to be the most cancer cell growth inhibitory antimitotic substances discovered to date.

The exigent financial assistance was obtained from Outstanding Investigator Grant CA 44344-01A1-05 awarded by the Division of Cancer Treatment, National Cancer Institute, DHHS; the Arizona Disease Control Research Commission; Eleanor W. Libby; Herbert and Diane Cummings; the Nathan Cummings Foundation, Inc.; Gary L. Tooker and the Robert B. Dalton Endowment Fund; the U.S. National Science Foundation (Grants CHE-8409644 and BBS-88-04992) and the Midwest Center for Mass Spectrometry, the University of Nebraska-Lincoln (NSF Regional Instrumentation Grants CHE-8620177 and DIR9017262).

Received, 29th March 1994; Com. 4/01901F

Footnotes

† For the preceeding contribution, refer to ref. 1.

‡ Subsequent to our first discovery[12] of spongistatin 1 and the novel spongipyran nucleus common to the series, others[16,17] have reported structures corresponding to spongistatins 1 and 4 from different sponge sources. In order to avoid obfuscation of the literature by use of the names altohyrtin (ref. 16) for spongistatin 1 (**1**) and cinachyrolide A (ref. 17) for spongistatin 4, we recommend that the spongistatin nomenclature be used for all members of the spongipyran series.

References

1 For part 299 consult C. Stanwell, A. Gescher, T. D. Bradshaw and G. R. Pettit, *Int. J. Cancer*, 1994, **56**, 585.
2 G. R. Pettit, J. F. Day, J. L. Hartwell and H. B. Wood, *Nature*, 1970, **227**, 962.
3 G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald, M. R. Boyd, J. M. Schmidt and J. N. A. Hooper, *J. Org. Chem.*, 1993, **58**, 1302.
4 G. R. Pettit, R. Tan, F. Gao, M. D. Williams, D. L. Doubek, M. R. Boyd, J. M. Schmidt, J-C. Chapuis, E. Hamel, R. Bai, J. N. A. Hooper and L. P. Tackett, *J. Org. Chem.*, 1993, **58**, 2538.
5 D. C. Radisky, E. S. Radisky, L. R. Barrows, B. R. Copp, R. A. Kramer and C. M. Ireland, *J. Am. Chem. Soc.*, 1993, **115**, 1632.
6 S. Kanazawa, N. Fusetani and S. Matsunaga, *Tetrahedron Lett .*, 1993, **34**, 1065.
7 J. Kobayashi, M. Tsuda, T. Nakamura, Y. Mikami and H. Shigemori, *Tetrahedron*, 1993, **49**, 2391.
8 G. R. Pettit, J. K. Srirangam, D. L. Herald, K. L. Erickson, D. L. Doubek, J. M. Schmidt, L. P. Tackett and G. J. Bakus, *J. Org. Chem.*, 1992, **57**, 7217.
9 M. Chbani, M. Païs, J.-M. Delauneux and C. Debitus, *J. Nat. Prod.*, 1993, **56**, 99.
10 A. D. Rodriguez and O. M. Cóbar, *Tetrahedron*, 1993, **49**, 319.
11 A. Casapullo, E. Finamore, L. Minale, F. Zollo, J. B. Carré, C. Debitus, D. Laurent, A. Folgore and F. Galdiero, *J. Nat. Prod.*, 1993, **56**, 105.
12 G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald, M. R. Boyd, J. M. Schmidt and J. N. A. Hooper, *J. Org. Chem.*, 1993, **58**, 1302.
13 G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald and M. R. Boyd, *J. Chem. Soc., Chem. Commun.*, 1993, 1166.
14 G. R. Pettit, C. L. Herald, Z. A. Cichacz, F. Gao, J. M. Schmidt, M. R. Boyd, N. D. Christie and Fred E. Boettner, *J. Chem. Soc., Chem. Commun.*, 1993, 1805.
15 G. R. Pettit, C. L. Herald, Z. A. Cichacz, F. Gao, M. R. Boyd, N. D. Christie and J. M. Schmidt, *Nat. Prod. Lett.*, 1993, **3**, 239.
16 M. Kobayashi, H. Uehara, K. Matsunami, S. Aoki and I. Kitagawa, *Tetrahedron Lett.*, 1993, **34**, 7925.
17 N. Fusetani, K. Shinoda and S. Matsunago, *J. Am. Chem. Soc.*, 1993, **115**, 3977.
18 M. R. Boyd, Status of the NCI preclinical antitumor drug discovery screen, in *Principles and Practices of Oncology Updates*, ed. V. T. DeVita, Jr., S. Hellman and S. A. Rosenberg, Lippincott, Philadelphia, 1989, vol. 10, No. 3, pp. 1–12.
19 M. R. Boyd, K. D. Paull and L. R. Rubinstein, Data display and analysis strategies from NCI disease-oriented *in vitro* antitumor drug screen, in *Cytotoxic Anticancer Drug Models and Concepts for Drug Discovery and Development*, ed. F. A. Valeriote, T. Corbett and L. Baker, Kluwer Academic Publishers, Amsterdam, 1992, pp. 11–34.
20 M. R. Boyd, The Future of New Drug Development, in *Current Therapy in Oncology*, ed. J. E. Niederhuber, Mosby Publishing, St. Louis, 1993, pp. 11–22.
21 E. Hamel, Interaction of Tubulin with Small Ligands, in *Microtubule Proteins*, ed. J. Avila, CRC Press, Boca Raton, FL. 1990, pp. 89–191.
22 R. Bai, G. R. Pettit and E. Hamel, *Biochem. Pharmacol.*, 1990, **39**, 1941.
23 R. Bai, Z. A. Cichacz, C. L. Herald, G. R. Pettit and E. Hamel, *Mol. Pharmacol.*, 1993, **44**, 757.