Pergamon

Tetrahedron Letters, Vol. 35, No. 50, pp. 9435-9438, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)02039-6

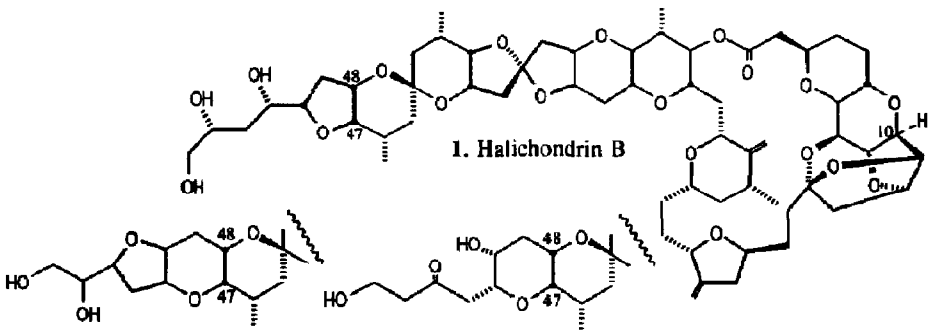
Isohomohalichondrin B, a New Antitumour Polyether Macrolide from the New Zealand Deep-Water Sponge *Lissodendoryx* sp.

Marc Litaudon, Joanne B Hart, John W Blunt,* Robin J Lake & Murray H G Munro.*

Department of Chemistry, University of Canterbury, Christchurch, NEW ZEALAND.

Abstract: Isohomohalichondrin B, a new structural type in the halichondrin group, has been isolated from the deep-water New Zealand sponge *Lissodendoryx* sp. Like some other members of the halichondrin family isohomohalichondrin B exhibited remarkable cytotoxicity against the P388 (murine leukaemia) cell line and selective cytotoxicity in the NCI's primary screen.

During our investigation of the biological potential of the New Zealand marine biota,[1] attention was drawn to a bright yellow sponge *Lissodendoryx* sp. (family Myxillidae, order Poecilosclerida) collected by dredging from deep water (100 m) off the Kaikoura Peninsula. Trial extracts from this sponge were strongly inhibitory against the murine leukemia cell line P388, a DNA virus (*Herpes simplex* Type I) and an RNA virus (*Polio* vaccine virus). Initial studies on the sponge established that the active components were stable, and furthermore, crude extracts of this sponge offered significant extensions of life-span in an *in vivo* murine P388 model (T/C ~250%). Extraction of the sponge (5 kg) with CH_3OH/CH_2Cl_2 (3:1) was followed by partitioning of the crude extract between CH_2Cl_2 and water. Further partitioning of the organic soluble portion between aqueous CH_3OH (80%) and hexane yielded a crude organic extract (3.8 g). This was subjected to bioassay-directed C-18 reverse phase flash chromatography, LH-20 gel permeation chromatography (2x) using CH_3OH/CH_2Cl_2 (6:4) as eluent, and preparative C-18 reverse phase high pressure liquid chromatography with CH_3CN/H_2O (1:1) as eluent to yield three biologically active components **1** (2 mg; $4x10^{-5}$%), **2** (3.5 mg; $7x10^{-5}$%) and **3** (4.5 mg; $9x10^{-5}$%) and several (>6) minor components. Based on HRFAB mass spectroscopy and extensive 1-D and 2-D nmr analysis **1** and **2** were identified as halichondrin B and homohalichondrin B respectively, while **3** was recognised as a new class of halichondrin.[2]



The halichondrins are a series of polyether macrolides originally isolated from the sponge *Halichondria okadai* Kadota.[3] Some of these, in particular halichondrin B, have shown potent *in vitro* and *in vivo* antitumor activity.[3-5] Subsequently, halichondrin B **1** and homohalichondrin B **2** have been isolated from two other Southern Hemisphere sponges,[4,5] along with halistatin 1 **4**, (10α-hydroxyhalichondrin B).[5] Halichondrin B **1** and halistatin 1 **4** have been determined to be noncompetitive inhibitors of the binding of vincristine to tubulin

and to inhibit nucleotide exchange on tubulin.[5,6] In 1992 halichondrin B 1 was selected by the NCI Decision Network Decision Committee for development as an anticancer drug,[7] indicating the importance of this family of compounds as potential anticancer agents. The finding of a new halichondrin therefore is of significance for structure-activity relationships.

HRFABMS on 3 (as MK^+) corresponded to $C_{61}H_{86}O_{19}$, isobaric with homohalichondrin B. Consequently, the new halichondrin was named isohomohalichondrin B. The structure of 3 was established using ^1H-^1H COSY (two different solvents, CD_3OD and $CDCl_3$/pyridine-d_5 (1 drop)), 1D and 2D TOCSY, HMQC and HMBC experiments. Proton connectivities from C1 to C48 were identical with those already defined for halichondrin B 1 and homohalichondrin B 2.[2,3-5] This region constitutes the unchanged section of the halichondrin system found through the norhalichondrin,[3] halichondrin 1 and homohalichondrin 2 series of compounds and their associated A, B and C families.[3,8] This unifying aspect of the halichondrin structure has been annunciated as the *halipyran* system by Pettit *et al.*[5] For isohomohalichondrin B 3 the "unique" region encompassing H48 to H55 was identified as three additional proton spin systems.[2] In the ^{13}C NMR spectrum at 125 MHz all 61 carbons were observed, but the ketone resonance for C53 was absent from a spectrum obtained at 75 MHz. The HMQC experiment confirmed the presence of 4 methines and 4 methylenes in the unique "left hand" end (from C47 to C55), while HMBC supported the unique sequence C51 to C55 by the observation of long-range correlations between the protons at positions 51, 52, 54 and 55 and the carbon at 209.5 ppm as shown in Figure 1. The sequence from H46 through to H51 was established by COSY and TOCSY correlations.

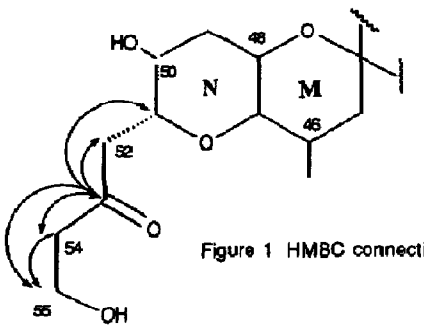


Figure 1 HMBC connectivities

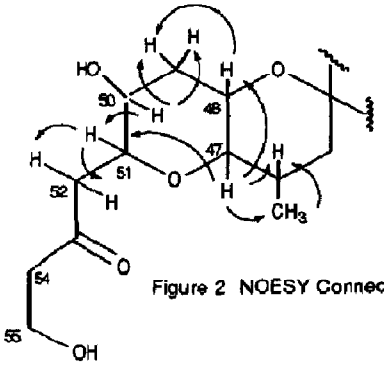


Figure 2 NOESY Connectivities

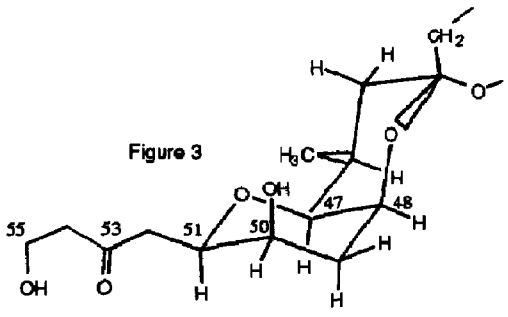


Figure 3

The relative stereochemistry of isohomohalichondrin 3 was established by NOESY experiments (see Figure 2). This confirmed the *cis* fusion of the M/N ring junction (H47 and H48 *cis*) as in all other halichondrins isolated to date. A strong correlation between H50 and H51, which was also correlated with H47, established the *cis* relationship of all 3 hydrogens. These NOESY results, supported by the derived coupling constants for the spin systems, are best interpreted in terms of a chair/chair conformation for this 1,5-bisoxadecalin system (Figure 3).

The absolute stereochemistry of isohomohalichondrin B, as depicted in 3 and Figure 3, is based on the defined stereochemistry of a norhalichondrin derivative by X-ray crystallography[3a] and the similarity in sign and magnitude of the optical rotation of 3[2] with that of homohalichondrin B 2, and other members of the halichondrin family of compounds.[3b]

The side-chain (C52-C55) of 3 has more degrees of motional freedom than the central core. This is shown by the low intensity resonance for C53 (δ_C 209.5 ppm), which can be attributed to a longer relaxation time in comparison to the other carbons present and supported by the measurement of the relaxation times for the C54 and C55 protons which were 2x the average of the T_1 values of the other methylene protons.

Testing of the halichondrins from *Lissodendoryx* sp. in our in-house murine leukaemia (P388 cell line) assay confirmed the exceedingly potent nature of this group of compounds. [Isohomohalichondrin B 3, halichondrin B 1 and homohalichondrin B 2 (IC_{50} 0.18, 0.78 and 0.22 ng/mL respectively)]. These results were confirmed when the New Zealand samples of the halichondrins (1, 2 and 3) were tested against the >60 cell lines in the US NCI's human tumour cell line *in vitro* screen.[9] The pattern of inhibition demonstrated by the isohomohalichondrin B 3 was highly characteristic and use of the COMPARE algorithm[10] showed that it was highly correlated with the other known halichondrins (see Table below). As noted previously for halichondrin B 1 and homohalichondrin B 2,[11] isohomohalichondrin B 3 was also highly correlated with tubulin-binding agents such as vincristine, maytansine and taxol. When tested in a tubulin binding assay[11] isohomohalichondrin B 3 was found to be an effective inhibitor of tubulin polymerisation.

Compound[a]	Mean Panel GI_{50} ($x10^{-10}M$)[b]	COMPARE Correlation Coeff.[c]
Halichondrin B 1	1.38	1.00
Homohalichondrin B 2	1.58	0.91
Isohomohalichondrin B 3	1.15	0.80

a Compounds 1, 2, 3 were tested in quadruplicate at each of 3 different concentration ranges.
b Standard errors averaged < 15% of the respective means.
c Coefficients were calculated using the TGI-centered mean graph profiles from the highest test concentration. The TGI mean graph profile of halichondrin B 1 was used as the "seed" for all comparisons.

The halichondrins have now been isolated from three quite diverse sponge sources and detected in a fourth. The first reported isolation was from a Halichondrid sponge, *Halichondria okadai* Kadota, found on the Pacific coast of Japan.[3] The next reported findings were from the Axinellid sponges, *Axinella* sp and *Phakellia carteri*, found in the Western Pacific Island of Palau[4] and in the Republic of Comoros in the Eastern Indian Ocean,[5] respectively. This most recent finding has been from the New Zealand Poecilosclerid sponge *Lissodendoryx* sp. We have also detected the halichondrins in yet another New Zealand sponge, the black shallow-water Axinellid, *Raspalia agminata*.[12] The finding of the same compounds in such geographically and biologically diverse series strongly suggests that these compounds are less likely to be biosynthesised *de novo* by the sponge cells and more likely to originate from a sponge symbiont. Considerable attention has been focused recently on sponge symbionts and the role they play in the production of many of the so-called sponge metabolites.[13] We are currently examining samples of the *Lissodendoryx* sp. for symbionts and have undertaken a series of cellular dissociation experiments in an attempt to determine the possible origin of the halichondrins.[14]

One notable difference between the other halichondrin-containing sponges and the *Lissodendoryx* sp. is the relative yield of the halichondrins. The yield from the New Zealand sponge is >10x more than that noted for the other sponges. Notwithstanding, the halichondrins are another example of a rare resource with potential as an anticancer agent. Despite the successful synthesis[15] and partial syntheses[16] of the halichondrin skeleton the most obvious source of the compound in the near future is from natural sources. *Lissodendoryx* sp. is found in a challenging environment at only one site in New Zealand. To protect this resource and establish an alternative source of supply, intensive studies on the aquaculture of this sponge have been initiated.[17]

Acknowledgments: Especial thanks to Dr M.R. Boyd, National Cancer Institute (NCI/DTP/LDDRD). To PharmaMar SA, Harbor Branch Oceanographic Institution/SeaPharm Project, University of Canterbury, New Zealand Universities Grants Committee, Canterbury Medical Research Foundation, New Zealand Lotteries Board (Scientific), Cancer Society of New Zealand our very grateful thanks for funding, past and present. We also wish to thank the following: Ms G. Barns & Mr R. Thow, Chemistry Department, University of Canterbury, Mr M.J. Page, NIWA, Wellington, Mr C. Spiers, Captain of the RV "Munida" & Dr D.J. Newman, Natural Products Branch, NCI, Frederick, USA.

References and Notes

1. Munro, M.H.G.; Blunt, J.W.; Barns, G.; Battershill, C.N.; Lake, R.J.; Perry, N.B. *Pure Appl. Chem.*, 1989, *61*, 529-34.
2. Isohomohalichondrin B obtained as a white amorphous powder; no UV maximum above 210 nm ; IR (NaCl pellet) 3450, 1732, 1695 (sh), 1648 cm^{-1} (hydroxyls, a lactone and an aliphatic ketone). LRFABMS showed MNa^+ and MK^+ at m/z 1145 and 1161. HREIMS; observed m/z 1161.5395. (MK^+, calc: 1161.53999; error - 0.4 ppm). $[\alpha]_D$ -21.6° (c = 1.02, MeOH). Nmr spectra obtained on a Varian VXR 500 spectrometer operating at 500 MHz for ^1H and 126 MHz for ^{13}C. ^{13}C nmr (CDCl3) δ C1, 171.1; C2, 40.4; C3, 73.6; C4, 30.6; C5, 30.0; C6, 68.2; C7, 77.6; C8, 74.3; C9, 73.8; C10, 76.5; C11, 82.1; C12, 81.0; C13, 48.3; C14, 110.0; C15, 34.4; C16, 28.1; C17, 75.3; C18, 38.7; C19, 151.7; C19=CH_2, 104.4; C20, 75.3; C21, 29.3; C22, 32.0; C23, 74.8; C24, 43.3; C25, 35.9; C25-CH_3, 18.0; C26, 151.5; C26=CH_2, 104.1; C27, 73.5; C28, 36.9; C29, 71.1; C30, 77.2; C31, 36.5; C31-CH_3, 15.0; C32, 77.5; C33, 66.4; C34, 29.0; C35, 75.1; C36, 76.2; C37, 43.3; C38, 112.4; C39, 42.5; C40, 71.2; C41, 79.0; C42, 25.6; C42-CH_3, 17.5; C43, 36.9; C44, 97.2; C45, 37.2; C46, 28.6; C46-CH_3, 16.9; C47, 75.9; C48, 66.4; C49, 34.4; C50, 66.4; C51, 76.4; C52, 45.2; C53, 209.5; C54, 46.1; C55, 57.8 ppm. ^1H nmr (CDCl3) δ (position, mult) H2, 2.36, (*J*=16.8); H2, 2.61 (dd, *J*=16.8, 8.1); H3, 3.89 (*J*=8.1); H4, 1.75 (m); H4, 1.38 (m); H5, 1.41 (m); H5, 2.12 (m); H6, 4.35 (bm); H7, 2.95 (*J*=10.6); H8, 4.33 (*J*=3.9); H9, 4.06 (dd, *J*=6.4, 3.9); H10, 4.22 (dd, *J*=6.4, 4.5); H11, 4.60 (t, *J*=4.5, 4.5); H12, 4.70 (dd, *J*=4.5, 4.75); H13, 1.95 (dd, *J*=13.1, 4.75); H13, 2.16 (*J*=13.1); H15, 2.18* (m); H15, 1.62* (m); H16, 2.16* (m); H16, 1.42* (m); H17, 4.10 (m); H18, 2.27 (m); H18, 2.80 (m); C19=CH_2, 5.01 (brs, *J*=1.7); C19=CH_2, 4.93 (brs, *J*=1.7); H20, 4.39 (m); H21, 1.42 (m); H21, 1.90 (m); H22, 1.62 (m); H22, 1.62 (m); H23, 3.55 (m); H24, 1.05 (m); H24, 1.72 (m); H25, 2.23 (m); C25-CH_3, 1.07 (d, *J*=6.4); C26=CH_2, 4.83 (brs; C26=CH_2, 4.78 (brs); H27, 3.56 (m); H28, 2.01 (m); H28, 1.95 (m); H29, 4.22 (m); H30, 4.66 (m); H31, 2.03 (m); C31-CH_3, 1.00 (d, *J*=6.7); H32, 3.20 (dd, *J*=8.1, 6.15); H33, 3.84 (m); H34, 1.81 (m); H34, 2.16 (m); H35, 4.12 (m); H36, 4.12 (m); H37, 2.37 (m); H37, 1.92 (m); H39, 2.22 (m); H39, 2.22 (m); H40, 3.94 (m); H41, 3.64 (m); H42, 2.29 (m); C42-CH_3, 0.95 (d, *J*=7.0); H43, 1.52* (m); H43, 1.33* (m); H45, 1.49 (m); H45, 1.52 (t, *J*=12.7, 12.7); H46, 2.18 (m); C46-CH_3, 0.90 (d, *J*=6.9); H47, 3.23 (d, *J*=2.3); H48, 3.75 (brs); H49, 1.84 (d, *J*=3.2); H49, 2.13 (d, *J*=13.2); H50, 3.52 (s); H51, 3.82 (dd, *J*=5.0, 8.0); H52, 2.62 (dd, *J*=5.0, 15.7); H52, 2.93 (dd, *J*=8.0, 15.7; H54/H54, 2.73 (t, *J*=5.4, 5.4); H55/H55, 3.86 (t, *J*=5.4, 5.4) ppm (* Assignments are tentative)
3. (a) Uemura, D.; Takahashi, K.; Yamamoto, T.; Katayama, C.; Tanaka, J.; Okumura, Y.; Hirata, Y. *J. Amer. Chem. Soc.*, 1985, *107*, 4796 -98. (b) Hirata, Y.; Uemura, D. *Pure Appl. Chem.*, 1986, *58*, 701-710.
4. Pettit, G.R.; Herald, C.L.; Boyd, M.R.; Leet, J.E.; Dufresne, C.; Doubek, D.L.; Schmidt, J.M.; Cerny, R.L.; Hooper, J.N.A.; Rutzler, K.C. *J. Med. Chem.* 1991, *34*, 3339-40.
5. Pettit, G.R.; Tan, R.; Gao, F.; Williams, M.D.; Doubek, D.L.; Boyd, M.R.; Schmidt, J.M.; Chapuis, J-C.; Hamel, E.; Bai, R.; Hooper, J.N.A.; Tackett, L.P. *J. Org. Chem.*, 1993, *58*, 2538-43.
6. Bai, R.; Paull, K.D.; Herald, C.L.; Malspeis, L.; Pettit, G.R.; Hamel, E. *J. Biol Chem.*, 1991, *266*, 15882-89.
7. Minutes, NCI Decision Network Committee, March 23, 1992.
8. The A family of the halichondrins is characterised by a 12β, 13β diol sytem; the C family are 12β hydroxy only.
9. (a) Boyd, M.R. in: "Principles & Practise of Oncology." Ed. by DeVita,V.T. Jr.; Hellman, S.; Rosenberg, S.A. Lippincott Co: Philadelphia, PA, 1989, Vol. 3, No 10, pp 1-12. (b) Monks, A.; Scuderio, D.A.;. Skehan, P; Shoemaker, R.H.; Paull, K.D.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Grey-Goodrich, M.; Campbell, H.; Boyd, M.R. *J. Natl. Cancer Inst.*, 83, 757 (1991). (c) Boyd M.R. in: "Current Therapy in Oncology." Ed. by Neiderhuber, J.E.; Decker, B.C. Philadelphia, 1992, pp 11-22.
10. Boyd, M.R.; Paul, K.D.; Rubenstein, L.R. in: "Antitumor Drug Discovery and Development." Ed. by Valeriote, F.A.; Corbett T.; Baker, L. Kluwer Academic Press, Amsterdam, 1992, pp 11-34.
11. Kellam, S.J.; Blunt, J.W.; Munro, M.H.G.; Perry, N.B. *J. Nat. Prod.* in press.
12. Internal research report, Dr R.J. Lake, Post-Doctoral Fellow, Marine Chemistry Group, University of Canterbury, 1988.
13. Garson, M.J.; Zimmermann, M.P.; Battershill, C.N.; Holden, J.L.; Murphy, P.T. *Lipids*, in press.
14. With Dr M.J. Garson, University of Queesland, Brisbane, Australia and Dr C.N. Battershill, NIWA, Wellington, New Zealand.
15. Aicher, T.D.; Buszek, K.R.; Fang, F.G. Forsyth, C.J.; Jung, S.H.; Kishi, Y.; Matelich, M.C.; Scola, P.M.; Spero, D.M.; Yoon, S.K. *J. Amer. Chem. Soc.* 1992, *114* , 3162-3164.
16. (a) Burke, S.D.; Buchanan, J.L.; Rovin, J.D. *Tetrahedron Lett.* 1991, *32*, 3961-3964. (b) Difranco, E.; Ravikumar, V.T.; Salomon, R.G. *Tetrahedron Lett.* 1993, *34*, 3247-3250. (c) Duan, J.J-W.; Kishi, Y. *Tetrahedron Lett.* 1993, *34*, 7541-7544. (d) Cooper, A.J.; Pan, W.X.; Salomon, R.G. *Tetrahedron Lett.* 1993, *34*, 8193-8196. (e) Horita, K.; Hachiya, S.; Nagasawa, M.; Hikota, M.; Yonemitsu, O. *Synlett.* 1994, 38-40. (f) Horita, K.; Hachiya, S.; Nagasawa, M.; Hikota, M.; Yonemitsu, O. *Synlett.* 1994, 40-43. (g) Horita, K.; Sakurai, Y.; Nagasawa, M.; Hachiya, S.; Yonemitsu, O. *Synlett.* 1994, 43-45. (h) Horita, K.; Sakurai, Y.; Nagasawa, M.; Maeno, K.; Hachiya, S.; Yonemitsu, O. *Synlett.* 1994, 46-48. (i) Burke, S.D.; Jung, K.W.; Phillips, J.R.; Perri, R.E. *Tetrahedron Lett.* 1994, *35*, 703-706.
17. Dr C.N.Battershill, NIWA, Wellington, NEW ZEALAND.

(*Received in UK* 22 *August* 1994; *accepted* 14 *October* 1994)