Pergamon

*Tetrahedron Letters*, Vol. 35, No. 28, pp. 5013-5014, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01013-7

# Halichomycin, a New Class of Potent Cytotoxic Macrolide Produced by an Actinomycete from a Marine Fish[1]

**Chika Takahashi, Tamie Takada, Takeshi Yamada, Katsuhiko Minoura, Kenshiro Uchida,[a] Eiko Matsumura and Atsushi Numata***

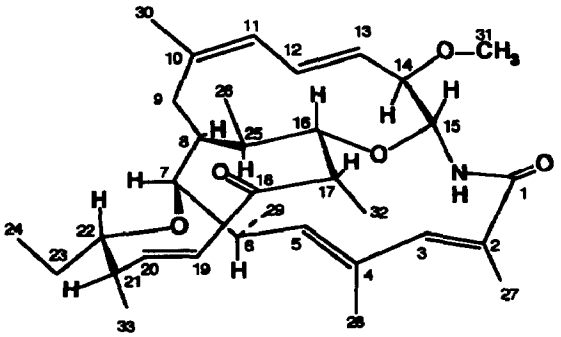
Osaka University of Pharmaceutical Sciences, Matsubara, Osaka 580, Japan
and [a]Bruker Japan Co., Ltd., Ninomiya, Tsukuba, Ibaraki 305, Japan

***Abstract*** : Halichomycin, produced by a strain of *Streptomyces hygroscopicus* from the marine fish *Halichoeres bleekeri*, is a novel class of macrolide with potent cytotoxicity against tumour cells in culture.

In our continuing search for antitumour and/or cytotoxic metabolites from microorganisms inhabiting the marine environment,[2] we have found that a structurally unique and potent cytotoxic macrolide (**1**), designated halichomycin, is produced by a strain of *Streptomyces hygroscopicus* which was isolated from the gastrointestinal tract of the marine fish *Halichoeres bleekeri* .

The producing microorganism was cultured at 27℃ for a week in a medium (20 l) containing 0.1% casein and 1% starch in artificial seawater adjusted to pH 7.5. The AcOEt extract of the culture filtrate was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and reverse phased HPLC to afford halichomycin(**1**)(4 mg) as an oily material.

Halichomycin (**1**) was assigned a molecular formula of $C_{33}H_{49}NO_5$ as deduced from HREIMS. A close inspection of the $^1H$ and $^{13}C$ NMR spectral data[3] of **1** by DEPT and $^1H$–$^{13}C$ COSY experiments revealed the presence of an amide, a conjugated ketone, five double bonds, a methoxy, an ethyl, seven methyls including three allylic methyls, a methylene, and ten $sp^3$ methines including five methines linked to an oxgen atom, one of which additionally bonded to a nitrogen atom.



**1** (relative stereochemistry)

The $^{1}H$-$^{1}H$ COSY analysis of **1** led to partial structures A (C2 to C15 and C27 to C30), B (C19 to C24 and C33) and C (C16, C17, C25, C26 and C32), which were supported by HMBC correlations. The geometry of the double bonds was deduced from chemical shifts of $^{13}C$ NMR signals of allylic methyls,[3,4] coupling constants of olefinic protons,[3] and NOEs between H3 and H5, H11 and H13, and H11 and H30. The connection of the partial structures (A to C) and the remaining functional groups (amide, ketone and methoxy functionalities) was determined on the basis of HMBC correlations. The typical correlations are as follows; H27 to C1 and C3, H31 to C14, H26 to C8 and C16, H15 to C1 and C16, H32 to C18, and H20 to C18. Based on this evidence, the planar structure of **1** was elucidated.

The relative stereochemistry for **1** was established by NOESY experiments. The main NOEs were observed between the following protons in addition to ones mentioned above; H3-H14, H5-H8, H6-H28, H7-H24 and H29, H9-H26, H12-H5, H8 and H14, H15-H13, H16, H17 and H31, H19-H32 and H33, and H22-H20. This evidence led to relative stereostructure **1** with the 6S*, 7R*, 8S*, 14R*, 15R*, 16S*, 17S*, 21S*, 22S* and 25S* configurations for halichomycin.

Halichomycin (**1**) exhibited potent cytotoxicity ($ED_{50}$ 0.13 μg/ml) in the P-388 lymphocytic leukemia test system in cell culture.[5]

**Acknowledgements:** We thank Dr. T. Hasegawa, Institute for Fermentation Osaka, for the actinomycete indentification and Mrs. M. Fujitake, this university, for MS measurements.

**References and Note**

1. This paper is dedicated to Professor Yoshifumi Maki on this occasion of his retirement from Gifu Pharmaceutical University in March 1994.
2. (a) A. Numata, C. Takahashi, T. Matsushita, T. Miyamoto, K. Kawai, Y. Usami, E. Matsumura, M. Inoue, H. Ohishi and T. Shingu, *Tetrahedron Lett.*, **33**, 1621 (1992); (b) A. Numata, C. Takahashi, Y. Ito, T. Takada, K. Kawai, Y. Usami, E.Matsumura, M. Imachi, T. Ito and T. Hasegawa, *Tetrahedron Lett.*, **34**, 2355 (1993).
3. Data for **1** : $[\alpha]^{20}$ + 3.8 ° (*c* 1.2, $CHCl_3$) ; UV λ max (EtOH) (log ε) nm 242.4 (4.31), 275.2 (3.88); IR υ max (liquid) $cm^{-1}$ 3445, 1693, 1674, 1622; $^{1}H$ NMR (300 MHz, $CDCl_3$) δ 7.22 (s, H3), 5.83 (d, $J_{5,6}$ 9.5 Hz, H5), 2.55 (dqd, $J_{6,5}$ 9.5, $J_{6,29}$ 7.2, $J_{6,7}$ 2.0Hz,H6), 3.30 (dd, $J_{7,8}$ 6.0, $J_{7,6}$ 2.0 Hz,H7), 1.94 (m, H8), 1.99 (m, H9), 2.13 (br d, $J_{9,9}$ 12.5 Hz, H9), 5.83 (d, $J_{11,12}$ 10.8 Hz, H11), 6.48 (dd, $J_{12,13}$ 15.0, $J_{12,11}$ 10.8 Hz, H12), 5.18 (ddd, $J_{13,12}$ 15.0, $J_{13,14}$ 9.2, $J_{13,15}$ 0.8 Hz, H13), 3.81 (dd, $J_{14,13}$ 9.2, $J_{14,15}$ 8.0 Hz, H14), 5.01 (dd, $J_{15,14}$ 8.0, $J_{15,13}$ 0.8 Hz, H15), 3.73 (dd, $J_{16,25}$ 8.4, $J_{16,17}$ 4.5 Hz,H16), 2.97 (qd, $J_{17,32}$ 7.2, $J_{17,16}$ 4.5 Hz, H17), 6.27 (d, $J_{19,20}$ 15.0 Hz, H19), 6.83 (dd, $J_{20,19}$ 15.0, $J_{20,21}$ 8.0 Hz, H20), 2.43 (ddq, $J_{21,22}$ 9.0, $J_{21,20}$ 8.0, $J_{21,33}$ 7.2 Hz, H21), 3.49 (dt, $J_{22,21}$ 9.0, $J_{22,23}$ 4.5 Hz, H22), 1.36 (m, H23), 1.54 (m, H23), 0.96 (t, $J_{24,23}$ 7.2 Hz, H24), 2.02 (m, H25), 0.95 (d, $J_{26,25}$ 7.2 Hz, H26), 2.06 (s, H27), 1.97 (s, H28), 1.08 (d, $J_{29,6}$ 7.2 Hz, H29), 1.92 (s, H30), 3.21 s, H31), 1.21 (d, $J_{32,17}$ 7.2 Hz, H32), 1.09 (d, $J_{33,21}$ 7.2 Hz, H33); $^{13}C$ NMR (75.4 MHz, $CDCl_3$) δ171.5 (s, C1), 122.5 (s, C2), 146.4 (d, C3), 134.3 (s, C4), 144.1 (d, C5), 36.8 (d, C6), 81.3 (d, C7), 39.6 (d, C8), 41.2 (t, C9), 142.5 (s, C10), 125.3 (d, C11), 132.4 (d, C12), 127.3 (d, C13), 83.8 (d, C14), 75.8 (d, C15), 72.8 (d, C16), 46.6 (d, C17), 203.3 (s, C18), 128.8 (d, C19), 149.1 (d, C20), 42.4 (d, C21), 76.0 (d, C22), 27.3 (t, C23), 10.8 (q, C24), 38.9 (d, C25), 21.6 (q, C26), 13.9 (q, C27), 15.3 (q, C28), 17.6 (q, C29), 20.0 (q, C30), 55.7 (q, C31), 10.4 (q, C32), 14.0 (q, C33); HR EIMS *m/z* 539.3606 ($M^+$), Δ -0.1 mmu.
4. G. Englert, *Helv. Chim. Acta*, **58**, 2367 (1975).
5. A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae and E. Fujita, *Chem. Pharm. Bull.*, **37**, 648 (1989).

(*Received in Japan* 28 *March* 1994; *accepted* 13 *May* 1994)