Tetrahedron Letters,Vol.27,No.18,pp 1979-1982,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain

HORMOTHAMNIONE, A NOVEL CYTOTOXIC STYRYLCHROMONE FROM THE MARINE CYANOPHYTE HORMOTHAMNION ENTEROMORPHOIDES GRUNOW

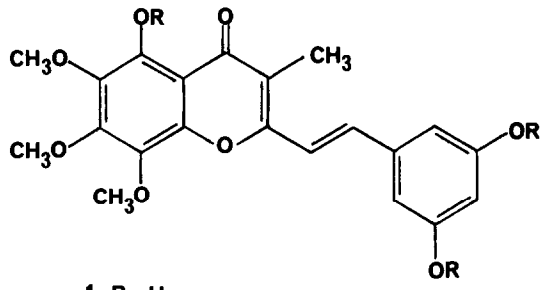
William H. Gerwick,*[a] Albert Lopez,[a] Gregory D. Van Duyne,[b] Jon Clardy,[b]
Wanda Ortiz,[c] and Adriana Baez[c]

[a]College of Pharmacy, Oregon State University, Corvallis, Oregon 97331,
[b]Department of Chemistry - Baker Laboratory, Cornell University, Ithaca, NY 14853,
[c]Department of Pharmacology, University of Puerto Rico, School of Medicine,
San Juan, Puerto Rico 00936

Abstract: The first naturally occurring styrylchromone, hormothamnione, was isolated from the marine cyanophyte Hormothamnion enteromorphoides and its structure determined by an x-ray experiment on its triacetate derivative. Hormothamnione is an exceptionally potent cytotoxin to cancer cells in vitro and appears to be a selective inhibitor of RNA synthesis.

Blue green algae have recently received considerable attention by academic researchers,[1,2] the National Cancer Institute[3] and industry[4] as a new source of novel bioactive natural products. We have therefore collected several cyanophytes as part of a survey of algal chemistry from the north coast of Puerto Rico and we report here the structure of a novel cytotoxin from one of these, Hormothamnion enteromorphoides. A $CHCl_3$/MeOH extract of fresh H. enteromorphoides demonstrated slight gram positive antimicrobial activity and showed the presence of a distinctive orange-charring (H_2SO_4) yellow pigment by thin layer chromatography.[5] The readily isolable pigment band contained a subtle mixture of several compounds, the major of which, 1, was isolated by careful reverse phased HPLC and its novel styrylchromone structure solved by x-ray crystallographic means. Trivially named hormothamnione, metabolite 1 is a potent cytotoxin to several human cancerous cell lines and appears to operate via inhibition of RNA synthesis (Table 1).

Senescent yellow-colored H. enteromorphoides was found in abundance on an exposed shallow reef (1-3m) at Playa de Vega Baja, Puerto Rico several times during the months of July and August in 1984 and 1985. The frozen or alcohol preserved seaweed was extracted in standard fashion ($CHCl_3$/MeOH 2:1) and the yellow pigment band containing 1 was isolated by vacuum chromatography over TLC grade silica gel. Preparative thick layer chromatography of this yellow band simplified the mixture to two components of 75:25 ratio. Small quantities of 1 were obtained from this mixture employing painstaking reverse phased HPLC conditions.[6]



1 R = H

2 R = $\overset{\overset{O}{\|}}{C}CH_3$

The major component (1) was more conveniently isolated from this mixture by first derivatizing to 2 (Ac_2O, pyr, 16 hrs, RT) and then employing normal phased HPLC conditions.[7] The natural product 1 was easily reformed (as determined by TLC, MS, NMR) via mild base treatment (K_2CO_3 in MeOH) of 2.

The natural product (1) was a yellow solid (m.p. 270°C dec) which analyzed for $C_{21}H_{20}O_8$ by high resolution election impact mass spectrometry (M^+ M/Z 400.1163, 46%) which upon derivatization to 2 increased in mass to $C_{27}H_{26}O_{11}$ (M^+ = base peak at m/Z 526 by low resolution CIMS in the negative ion mode) and thus, 2 was the triacetate derivative of 1. The natural product had typical chromone[8] UV absorptions at 295 and 353 nm (MeOH; ε=6,200; 8,900 respectively) and IR absorptions (KBr) broadly centered at 3400 cm^{-1} (phenol) and at 1640 cm^{-1} (pyrone carbonyl). The 1H NMR (80 MHz, $CDCl_3$) of 2 confirmed its triacetate nature [δ 2.34 (6H,s) and 2.50 (3H,s)].

Of the remaining seventeen hydrogens in the 1H NMR spectrum of 2, twelve were present as methyl singlets, three at shifts consistent with aromatic methoxyls [δ 3.89 (3H,s), 4.06 (3H,s) and 4.11 (3H,s)] and one consistent with a deshielded olefinic methyl group [δ 2.17 (3H,s)]. The other 5 protons were separated into two deshielded spin systems, one consistent with a trans disubstituted olefin [δ 7.08 (1H,d,J=16) and δ 7.58 (1H,d,J=16)] and the other interpreted as forming the proton component of a symmetrical 1,3,5 trisubstituted aromatic ring [δ 6.94 (1H,t,J=1.9) and 7.23 (2H,d,J=1.9)]. The nature of the ring system and placement of these proton, methyl, methoxy and acetoxy substituents was not readily determinable, and more definitive NMR experiments were precluded due to limited resources of compound. Hence, an x-ray experiment was performed to unambiguously assign the structure of crystalline 2 (mp 198-201°C).

Preliminary x-ray photographs of the triacetate 2 displayed monoclinic symmetry and accurate lattice constants of a=18.472(5), b=5.769(3), c=24.096(6) Å, and β=93.40(2)° were determined from a least squares analysis of fifteen moderate, diffractometer measured 2θ-values. The systematic extinctions and crystal density indicated space group $P2_1/a$ with one molecule of composition $C_{27}H_{26}O_{11}$ forming the asymmetric unit (Z=4). All diffraction maxima with $2\theta \leq 114°$ were measured on a computer controlled four-circle diffractometer with graphite monochromated Cu Kα radiation (1.54178 Å) and variable speed, 1° ω-scans. Of the

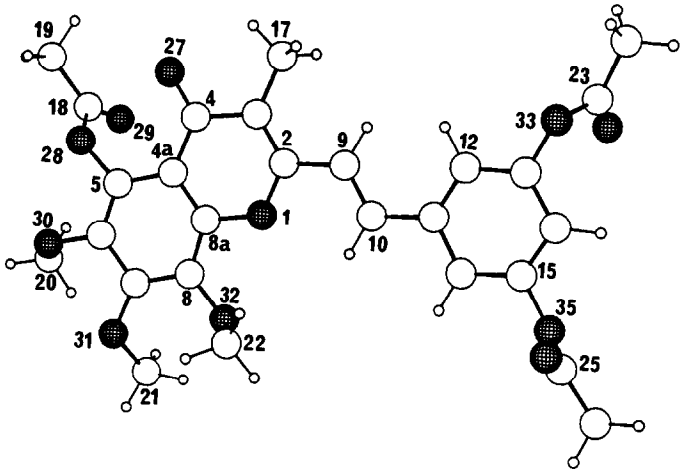


Figure 1. A computer generated perspective drawing of the final x-ray model of hormothamnione triacetate (2). The chromone fragment (atoms O1 to C8a) is planar, and the substituents on the fully substituted aromatic ring are all rotated out of the plane. The relevant torsional angles vary from 50° for C8-C7-O31-C21 to 87° for C7-C8-O32-C22. The acetate substituents on the 1,3,5-substituted ring are also rotated by roughly 77°.

3459 reflections surveyed, only 1211 (35%) were judged observed ($F_o \geq 3\sigma(F_o)$) after correction for Lorentz, polarization and background effects.[9] The centrosymmetric structure was solved easily. Hydrogen atoms were located on a ΔF-synthesis after partial refinement of the nonhydrogen atoms. Block diagonal least-squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.081 for the observed reflections. Crystallographic parameters have been deposited with and are available from the Cambridge Crystallographic Data File, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, England.

The styrylchromone structure of hormothamnione is unprecedented in natural products although the carbon skeleton is known from synthetic studies.[10] It is equally plausible that hormothamnione is biogenetically derived from acetate or shikimate pathways, though the oxidations at C-13 and C-15 are inconsistent with the expected oxidation patterns resulting from either sequence.

As indicated in Table I, the natural product 1 was found to be a potent cytotoxic agent to P388 lymphocytic leukemia and HL-60 human promyelocytic leukemia cell lines. Macromolecule biosynthesis in the presence of 1 was measured via radioactive precursor incorporation studies using HL-60 and KB cell lines (Table I). A major mode of cytotoxic action of hormothamnione appears to be by inhibition of RNA synthesis. Further studies on the site of cytotoxic action of this novel marine natural product are underway.[11]

Table I. Inhibition of Cell Growth and Macromolecule Biosynthesis in Several Cancerous Human Cell Lines by Hormothamnione (1)

Cell-line	ID_{50} (ng/ml)[a] Cell Growth	ID_{50} (ng/ml)[bc] DNA	ID_{50} (ng/ml)[bd] RNA	ID_{50} (ng/ml)[be] Protein
P-388	4.6	---	---	---
HL-60	0.1	300	1.0	> 500
KB	---	530	140	> 500

[a]ID_{50} is the drug concentration required to inhibit cell growth to 50% of control levels.
[b]The percentages of macromolecule biosynthesis inhibition were calculated from the specific activities of incorporated precursors (c-e) into 10^6 cells in drug treated as versus control cells. ID_{50} values were obtained by plotting the logarithmic drug concentrations against the percentage inhibition of macromolecule biosynthesis.
[c]Exponentially growing cells pulse labeled with 1 μCi of ^{3}H-thymidine (16.6 Ci/mmole) per mL of cell suspension.
[d]Exponentially growing cells pulse labeled with 2 μCi [5-^{3}H]-uridine (28 Ci/mmole) per mL of cell suspension.
[e]Exponentially growing cells pulse labeled with 1 μCi of ^{3}H-L-leucine (130 Ci/mmole) per mL of cell suspension.

References

(1) Carter, D.C., Moore, R.E., Mynderse, J.S., Niemczura W.P. and Todd, J.S. J. Org. Chem. 1984, 49, 236-244.
(2)(a) Moore, R.E., Cheuk, C. and Patterson, G.M.L. J. Am. Chem. Soc. 1984 106, 6456-6457.
(b) Barchi, J.J. Jr., Moore, R.E. and Patterson G.M.L. J. Am. Chem. Soc. 1984, 106, 8193-8197.
(3)(a) Cragg, G.M. and Suffness, M. Am. Soc. Pharmacog. Proceedings 1985, abs. no. 185.
(b) NIH RFP #NCI-CM-57745-16 "Cultivation of Blue-Green Algae" to begin August 1986.
(4) 1984 Prospectus, Sea Pharm, Inc., Ft. Pierce, Florida.
(5) Paper disc-agar plate method, using 100 μg and 25 μg of 1 per 6 mm paper disc, tested against Staphylococcus aureus (-), Bacillus subtilus (10 mm zone at 100 μg, 8 mm zone at 25 μg), Escherichia coli (-) and Candida albicans (-). Hormothamnione was inactive in our goldfish toxicity experiments (25 μg/ml H_2O).
(6) Two Waters 3.9 mm x 25 cm μ-Bondapak C-18 columns in series, 40% H_2O/MeOH as mobile phase, 1.5 ml min, 35 min retention time, 0.5 mg per injection.
(7) Waters 8 mm x 10 cm μ-Porasil Radial Compression Column, 40% EtOAc/isooctane, 3.0 ml/min, 7 min retention time, 1 mg/injection.
(8) Ellis, G.P. "Chromone and its Benzo Derivatives" in Heterocyclic Compounds: Chromenes, Chromanones and Chromones, G.P. Ellis, ed., John Wiley and Sons, New York, 1977; pp. 557-580.
(9) All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M.E. Leonowicz, Cornell University, 1978; MULTAN 78, MULTAN 80, and RANTAN 80, systems of computer programs for the automatic solution of crystal structures from x-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S.E. Hull, L. Lessinger, G. Germain, J.P. Declercq and M.M. Woolfson, University of York, England, 1978 and 1980; BLS78A, an anisotropic block diagonal least squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a crystallographic illustration program by W.D.S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.
(10) Ellis, G.P. "Alkylchromones" in Heterocyclic Compounds: Chromenes, Chromanones and Chromones, G.P. Ellis, ed., John Wiley and Sons, New York, 1977; pp. 581-631.

(11) Our gratitude goes to Dr. David Ballantine for taxonomic identifications of Hormothamnion enteromorphoides. Work at Cornell University was supported by NIH CA24487 and the New York State Sea Grant Program. We acknowledge at Oregon State University the mass spectral facilities in the Department of Agricultural Chemistry run by Dr. Max Deinzer and the NMR facility in the Department of Chemistry. Collecting efforts in Puerto Rico were supported by the Puerto Rico Sea Grant Program while work conducted at Oregon State University was supported by the Oregon Sea Grant Program and the OSU Research Council.

(Received in USA 24 January 1986)