Journal of Natural Products
Vol. 52, No. 2, pp. 252-256, Mar-Apr 1989

6-DESMETHOXYHORMOTHAMNIONE, A NEW CYTOTOXIC STYRYLCHROMONE FROM THE MARINE CRYPTOPHYTE *CHRYSOPHAEUM TAYLORI*

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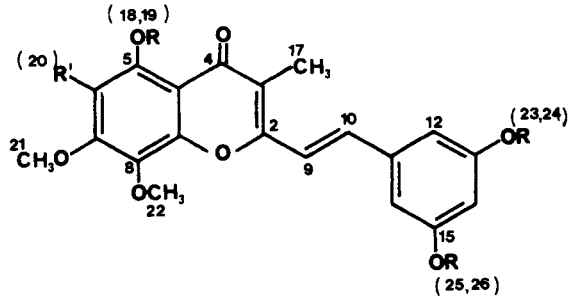
ABSTRACT.—Our previous chemical investigations of a yellow marine alga from Puerto Rico, tentatively identified as the tuft-forming cyanobacterium *Hormothamnion enteromorphoides*, led to the isolation of a structurally novel cytotoxic styrylchromone natural product, hormothamnione [**2**]. Continued chemical work with this organism has resulted in the isolation and spectroscopic structure elucidation of a closely related styrylchromone, 6-desmethoxyhormothamnione [**1**], which is also cytotoxic to cancer cells. The substitution pattern of proton, hydroxyl, and methoxyl groups on the chromone ring was established by long range ^{1}H-^{13}C heteronuclear correlation spectroscopy. The taxonomy of the source organism for these styrylchromone natural products, **1** and **2**, is revised to the marine cryptophyte *Chrysophaeum taylori* as a result of detailed microscopic and cultural efforts.

As part of an ongoing project to evaluate the biomedicinal potential of marine organisms from the environs of Puerto Rico (1), we previously identified the crude lipid extract of a "yellow slime" from a small bay on the North Coast as containing a complex mixture of structurally unique styrylchromone natural products (2). We erroneously identified this material as the cyanobacterium *Hormothamnion enteromorphoides* in a senescent stage. Subsequently, by light microscopy with fresh and preserved materials, culture work, and comparison with the original descriptions for this organism (3), we have revised the taxonomy of this source material to the marine Cryptophycean alga *Chrysophaeum taylori* Lewis and Bryan. [We have since found that the emerald green cyanobacterium, *Hormothamnion enteromorphoides*, also collected from Puerto Rico, is a rich source of cytotoxic cyclic peptides (4).] Upon continued chemical investigation of this eukaryotic alga, we have isolated a new styrylchromone **1** closely related to hormothamnione [**2**] (3), which we previously described and which was recently synthesized (5,6). The structure of this new compound was deduced by comparisons of spectroscopic features with the parent metabolite, hormothamnione, as well as several 2D nmr experiments (^{1}H-^{13}C HETCOR and ^{1}H-^{13}C LR HETCOSY).

Since June 1984, we have made nearly a dozen collections of a slimy, yellow material (up to 10 cm) found in variable abundance on a limestone rock reef in a small bay at Vega Baja, Puerto Rico. The cellular structure of this material does not preserve well by



1 R=R′=H
2 R=H, R′=OMe
3 R=OAc, R′=H
4 R=OAc, R=OMe

storage either frozen or in EtOH or 5% formalin/seawater. Further, fresh material placed either in liquid seawater media or on seawater Petri dishes rapidly changes pigmentation, changing from a sulfur yellow to a rusty brown, and also loses any recognizable multicellular structure. Light microscopic examination of any of these preservations shows mainly translucent "sheath" material and numerous small "granules" similar to simple cytoplasmic debris. However, by employing light microscopy on fresh material in the field (<2 h old) the cellular nature of this organism was clearly observed. The small "blades" or "slime steamers" were in fact a loose network of stalked cells, teardrop in shape with a large vacuole, that frequently undergo slow contractile movements followed by rapid expansions. Subsequently, we have found that storage in glutaraldehyde preserves intact a modest proportion of these delicate cells. Comparisons of size, shape, cellular anatomy, color, movement, habitat, and propensity to "brown" upon collection all match closely the original descriptions for the Cryptophycean alga *C. taylori* (3).

Si gel vacuum chromatography of the $CHCl_3$/MeOH extract from a large collection (July 1984) of *C. taylori* gave a yellow pigment band that was a mixture of at least two compounds (**1** and **2**). The major of these, hormothamnione [**2**], could be separated from the mixture in small quantities by analytical reversed-phase hplc or in larger quantities as the triacetate **4**, by converting this mixture to the corresponding peracetates and then separating using normal-phase hplc. A second and more minor peak from hplc of the peracetates was principally derivative **3**, and upon concentration, this derivative crystallized in pure form from a mixture of $CHCl_3$-EtOAc-isooctane (1:0.9:2.1).

This crystalline derivative (mp 184–186°) gave a parent ion by hreims which afforded a molecular formula of $C_{26}H_{24}O_{10}$ (15 degrees of unsaturation). This differed from the molecular formula for the peracetate of hormothamnione only by the elements $-OCH_2-$. Further, pure **3** displayed absorption bands in its ir, uv, and nmr spectra of close similarity to those of hormothamnione triacetate [**4**]. Specifically, a 2H doublet at δ 7.20 was coupled to a 1H triplet at δ 6.93, suggestive of a C-2 axis symmetrical 1,3,5-trisubstituted aromatic compound. Two overlapping acetate methyl groups at δ 2.32 were identical to those observed in **4** and suggested that atoms 11–16 in derivative **3** were the same, including substituents, as in derivative **4**. In further similarity with **4**, the new derivative **3** has resonance lines describing a *trans* disubstituted olefin, a vinyl methyl, a third aromatic acetate methyl, and two aromatic methoxyl functions (Table 1). Thus, the new compound, **1**, differed from the parent compound, hormothamnione [**2**] only in that one of the A-ring methoxyl groups was replaced by an aromatic proton (Table 1) that was, predictably, a sharp singlet.

The location of this substituent change was explored by an analysis of the long range ^1H-^{13}C couplings in derivative **3**. The LR ^1H-^{13}C HETCOSY (7) spectrum was richly detailed showing a multitude of 2-, 3-, and 4-bond couplings. Interpretation of most of these, in conjunction with the ^1H-^{13}C HETCOR data, helped to confirm the structural assignments in common with hormothamnione triacetate [**4**]. However, uncertainty about the 2-, 3-, or 4-bond nature of the couplings observed between the new A-ring aromatic proton and various carbon atoms around the ring precluded firm structure assignment on the basis of these data alone. Hence, the acetate ester groups were removed from derivative **3** with K_2CO_3 in MeOH, and a LR ^1H-^{13}C HETCOSY experiment was obtained for this reformed natural product **1** in DMSO-d_6. We thought that in this solvent exchange of the hydrogen-bonded phenolic proton at C-5 would be sufficiently slow to allow visualization of long range couplings between it and various carbon atoms in the ring. Indeed, this was the case and, most notably, the carbon atom bearing the A-ring proton was coupled to this phenolic proton. Additional couplings in the reformed natural product **1** helped to establish that it was the C-6 methoxyl group of hor-

TABLE 1. Nmr Data for Styrylchromones **1**, **3**, and **4** (9.398T).

Carbon	6-Desmethoxyhormothamnione [1] (DMSO)				6-Desmethoxyhormothamnione triacetate [3] ($CDCl_3$)				Hormothamnione triacetate [4] ($CDCl_3$)		
	^1H	Multiplicities	^{13}C	Long-range couplings	^1H	Multiplicities	^{13}C	Long-range couplings	^1H	Multiplicites	^{13}C
2			157.74				155.26				155.26
3			114.41				118.25				118.37
4			181.70				176.67				176.68
4a			103.21				110.19				112.23
5			156.55				145.19				139.28[b]
6	6.52	s	95.43	C-4a,8,5	6.60	s	104.08	C-4a;7;5;8			142.78[b]
7			158.28				155.89				146.90[b]
8			127.89				134.44				137.47[b]
8a			136.65				137.85				137.79[b]
9	7.17	d,16	117.56		7.06	d,16	119.99	C-8a;11;2	7.07	d,15.7	120.02
10	7.36	d,16	137.40		7.53	d,16	134.27	C-13,16;9;2	7.52	d,15.7	134.36
11			148.11				150.39				151.26
12	6.62	bs	106.19	C-14	7.20	d,3	118.03	C-14;10;13,15	7.21	d,2.0	118.00
13			158.78				151.42				151.49
14	6.33	bs	104.55	C-13,15	6.93	t,3	116.32	C-12,16;13,15	6.94	t,2.0	116.36
15			158.78				151.42				151.49
16	6.62	bs	106.19	C-14	7.20	d,3	118.03	C-14;10;13,15	7.21	d,2.0	118.00
17	2.10	s	8.19	C-3;2,4	2.13	s	9.38	C-3,2,4	2.14	s	9.44
18	12.80	s		C-6;5			169.96				169.76
19		—			2.47	s	21.18		2.49	s	21.00
20		—							4.10[a]	s	61.58[a]
21	3.88	s	56.41	C-7	3.92	s	56.38	C-7	3.87[a]	s	61.84[a]
22	3.81	s	60.98	C-8	3.98	s	61.47	C-8	4.04[a]	s	62.02[a]
23	9.52	s		C-13,15			168.91				168.90
24		—			2.32	s	21.10		2.32	s	21.06
25	9.52	s		C-13,15			168.91				168.90
26		—			2.32	s	21.10		2.32	s	21.06

[a,b]Assignments may be interchanged.

mothamnione [**2**] that was replaced with a proton in the new natural product, 6-desmethoxyhormothamnione [**1**] (Table 1).

Pure 6-desmethoxyhormothamnione [**1**], obtained by base hydrolysis of peracetate **3**, showed good cytotoxicity to 9 KB cells (LD_{50}~1 μg/ml). Antimicrobial activity was not detected. Compound **1** was screened for antimicrobial activity to *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans* using paper discs impregnated with 100 μg of compound.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on an Aminco DW-2a UV-Vis spectrophotometer and ir on a Beckman AccuLab 7 spectrophotometer. Nmr spectra were recorded on Varian EM 360, FT-80A, and Brucker AM 400 nmr spectrometers, and all shifts are reported relative to an internal TMS standard. Low resolution mass spectra (lrms) were obtained on a Varian MAT CH7 spectrometer, while high resolution mass measurements (hrms) were obtained on a Kratos MS 50 TC. Hplc employed a Waters M-6000 pump, U6K injector and R 401 differential refractometer, and tlc used Merck aluminum-backed tlc sheets (Si gel 60 F_{254}).

COLLECTION, EXTRACTION, AND ISOLATION.—*C. taylori* was first collected from a small limestone reef at a depth of −0.5 to −2 m offshore of Playa de Vega Baja in June 1984. Subsequently, this alga was recollected several times during the summer months of 1984 through 1987 and preserved for chemical studies either frozen or in solvent (iPrOH or MeOH). A large collection of fresh *C. taylori* from July 1984 (596.1 g air-dried and extracted weight) yielded, upon $CHCl_3$/MeOH extraction, 3.59 g of a dark brown oil. Vacuum chromatography of this extract over normal phase Si gel and eluting with mixtures of Et_2O in C_6H_6 gave 8 fractions. Tlc showed that fractions 5–7 contained nearly the same mixture of a carotenoid pigment and a new uv-absorbing yellow compound which intensified in color upon acidification and charring. A portion of these fractions (46 mg) was acetylated over a 23-h period at room temperature in 3 ml of Ac_2O-pyridine (1:1). The reaction was terminated with the addition of ice and then H_2O and extracted three times with Et_2O. The combined Et_2O layers were sequentially washed with 5% HCl (2×), saturated HCO_3^- (2×), distilled H_2O (1×), and then dried over $MgSO_4$. Following filtration the solvents were removed in vacuo to yield ca. 50 mg of two major products, compounds **3** and **4**. These were initially separated employing preparative tlc [Et_2O-C_6H_6 (1:1)] and finally cleanly resolved employing hplc (8 mm × 10 cm μ-Porasil Radial Compression Column, 50% EtOAC in isooctane) to give ca. 10 mg of **3** and 35 mg of **4**.

6-DESMETHOXYHORMOTHAMNIONE TRIACETATE [**3**].—Mp 184–186°; ir ($CHCl_3$) 2990, 2960, 1770, 1620, 1510, 1440, 1415, 1395, 1372, 1345, 1310, 1270, 1190, 1150, 1130, 1090, 1030, 970 cm^{-1}; uv ($CHCl_3$) λ max 326 nm, ε = 56,800; lreims (70 eV) $[M]^+$ 496 (6.9%), 454 (87.8%), 439 (100%), 397 (17.1%); hreims *m/z* $[M]^+$ 496.13893 ($C_{26}H_{24}O_{10}$) 2.0 mamu dev.; ^1H and ^{13}C nmr see Table 1.

BASE (K_2CO_3) TREATMENT OF TRIACETATE **3** TO REFORM NATURAL PRODUCT **1**.—Compound **3** (13.9 mg, 0.028 mmol) was solubilized in 1.0 ml $CHCl_3$ and then treated with 2 ml of 5% K_2CO_3 in MeOH at room temperature with stirring. Within 0.5 h the solution was a dark orange, at which time it was neutralized with 5% HCl in MeOH and turned a light orange color. This orange color was successively extracted with small portions of $CHCl_3$ (4 × 25 ml) which were combined to give mainly pure **1** contaminated with a polar orange pigment. This contaminant was efficiently removed by washing the solid residue following in vacuo removal of solvents with small portions of MeOH and yielded 9.9 mg (0.27 mmol, 96% of pure 6-desmethoxyhormothamnione [**1**] as a light yellow crusty solid. This material was identical by tlc to the more polar compound (R_f 0.65, 20% MeOH in $CHCl_3$) in crude underivatized fractions containing a mixture of natural products **1** and **2**. Uv (MeOH) λ max = 270, 342 nm, ε = 1340, 3000, respectively; ^1H and ^{13}C nmr see Table 1.

ACKNOWLEDGMENTS

I thank Dr. A. Baez (University of Puerto Rico) for assays to 9 KB cells, Mr. R. Kohnert for nmr data [OSU Department of Chemistry's Bruker AM 400, purchased through grants from NSF (CHE-8216190) and the M.J. Murdock Charitable Trust], Ms. C. Mrozek (OSU) for light microscopy, and Dr. S.J. Gould (OSU) for spectroscopic discussions. Mr. B. Arbogast and D. Griffin helped obtain mass spectra on a Kratos MS 50 TC (OSU College of Agricultural Chemistry) purchased with grants from the NIH Division of Resources (DRR 1S10RR01409). This work was supported by NIH (CA 42850).

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Received 5 July 1988