Pergamon

Tetrahedron Letters, Vol. 37, No. 51, pp. 9131-9134, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)02157-0

Tridentatols A-C, Novel Natural Products of the Marine Hydroid *Tridentata marginata*

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Abstract: Three novel phenolic metabolites, tridentatols A-C (**1-3**), with an uncommon sulfur-containing functional group have been isolated from the marine hydroid *Tridentata marginata*. The structures of the compounds were determined by interpretation of spectral data and a single crystal X-ray diffraction study on tridentatol C. Ecological studies showed that tridentatol A deters feeding by a common hydroid predator. These metabolites also may function to protect *T. marginata* from damaging solar ultraviolet radiation. Copyright © 1996 Elsevier Science Ltd

Few secondary metabolites have been isolated from marine hydroids,[1] and no ecological roles for these few compounds have been reported. A recent study focused on predator-prey interaction among animals of the pelagic *Sargassum* communities of the western Atlantic Ocean found that among four common hydroids growing on the *Sargassum*, only *Tridentata marginata* was not eaten by the most abundant *Sargassum*-associated predator, the Planehead filefish.[2] Bioassay-guided fractionation of the DCM/MeOH extract of *T. marginata* yielded tridentatol A (**1**) as the only metabolite to significantly reduce filefish feeding.[2] Two related metabolites, tridentatols B and C (**2** and **3**), were also isolated. In this paper, we report the structures of tridentatols A-C (**1-3**, Figure).

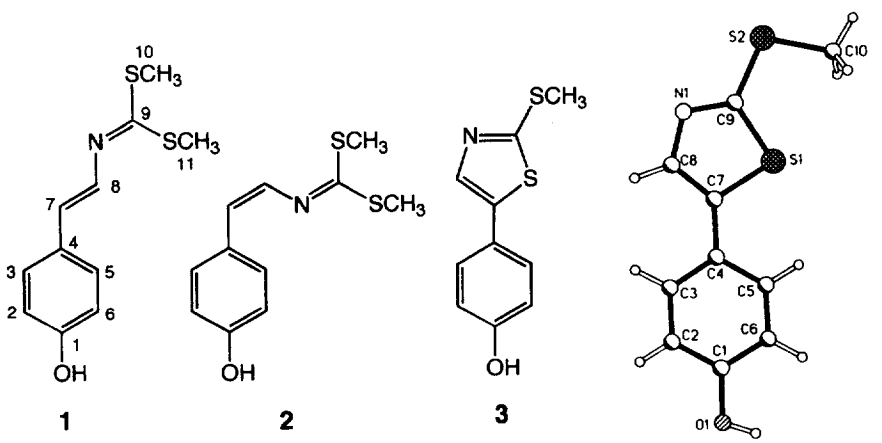

Figure. Structure of tridentatols A-C (**1-3**) and the X-ray structure of **3**.

T. marginata for our investigations was taken from floating *Sargassum* plants collected in October 1994 20-30 km offshore from Morehead City, North Carolina, USA. The tridentatols were isolated by silica thick-plate chromatography and silica HPLC (92:8 iso-octane/ethyl acetate). Concentrations of **1-3** were 7.8, 1.4, and 1.7 mg/mL of hydroid tissue, respectively. The tridentatols comprised nearly 10% of the hydroid's dry mass.

Tridentatol A (**1**) analyzed for $C_{11}H_{13}NOS_2$ by HREIMS in conjunction with ^1H and ^{13}C NMR data.[3] The high percentage of sulfur in **1** was confirmed by the intensity of the $M^+ + 2$ ion (9.5% of the M^+ ion) in the high resolution EI mass spectrum. Of the six units of unsaturation in **1**, four were attributed to a 1,4 disubstituted benzene by the characteristic ortho coupling (8.6 Hz) between the degenerate aromatic protons at δ 7.32 (2H) and 6.67 (2H). A bathochromic shift of 18 nm in the UV spectrum of **1** upon the addition of base, and the relative upfield shift (δ 115.6) of the spectroscopically indistinguishable C2 and C6 carbons in the ^{13}C NMR spectrum of **1**, indicated that this aromatic ring was phenolic. Additionally, either of the two downfield non-protonated carbons (δ 160.5 or 155.0) could be assigned as an aromatic carbon bearing hydroxyl. A trans carbon-carbon double bond was assigned to the two olefinic protons δ 7.55 and 6.62 based on their 13.3 Hz coupling constant. The extended conjugation of **1** (λ_{max} 337 nm) suggested that the C7-C8 double bond was connected to the aromatic ring at the 4 position. The remainder of the molecule ($C_3H_6NS_2$) possessed one degree of unsaturation, and contained two methyl groups, as indicated by the corresponding signals in the NMR spectra. The identity of the remaining functionality could not be confidently assigned from available spectral data.

Tridentatol B (**2**) has the same molecular formula as **1** and very similar spectra.[3] The most obvious difference between the ^1H NMR spectra of **1** and **2**, was the chemical shifts and coupling constants of the olefinic protons. The coupling constant of the olefinic protons in **2** (8.5 Hz) indicated that its C7-C8 double bond has the cis geometry.

Tridentatol C (**3**) analyzed for $C_{10}H_9NOS_2$ by HREIMS in conjunction with ^1H NMR data. Relative to **1** and **2**, this metabolite has: (1) an additional degree of unsaturation, (2) only a single, uncoupled olefinic proton, and (3) one, not two, singlet methyl groups in the ^1H NMR spectrum. Thus **3** must have an additional ring. The structure of **3** was solved by a single crystal X-ray diffraction study (Figure).[4] This analysis revealed that **3** was indeed a para-substituted phenolic, with a second ring containing a dithio-carbamate functional group conjugated to the aromatic ring through the endocyclic C7-C8 double bond.

Using the X-ray structure of **3** in conjunction with spectral data for **1-3**, we assigned the undetermined portion of **1** and **2** as a dimethyl-dithio-carbamate. This assignment was consistent with the extended UV chromophore of **1-3**,[3] and, in the ^{13}C NMR spectrum of **1**, accounted for the second downfield-shifted quaternary carbon (likely δ 160.5), and two upfield-shifted methyl signals (δ 15.2 and 14.1).

The tridentatols represent only the second group of marine secondary metabolites to possess the dithio-carbamate functional group. The bacterium *Micrococcus* sp. isolated from the Caribbean sponge *Tedania ignis* was the first reported marine species to produce this functional group.[5] This functional group, however, is more common among terrestrial natural products.[6]

Tridentata marginata commonly grows on *Sargassum* floating at the sea surface. Given the UV-absorbing characteristics of the tridentatols[3] and their high tissue concentrations, the tridentatols may function to protect *T. marginata* from intense levels of damaging solar radiation typical of near-surface

oceanic habitats.[7] The mycosporin-like amino acids, which are commonly found in many marine species, have also been proposed to function as sunscreens,[8] but these compounds do not occur in *T. marginata*.[2]

The floating *Sargassum* communities are home to a large number of small predatory fishes, primarily filefish, that commonly consume hydroids.[2] However, *Tridentata marginata* is not consumed by filefish, and tridentatol A was determined to be responsible for its unpalatability.[2] When each of 13 filefish were offered a bite-sized pellet of a squid-based food containing tridentatol A at the volumetric concentration at which it occurs in the hydroid, only two of the 13 fish ate the pellet; all 13 fish consumed a "control" pellet which was identical to the "treatment" pellet except that it did not contain tridentatol A. The difference between the consumption levels of treatment and control pellets was highly significant ($P < 0.0001$, Fisher's exact test). Additionally, the filefish readily fed on squid pellets containing the hydroid crude extract from which tridentatol A had been removed chromatographically.[2] Multiple ecological roles for secondary metabolites (e.g., predator deterrence and sunscreening) can have significant implications for the evolution of secondary compounds, however, multiple roles for marine secondary metabolites rarely have been reported.[9]

Acknowledgments

Funding to support field operations was provided by a grant from the Cooperative Institute for Fisheries Oceanography (CIFO-NOAA) to C.H. Peterson, R.A. Luettich, N. Lindquist and M.E. Hay. Ship time and support on the R/V Elusive was provided by NOAA/NURC at the University of North Carolina at Wilmington. Funding for the chemical studies was provided by NSF grant 92-02847 to M.E. Hay and NIH CA24487 to J. Clardy.

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3. Spectral data: For **1**: amorphous white solid; UV (MeOH) 337 (ε 18,000); IR ($CHCl_3$) 3595, 2929, 1608, 1521, 1509, 1432, 1259, 1170 cm^{-1}; HREIMS obsd. (M^+) 239.0430, $C_{11}H_{13}NOS_2$, requires 239.0439; ^1H NMR ($CDCl_3$) δ 7.55, 1H, d, J = 13.3 Hz; 7.32, 2H, d, J = 8.6; 6.78, 2H, d, J = 8.6; 6.62, 1H, d, J = 13.3; 4.87, 1H, bs; 2.59, 3H, s; 2,50, 3H, bs; $^{13}CNMR$ ($CDCl_3$) δ 160.5 (c), 155.0 (c), 132.2 (CH), 129.6 (CH), 127.8 2C (CH), 126.3 (CH), 115.6, 2C (CH), 15.2 (CH_3), 14.1 (CH_3). For **2**: amorphous white solid; UV (MeOH) 332 (ε 11,000); IR ($CHCl_3$) 3596, 2928, 1608, 1522, 1508, 1261, 1170 cm^{-1}; HREIMS obsd. (M^+) 239.0435, $C_{11}H_{13}NOS_2$, requires 239.0439; ^1H NMR ($CDCl_3$) δ 7.71, 2H, d, J = 8.4 Hz; 6.89, 1H, d, J = 8.5; 6.78, 2H, d, J = 8.4; 5.91, 1H, d, J = 8.5; 4.82, 1H, bs; 2.59, 6H, s. For **3**: clear plates; UV (MeOH) 313 (ε 3,000); IR ($CHCl_3$) 3690, 3594, 2928. 1610,

1496, 1264, 1173 cm^{-1}; HREIMS obsd. (M^+) 223.0132, $C_{10}H_9NOS_2$, requires 223.0126; ^1H NMR ($CDCl_3$) δ 7.69, 1H, s; 7.37, 2H, d, J = 8.7 Hz; 6.86, 2H, d, J = 8.7; 5.53, 1H, bs; 2.73, 3H, s.

4. Tridentatol C (**3**) crystallizes with two molecules in the asymmetric units of the centrosymmetric triclinic space group with a = 7.309(7), b = 9.315(8), c = 17.865(3) A, α = 86.89 (6), β = 99.72(7), and γ = 104.10(7)^o (Z = 4). Only 1363 out of the 2401 independent reflections measured that had F > 4σ(F) and were used in solution and refinement. The final model has two independent molecules of **3** and a disordered $CHCl_3$ at the inversion center with an R of 7.3%. Archival data have been deposited with the Cambridge Crystallographic Data Centre and are available from them.
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(*Received in USA* 19 *September* 1996; *revised* 24 *October* 1996; *accepted* 25 *October* 1996)