Pergamon

Tetrahedron Letters, Vol. 35, No. 15, pp. 2421-2422, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0262-V

The Unique 6-(*p*-Hydroxyphenyl)-2H-3,4-Dihydro-1,1-Dioxo-1,4-Thiazine and the New Tripeptide L-Glu-Gly-4-Hydroxystirylamine from the Marine Sponge *Anchinoe tenacior.*

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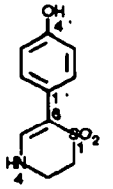
Abstract. 6-(*p*-Hydroxyphenyl)-2H-3,4-dihydro-1,1-dioxo-1,4-thiazine (**1**) has been isolated from the marine sponge *Anchinoe tenacior*, along with a novel tripeptide with a C-terminal *trans*-4-hydroxystirylamino residue (**2**).

In our continuing search for bioactive compounds from marine organisms, we reported previously the isolation of a dimeric peptide alkaloid, anchinopeptolide A, from the Mediterranean sponge *Anchinoe tenacior*.[1] Here we describe the isolation and structure elucidation of an unique metabolite **1**, which incorporates a 1,1-dioxo-1,4-thiazine ring, and of a new tripeptide (**2**), from the sponge *A. tenacior*.

Anchinoe tenacior Topsent (1.5 Kg), collected along the coast of Tunisia in June '91 (Ardoukoba expedition), was homogenized in acetone and extracted at room temperature for two days. The acetone filtrate was evaporated *in vacuo* to give a residue that was suspended in water and sequentially extracted with ethyl ether and n-butanol. Purification of the *n*-butanol fraction by sequential application of Sephadex LH-20 (eluent methanol), droplet counter-current chromatography (DCCC; *n*-butanol-MeOH-H_2O, 3:1:5) and HPLC (C_{18} μ-bondapak; MeOH-H_2O)



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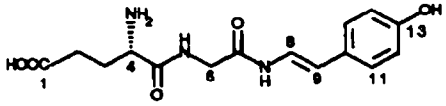

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gave, along with the major anchinopeptolides, two minor compounds **1** (4.4 mg) and **2** (5.6 mg).

A molecular formula $C_{10}H_{11}NO_3S$ was established for **1** by HREIMS, m/z 225.045883 (calc. m/z 225.045965). A strong peak at m/z 160.07611 (calc. for $C_{10}H_{10}NO$, m/z 160.07600) due to the loss of SO_2 + H, was suggestive for the presence of a sulfone functionality. A *para* substituted phenol was suggested by two mutually coupled two-proton doublets at δ 7.22 (2H, d, J = 8.6 Hz) and 6.77 (2H, d, J = 8.6 Hz) in CD_3OD. Two methine carbon signals at 131.0 and 116.9, and the quaternary carbon signals at 157.6 and 124.4 ppm supported the presence of a *para* substituted phenyl residue in the molecule. Two olefinic carbons at 140.3 (CH, δ_H 6.85, s), and at 108.7 (quaternary) and two methylene carbons at 41.3 (δ_H 3.26, m) and 49.9 (δ_H 3.87, m) ppm completed the ^{13}C NMR spectrum. The ^1H NMR spectrum in DMSO-d_6[2] disclosed two exchangeable signals for one phenolic group at δ 9.38 and for one N-H group at δ 7.18 m, this latter coupled with the olefinic signal at δ 6.87, now doublet, J = 6.4 Hz, and with the methylene multiplet at δ 3.68, which in turn was coupled with the multiplet at δ 3.18. A strong NOE effect between the olefinic proton resonating at δ 6.85 and the aromatic protons at C-2' and C-6' (δ 7.22, d) confirmed that the trisubstituted double bond was attached to the phenol ring (UV, λ_{max} 274 nm, ε = 8100, in CH_3OH). Thus, the structure

of 6-(*p*-hydroxyphenyl)-2H-3,4-dihydro-1,1-dioxo-1,4-thiazine was determined for the compound **1**. The sulfone functionality is rare among marine natural products and has previously been found in agelasidines, from a sponge of the genus *Agelas* [3,4] and in xesto- and halenoquinone derivatives, from a sponge of the genus *Adocia* [5]. The biogenetic formation of **1** is an intriguing matter.

Structure elucidation of peptide **2** [6] was begun by analysis of the nmr spectra (^{1}H, ^{13}C, 2D-COSY, HETCOR and HMBC). A *para* substituted phenol was indicated by two mutual coupled doublets at δ 7.19 (2H, d, J = 8.8 Hz) and 6.74 (2H, d, J = 8.8 Hz) each attacched to carbons at 127.8 and 116.6 ppm, respectively (CD_3OD; HETCOR). In HMBC both aromatic proton signals displayed correlations to C-13 (157.7 ppm), while H-12 and H-14 correlated to C-10 (129.2 ppm). Two olefinic methine carbons (121.2 and 115.5 ppm) remained to be placed. The coupling constants of the attacched protons (δ 7.30 d, J = 14.6 Hz; 6.21 d, J = 14.6 Hz) indicated *trans* double bond. HMBC correlations of H-9 (δ 6.21) to C-11 and C-15 (127.8 ppm) and H-8 (δ 7.30) to C-10 (129.2 ppm) linked the olefin to the aromatic ring. In DMSO-d_6, the ^{1}H NMR of the peptide **2** disclosed one exchangeable doublet (J = 9.8 Hz) at δ 10.03 coupled with the olefinic proton at C-8 (δ 7.18 dd, J = 9.8, 14.2 Hz), thus the presence of a *trans* 4-hydroxystirylamino residue was demonstrated. The ^{13}C NMR spectrum also showed two amide carbonyls (168.6 and 175.5 ppm) and one carboxy carbonyl at 181.5 ppm. These data together with the occurence of two amide (CONH) protons in **2** [δ's (DMSO-d_6) 10.03 d and 8.3 t] led to the assumption that compound **2** was a linear tripeptide with a C-terminal *trans* 4-hydroxystirylamino residue. Specific chemical shift assignments revealed the individual amino acids as glycine and glutamic acid. An HMBC correlation of H-8 to the carbonyl carbon of glycine (168.6 ppm) connected the stirylamno and glycine portions. HMBC correlations of H_2-6 to both carbonyl carbons at 168.6 and 175.5 and of H_2-2 and H_2-3 to carboxy carbonyl at 181.5 ppm secured the structure **2** for the new peptide. A NOE between the glycine NH proton and H-4 confirmed that the glycine residue was attached via an amide linkage to the α-carbonyl of glutamic acid residue. The L- configuration of glutamic acid is derived from hydrolysis with 6N HCl followed by derivatization with Marfey's reagent and HPLC analysis [7]. Attempts to obtain a molecular ion peak by FABMS or EIMS failed; thus we treated our tripeptide with diazomethane and the EIMS spectrum of the dimethylated sample was consistent with the structure shown **2**, exhibiting a small peak at m/z 349 (M^+).

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2. Compound **1**, ^{1}H-NMR (DMSO-d_6): δ H_2-2 (3.18 m), H_2-3 (3.68 m), H-4 (7.18 m), H-5 (6.87 d, J = 6.4 Hz), H-2' and H-6' (7.12 d, J = 8.7 Hz), H-3' and H-5' (6.69 d, J = (8.7 Hz).
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6. Compound **2**; $[\alpha]_D$ = - 4.6° (MeOH, c = 1); UV (CH_3OH), λ_{max} 220 (ε = 7320), 283 (ε = 9650), 290 sh. and 320 sh. nm; IR, ν_{max} (KBr pellet) 3338-3193 broad, 1653, 1616, 1610, 1591, 1539, 1509, 1254 cm^{-1}; ^{1}H-NMR (CD_3OD): δ H_2-2 (2.35 m and 2.55 m), H_2-3 (2.18 m and 2.48 m), H-4 (4.29 m), H_2-6 (4.02 s), H-8 (7.30 d, J = 14.6 Hz), H-9 (6.21 d, J = 14.6 Hz), H-11 and H-15 (7.19 d, J = 8.8 Hz), H-12 and H-14 (6.74 d, J = 8.8 Hz); ^{1}H-NMR (DMSO-d_6): δ H_2-2 (2.10 m), H_2-3 (1.90 m and 2.25 m), H-4 (4.09 m), NH_2 glu (9.35 m), NH gly (8.30 t, J = 5.4 Hz), H_2-6 (3.80 AB part of an ABX; J_{AB} = 16.6 Hz; J_{AX} = J_{BX} = 5.4 Hz), NHCH=CH (10.03 d, J = 9.8 Hz), H-8 (7.18 dd, J = 9.8, 14.2 Hz, partially overlapped with H-11 and H-15), H-9 (6.10 d, J = 14.2 Hz), H-11 and H-15 (7.15 d, J = 8.5 Hz), H-12 and H-14 (6.67 d, J = 8.5 Hz), -OH (7.8 s); ^{13}C-NMR (CD_3OD): C-1 (181.5), C-2 (30.4), C-3 (26.7), C-4 (58.3), C-5 (175.5), C-6 (43.3), C-7 (168.6), C-8 (121.2), C-9 (115.5), C-10 (129.2), C-11 and C-15 (127.8), C-12 and C-14 (116.6), C-13 (157.7).
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Acknowledgment. Work supported by P.F. Chimica Fine II, C.N.R. and M.U.R.S.T. - ROME.

(*Received in UK* 31 *December* 1993; *revised* 31 *January* 1994; *accepted* 4 *February* 1994)