

Fulvinol, a New Long-Chain Diacetylenic Metabolite from the Sponge *Reniera fulva*

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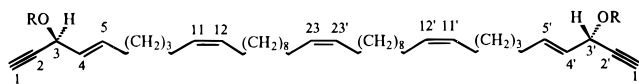
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The sponge *Reniera fulva* from Algeciras Bay, Spain, contains, in addition to the five acetylenic compounds described previously, a new long-chain acetylene named fulvinol (**1**). Its highly symmetric structure was elucidated by interpretation of spectral data, and its absolute configuration was established using the Mosher method. Fulvinol (**1**) exhibited cytotoxicity against four tumor cell lines (ED₅₀ = 1 μg/mL).

In our ongoing efforts toward the search for biologically active compounds from marine organisms of the southern coast of Spain, we obtained specimens of the orange sponge *Reniera fulva* Topsent (Chalinidae) collected in Algeciras Bay near Gibraltar Strait. Sponges of this genus have been a source of isoquinoline quinones, aryl carotenoids, sesquiterpene hydroquinones, pentacyclic alkaloids, and diacetylene metabolites.^{2,3}

In 1977, Cimino and De Stefano⁴ reported the isolation and characterization of five new polyacetylenes called renierin-1, debromorenierin-1, 18-hydroxyrenierin-1, renierin-2, and 18-hydroxyrenierin-2 from the sponge *Reniera fulva* collected in the bay of Naples, Italy. Later, a reinvestigation of this sponge from Egadi Islands, Sicily, surprisingly led to the isolation of two new sesquiterpenes as well as four known compounds of the panicein family.⁵ Our specimens of *Reniera fulva* contain the five acetylenic compounds mentioned above, together with a new long-chain diacetylenic compound, which we have named fulvinol (**1**). In this paper we describe the isolation and characterization of this new compound, its absolute configuration and the *in vitro* cytotoxicity assay results of fulvinol (**1**) against several tumor cell lines.

The sponge *R. fulva* was collected by hand using scuba and immediately frozen. The sponge was extracted with Me₂CO and concentrated to form an aqueous residue that was extracted with Et₂O. Subsequent normal- and reversed-phase chromatography of the organic phase led to the isolation of fulvinol (**1**) as colorless crystals, mp 35–37 °C (0.035% dry wt). FABMS showed a molecular ion peak [M + Na]⁺ at *m/z* 683. This finding, together with elemental analysis, indicated a molecular formula of C₄₆H₇₆O₂. The IR absorptions at 3300–3600 and 1660 cm⁻¹ indicated the presence of hydroxyl groups and double bonds in the structure of **1**.



- 1** R = H
1a R = (*R*)-MTPA
1b R = (*S*)-MTPA

Because both the ¹H- and the ¹³C-NMR spectra contained a smaller number of signals than those

expected from the molecular formula, it was concluded that fulvinol (**1**) possessed a highly symmetric structure, and each resonance of the spectrum was attributable to two magnetically equivalent nuclei.

The presence of the (*E*)-3-hydroxypent-1-en-4-ynyl moieties previously described in other sponge metabolites^{6–17} was ascertained by both ¹H and ¹³C NMR. Thus, the ¹H NMR contained a signal attributable to acetylenic protons observed at δ 2.59 (2H, d, *J* = 2 Hz), which showed long-range coupling in the COSY spectrum with the signal of protons geminal to hydroxyl groups at δ 4.86 (2H, br d, *J* = 5.2 Hz). This signal also showed vicinal and allylic couplings with the olefinic protons signals at δ 5.64 (2H, ddt, *J* = 15.2, 6.0, 1.6 Hz) and δ 5.97 (2H, ddt, *J* = 15.2, 6.8, 1.2 Hz). The coupling constant of 15.2 Hz indicated an *E* geometry for the double bond. The ¹³C-NMR resonances at δ 134.6 (d), 128.3 (d), 83.3 (s), 79.9 (d), and 62.8 (d) confirmed that fulvinol (**1**) contained the moieties mentioned above. These moieties account for six of the nine degrees of unsaturation indicated by the molecular formula. The three unsaturations remaining are due to three *Z* double bonds as indicated by the ¹H-NMR signal at δ 5.38 (6H, m) and by the ¹³C-NMR doublets at δ 130.0, 129.8, and 129.7.¹⁸ Because the molecule is highly symmetric, one of the double bonds must be central. The remaining two double bonds were located on C-11, C-12, and C-11' and C-12' based upon observation of the base peak at *m/z* 177 corresponding to the fragment C₁₂H₁₇O⁺ on FABMS. The structure shown was therefore proposed for fulvinol (**1**).

The absolute stereochemistry was assigned using the Mosher method.^{19,20} Because the compound is optically active, only the (*3R,3'R*) and (*3S,3'S*) possibilities have to be considered. The (*R*)- and (*S*)-MTPA esters (**1a** and **1b**) were prepared by treatment of fulvinol (**1**) with (*S*)- and (*R*)-α-methoxy-α-trifluoromethylphenylacetic chloride, respectively. The Δ (δ_S – δ_R) values found for H-1, H-4, H-5, and H-6 were +0.04, –0.11, –0.06, and –0.05 ppm, respectively. Following the MTPA rules, these data indicated *S* configurations for C-3 and C-3' and therefore an absolute stereochemistry as depicted in formula **1**.

The presence of polyacetylenes in *R. fulva* represents the only example of this kind of compound, in the genus *Reniera*. Phylogenetic studies using ribosomal RNA

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analyses have suggested that *R. fulva* is more closely related to the *Petrosia* genus than it is to *Reniera mucosa*.²¹ Interestingly, fulvinol (**1**) closely resembles the long-chain acetylenes isolated from some *Petrosia* sponges.⁶⁻⁹

Fulvinol (**1**) showed in vitro cytotoxicity against P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma (ED₅₀ = 1 µg/mL). These ED₅₀ values are in the range of other acetylenic compounds obtained from marine sources.⁶⁻¹⁷

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ¹H- and ¹³C-NMR spectra were recorded on a Varian 400 at 400 MHz and 100 MHz, respectively, using CDCl₃ as solvent. The resonances of residual CHCl₃ at δ_H 7.26 and δ_C 77.0 were used as internal reference for ¹H- and ¹³C-NMR spectra, respectively. An asterisk means interchangeable signals. MS were measured on a VG 12250 or on a Kratos MS 80RFA spectrometer. Elemental analysis was performed on a Carlo Erba 1106-N apparatus. In HPLC separations LiChrosorb Si-60 was used in normal-phase mode and LiChrosorb RP-18 in reversed-phase mode, using a differential refractometer. All solvents were distilled from glass prior to use.

Collection, Extraction, and Isolation Procedures. The sponge *Reniera fulva* was collected by hand using scuba in Algeciras Bay, Spain, and immediately frozen. A voucher specimen is available at Laboratorio de Biología Marina, Universidad de Sevilla (no. LBM-525). The frozen sponge was extracted exhaustively with Me₂CO at room temperature. After extraction, the sponge was dried, affording 20 g of dry wt. The filtered Me₂CO solution was evaporated under reduced pressure, and the aqueous residue was extracted with Et₂O. The organic layer was dried over anhydrous Na₂SO₄, and the solvent was evaporated to give an orange oil (1.9 g), which was chromatographed on a silica column eluted with solvents of increasing polarity from petroleum ether to Et₂O and subsequently to CHCl₃-MeOH. Selected nonpolar fractions were eluted with petroleum ether-Et₂O (9:1 and 3:1) and contained renierin-1 (155 mg, 0.775% dry wt), debromorenierin-1 (75 mg, 0.375% dry wt), and a mixture of 18-hydroxyrenierin (23 mg, 0.115% dry wt) and renierin-2 (32 mg, 0.160% dry wt), respectively. This mixture was further separated by HPLC in normal-phase mode (LiChrosorb 10 µ, 10 mm × 25 cm; petroleum ether-AcOEt, 9:1). The more polar fractions were eluted with petroleum ether-Et₂O (3:7) and contained fulvinol (**1**) (7 mg, 0.035% dry wt), which was purified by HPLC in reversed-phase mode (LiChrosorb 7 µm, 10 mm × 25 cm column; MeOH), and 18-hydroxyrenierin-2 (20 mg, 0.100% dry wt). The known compounds were identified by comparison of their spectroscopic data with those reported previously.⁴

Fulvinol (1): colorless crystals (petroleum ether-EtOAc): mp 35-37 °C; [α]_D²⁵ -14.8° (c 0.37, CHCl₃); IR (dry film) ν max 3600-3300 (OH), 2900 and 2850 (C-H, aliphatic), 1660 (C=C) cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 5.95 (2H, ddt, *J* = 15.2, 6.8, 1.2 Hz, H-5 and H-5'), 5.64 (2H, ddt, *J* = 15.2, 6.0, 1.6 Hz, H-4 and H-4'),

5.38 (6H, m, H-11, H-12, H-23, H-11', H-12', and H-23'), 4.86 (2H, br d, *J* = 5.2 Hz, H-3 and H-3'), 2.59 (2H, d, *J* = 2 Hz, H-1 and H-1'), 2.09 (4H, q, *J* = 6.8 Hz, H-6 and H-6'), 2.05 (12H, m, H-10, H-13, H-22, H-10', H-13', and H-22'), 1.38-1.29 (44H, m, H-7, H-8, H-9, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-7', H-8', H-9', H-14', H-15', H-16', H-17', H-18', H-19', H-20', and H-21'); ¹³C NMR (CDCl₃, 100 MHz) δ 134.6 (2 × d, C-5 and C-5'), 130.0 (2 × d, C-11 and C-11')*, 129.8 (2 × d, C-12 and C-12')*, 129.7 (2 × d, C-23 and C-23')*, 128.3 (2 × d, C-4 and C-4'), 83.3 (2 × s, C-2 and C-2'), 79.9 (2 × d, C-1 and C-1'), 62.8 (2 × d, C-3 and C-3'), 31.9 (2 × t, C-6 and C-6'), 29.8-28.8 (22 × t, C-7, C-8, C-9, C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21, C-7', C-8', C-9', C-14', C-15', C-16', C-17', C-18', C-19', C-20', and C-21'), 27.2-27.1 (6 × t, C-10, C-13, C-22, C-10', C-13', and C-22'); FABMS *m/z* [M + 23]⁺ 683 (70), 177 (100). Anal. Calcd for C₄₆H₇₆O₂: C, 83.56; H, 11.60. Found: C, 82.88; H, 12.40.

Synthesis of (R)-MTPA Ester (1a). A solution of **1** (1.5 mg) in dry pyridine (1 mL) was treated with (*S*)-MTPA chloride (15 µL), and the mixture was stirred at room temperature for 12 h. After evaporation of the solvent under reduced pressure the residue was purified on a Si gel TLC plate to obtain the (*R*)-MTPA ester **1a** (1.0 mg): ¹H NMR (CDCl₃, 400 MHz) δ 7.52-7.40 (10H, m, ArH), 6.07 (2H, ddt, *J* = 15.2, 6.7, 1.2 Hz, H-5 and H-5'), 6.01 (2H, br d, *J* = 6.0 Hz, H-3 and H-3'), 5.60 (2H, ddt, *J* = 15.2, 6.0, 1.8 Hz, H-4 and H-4'), 5.34 (6H, m, H-11, H-12, H-23, H-11', H-12', and H-23'), 3.55 (6H, br s, -OCH₃), 2.58 (2H, d, *J* = 2.0 Hz, H-1 and H-1'), 2.08 (4H, q, *J* = 7.0 Hz, H-6 and H-6'), 2.01 (12H, m, H-10, H-13, H-22, H-10', H-13', and H-22'), 1.35-1.25 (44H, m, H-7, H-8, H-9, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-7', H-8', H-9', H-14', H-15', H-16', H-17', H-18', H-19', H-20', and H-21').

Synthesis of (S)-MTPA Ester (1b). Treatment of **1** (1.5 mg) with (*R*)-MTPA chloride (15 µL) in pyridine as described above yielded the (*S*)-MTPA ester **1b** (1 mg): ¹H NMR (CDCl₃, 400 MHz) δ 7.52-7.39 (10H, m ArH), 6.02 (2H, br d, *J* = 6.0 Hz, H-3 and H-3'), 6.01 (2H, ddt, *J* = 15.2, 6.7, 1.2 Hz, H-5 and H-5'), 5.49 (2H, ddt, *J* = 15.2, 6.0, 1.8 Hz, H-4 and H-4'), 5.34 (6H, m, H-11, H-12, H-23, H-11', H-12', and H-23'), 3.59 (6H, br s, -OCH₃), 2.62 (2H, d, *J* = 2 Hz, H-1 and H-1'), 2.03 (16H, m, H-6, H-10, H-13, H-22, H-6', H-10', H-13', and H-22'), 1.35-1.25 (44H, m, H-7, H-8, H-9, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-7', H-8', H-9', H-14', H-15', H-16', H-17', H-18', H-19', H-20', and H-21').

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