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

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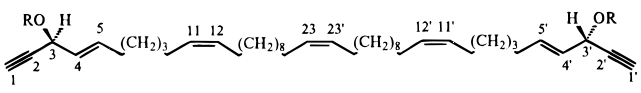
The sponge *Reniera fulva* from Algeciras Bay, Spain, contains, in addition to the five acetylenic compounds described previously, a new long-chain acetylene named fulvinol (**1**). Its highly symmetric structure was elucidated by interpretation of spectral data, and its absolute configuration was established using the Mosher method. Fulvinol (**1**) exhibited cytotoxicity against four tumor cell lines ($ED_{50} = 1$ 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 Stefano[4] 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 Me_2CO and concentrated to form an aqueous residue that was extracted with Et_2O. 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 $C_{46}H_{76}O_2$. 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 ^{1}H- and the ^{13}C-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 ^{1}H and ^{13}C NMR. Thus, the ^{1}H 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 ^{13}C-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 ^{1}H-NMR signal at δ 5.38 (6H, m) and by the ^{13}C-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 $C_{12}H_{17}O^+$ 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 (3*R*,3′*R*) and (3*S*,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 $\Delta(\delta_S - \delta_R)$ 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 ($ED_{50} = 1$ μg/mL). These ED_{50} 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. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 400 at 400 MHz and 100 MHz, respectively, using $CDCl_3$ as solvent. The resonances of residual $CHCl_3$ at δ_H 7.26 and δ_C 77.0 were used as internal reference for ^{1}H- and ^{13}C-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 Me_2CO at room temperature. After extraction, the sponge was dried, affording 20 g of dry wt. The filtered Me_2CO solution was evaporated under reduced pressure, and the aqueous residue was extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4, 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 Et_2O and subsequently to $CHCl_3$−MeOH. Selected nonpolar fractions were eluted with petroleum ether−Et_2O (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−Et_2O (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; $[\alpha]^{25}_D$ −14.8° (*c* 0.37, $CHCl_3$); IR (dry film) ν max 3600−3300 (OH), 2900 and 2850 (C-H, aliphatic), 1660 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 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′); ^{13}C NMR ($CDCl_3$, 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 $C_{46}H_{76}O_2$: 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): ^{1}H NMR ($CDCl_3$, 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, −OCH_3), 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): ^{1}H NMR ($CDCl_3$, 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, −OCH_3), 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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References and Notes

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(2) Faulkner, D. J. *Nat. Prod. Rep.* **1995**, *12*, 223−269; and previous reviews of this series.
(3) Zubía, E.; Ortega, M. J.; Carballo, J. L.; Salvá, J. *Tetrahedron* **1994**, *50*, 8153−8160.
(4) Cimino, G; De Stefano, S. *Tetrahedron Lett.* **1977**, 1325−1328.
(5) Casapullo, A.; Minale, L.; Zollo, F. *J. Nat. Prod.* **1993**, *56*, 527−533.

(6) Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *Tetrahedron Lett.* **1989**, *30*, 3563−3566.
(7) Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *J. Nat. Prod.* **1990**, *53*, 345−353.
(8) Guo, Y.; Gavagnin, M.; Trivellone, E.; Cimino, G. *Tetrahedron* **1994**, *50*, 13261−13268.
(9) Guo, Y.; Gavagnin, M.; Trivellone, E.; Cimino, G. *J. Nat. Prod.* **1995**, *58*, 712−722.
(10) Fusetani, N.; Shiragaki, T.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1987**, *28*, 4313−4314.
(11) Ochi, M.; Ariki, S.; Tatsukawa, A.; Kotsuki, H.; Fukuyama, Y.; Shibata, K. *Chem. Lett.* **1994**, 89−92.
(12) Quiñoá, E.; Crews, P. *Tetrahedron Lett.* **1988**, *29*, 2037−2040.
(13) Wright, A. E.; McConnell, O. J.; Kohmoto, S.; Lui, M. S.; Thompson, W.; Snader, K. M. *Tetrahedron Lett.* **1987**, *28*, 1377−1380.
(14) Gunasekera, S. P.; Faircloth, G. T. *J. Org. Chem.* **1990**, *55*, 6223−6225.
(15) Aiello, A.; Fattorusso, E.; Menna, M.; Pansini, M. *J. Nat. Prod.* **1992**, *55*, 1275−1280.
(16) Hallock, Y. F.; Cardellina, J. H., II; Balaschak, M. S.; Alexander, M. R.; Prather, T. R.; Shoemaker, R. H.; Boyd, M. R. *J. Nat. Prod.* **1995**, *58*, 1801−1807.
(17) Dai, J. R.; Hallock, Y. F.; Cardellina, J. H., II; Boyd, M. R. *J. Nat Prod.* **1996**, *59*, 88−89.
(18) E. Breitmaier, and W. Voelter, Eds. *Carbon-13 NMR Spectroscopy*, VCH: New York, 1990; 3rd Ed., p. 192.
(19) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(20) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296−1298.
(21) Lafay, B.; Boury-Esnault, N.; Vacelet, J.; Christen, R. *BioSystems* **1992**, *28*, 139−151.

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