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BIOACTIVE METABOLITES FROM SICILIAN MARINE FENNEL, *CRITHMUM MARITIMUM*

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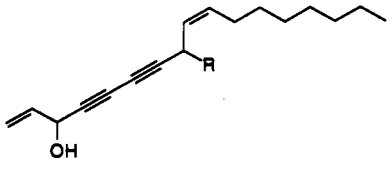
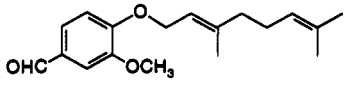
ABSTRACT.—Bioactivity-guided fractionation of the lipid extract of *Crithmum maritimum* using the brine shrimp lethality assay led to the isolation of three bioactive compounds. Two of these are known C_{17} polyacetylenic metabolites, falcarinol [**1**] and falcarindiol [**2**], previously isolated from several species of the Umbelliferae and Araliaceae. The third active principle was identified as *O*-geranylvanillin [**3**], an aromatic ether described in the literature as a synthetic compound but unknown as a natural product. Cytotoxic activity of the pure compounds was significant for **1** and **2**, much less intense for **3**.

Crithmum maritimum L. (Umbelliferae), popularly known as marine fennel, is a shrub which grows on maritime rocks, piers, and breakwaters, more rarely on sandy beaches along the European coasts (1,2). The whole plant is eaten raw for its tonic and depurative qualities, while an infusion of leaves reputedly aids digestive and diuretic activities (3). Owing to the antiscorbutic property, in times past sailors used to consume food preparations based on leaves of *C. maritimum* to prevent scurvy (4). For its particular taste, marine fennel is also used locally as a substitute for capers (5). Since present phytochemical knowledge of this plant is scant (6–10), in the course of a program aimed at the identification of bioactive constituents of plants of the Sicilian flora we have examined the lipid extract of *C. maritimum* and here we report the results of this investigation.



1 R=H
2 R=OH



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Si gel chromatography of the CH_2Cl_2 extract of the freeze-dried leaves of *C. maritimum* gave several fractions active in the *Artemia salina* assay, which is known to give information on cytotoxic and insecticidal properties. Further purification by preparative layer chromatography (plc) of the main bioactive fractions yielded three pure compounds, **1–3**.

Two of these were easily identified as falcarinol [**1**] (also known as panaxynol) and falcarindiol [**2**], originally isolated, respectively, from *Panax ginseng* C.A. Meyer (11) and *Falcaria vulgaris* Bernh. (12), and subsequently identified in many species of the Umbelliferae (13,14) and Araliaceae (15). The third bioactive metabolite was an optically inactive oil, $C_{18}H_{24}O_3$ (hreims). Its ir spectrum contained an intense carbonyl absorption, while the uv spectrum showed bands indicative of an aromatic chromophore. The ^{1}H-nmr spectrum displayed resonances for three aromatic protons whose coupling allowed us to deduce the substitution pattern on the benzene ring, as well as a formyl and a methoxyl group. The third substituent was identified as a linear monoterpene chain bound to the aromatic nucleus through an oxygen atom. Assignments and couplings were deduced

from a ^{1}H-^{1}H COSY spectrum. The ^{13}C-nmr spectrum exhibited signals for a trisubstituted benzene ring, a carbonyl group, a methoxyl, two unconjugated olefin groups, three methylenes, and three methyls. On the basis of these data, the metabolite was identified as 4-[(3,7-dimethyl-2,6-octadienyl)oxy]-3-methoxybenzaldehyde (*O*-geranylvanillin), a compound reported in the literature as a synthetic product (16) but never found before in nature. Its identity was definitely confirmed by comparison with a synthetic sample obtained by alkylation of vanillin with geranyl chloride.

Falcarinol and falcarindiol showed a significant cytotoxic activity against *A. salina* (LC_{50} 0.67 and 3.32 μg/ml, respectively), while the aromatic ether **3** was much less active (LC_{50} 85.82 μg/ml).

The occurrence of bioactive polyacetylenic compounds and aromatic ethers in members of the Umbelliferae is very common. Falcarinol and falcarindiol, for instance, are recognized as toxic, allergenic, and antifungal (17–20), whereas a hallucinogenic activity is attributed to the aromatic ethers (21).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Eims was determined at 70 eV on a Kratos MS-50S instrument. Uv and ir spectra were recorded on Perkin-Elmer model 330 and 684 spectrophotometers, respectively. Nmr spectra were measured on a Bruker AC-250 instrument, operating at 250 and 62.9 MHz for ^{1}H and ^{13}C, respectively. Multiplicities of ^{13}C-nmr resonances were determined by DEPT experiments. 2D nmr experiments were performed using standard Bruker microprograms. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Plc was carried out on a Jobin-Yvon LC Miniprep (LiChroprep Si 60, 25-40 μ as the stationary phase).

PLANT MATERIAL.—*C. maritimum* was collected along the Ionian coast of Sicily, near Catania, in September 1990. A voucher specimen was deposited at the Herbarium of the Department of Botany, Catania, Italy.

EXTRACTION AND PURIFICATION.—Shade-dried and ground plant material (800 g) was extracted three times with CH_2Cl_2 at room temperature with continuous stirring. The extracts were pooled and evaporated to give a dark green oil (20 g), which was applied to an open column (4.5×100 cm) of Si gel using eluents of increasing polarity from hexane to Et_2O. Fractions of 200 ml were collected, and those exhibiting similar tlc profiles were combined. Fraction 7 was subjected to plc [Si gel; hexane-MeCOEt (9:1)] to yield falcarinol [**1**]. Fraction 8 was evaporated to give a viscous residue which was purified by plc [Si gel; hexane-Et_2O (9:1)] to give *O*-geranylvanillin [**3**]. Fractions 16–17 were pooled and subjected to plc [Si gel; CH_2Cl_2-Et_2O (97:3)] to yield falcarindiol [**2**].

Falcarinol [**1**].—Oily (40 mg, 0.05% dry wt). Spectroscopic properties in accordance with published data.

O-Geranylvanillin [**3**].—Oily (15 mg, 0.018% dry wt): ir ν max ($CHCl_3$) cm^{-1} 1681, 1587; uv λ max (C_6H_{14}) 300 nm (ε=16850), 264 (ε=18500), 230 (ε=19000); hreims $[M]^+$ 288.1721 (calcd for $C_{18}H_{24}O_3$, 288.1725); ms *m/z* (%) 288 (10), 245 (5), 219 (8), 203 (11), 191 (11), 153 (55), 152 (94), 151 (18), 135 (16), 136 (60), 121 (12), 107 (7), 95 (18), 93 (68), 92 (50), 82 (7), 81 (75), 80 (20), 70 (11), 69 (100), 68 (10), 55 (8), 43 (4), 41 (10); ^{1}H nmr ($CDCl_3$) δ 1.58 (3H, s, H-9′), 1.65 (3H, s, H-8′), 1.74 (3H, s, H-10′), 2.08 (4H, m, H-4′ and H-5′), 3.92 (3H, s, OMe), 4.71 (2H, d, *J*=6 Hz, H-1′), 5.20 (1H, m, H-6′), 5.50 (1H, t, *J*=6 Hz, H-2′), 6.95 (1H, d, *J*=6 Hz, H-6), 7.40 (1H, s, H-2), 7.49 (1H, d, *J*=6 Hz, H-5), 9.70 (1H, s, CHO); ^{13}C nmr ($CDCl_3$) δ 141.6 (s, C-1), 126.7 (d, C-2), 149.8 (s, C-3), 153.8 (s, C-4), 108.9 (d, C-5), 111.4 (d, C-6), 66.6 (t, C-1′), 118.5 (d, C-2′), 131.8 (s, C-3′), 39.4 (t, C-4′), 26.1 (t, C-5′), 123.4 (d, C -6′), 129.8 (s, C-7′), 25.6 (q, C-8′), 17.6 (q, C-9′), 16.7 (q, C-10′), 55.6 (q, OMe), 190.7 (d, CHO).

Falcarindiol [**2**].—Oily (250 mg, 0.3% dry wt). Spectroscopic properties in accordance with published data.

REACTION OF VANILLIN WITH GERANYL CHLORIDE TO OBTAIN **3**.—To a solution of vanillin (100 mg) in EtOH (5 ml), geranyl chloride (150 mg) and K_2CO_3 (90 mg) were added. The suspension was kept at room temperature for 24 h with continuous stirring. The reaction mixture was filtered and the filtrate evaporated to dryness. The residue was subjected to plc [Si gel; hexane-Et_2O (88:12)] to give 106 mg (56%) of **3**.

BIOLOGICAL EVALUATIONS.—For the brine shrimp (*A. salina*) lethality assay, chromatographic fractions and pure compounds were dissolved in DMSO and experiments performed in triplicate using 10 animals for each test, suspended in artificial sea water, as previously described (22). Sur-

vivor shrimp were counted after 24 h, and the data were statistically analyzed using the probit analysis method described by Finney (23), to yield LC_{50} values with 95% confidence levels.

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