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Oxygenated C_{17} polyacetylene metabolites from Algerian *Eryngium tricuspidatum* L. roots: Structure and biological activity

A. Djebara[a,b], M.L. Ciavatta[a,*], V. Mathieu[c,d], M. Colin[c], F. Bitam[e], M. Carbone[a], M. Gavagnin[a]

[a] Consiglio Nazionale delle Ricerche (CNR), Istituto di Chimica Biomolecolare (ICB), Via Campi Flegrei, 34, 80078 Pozzuoli, Napoli, Italy
[b] Laboratoire de Chimie et Chimie de l'Environment, Faculté des Sciences de la Matiere, Departament de Chimie, Universitè de Batna-1, 05000, Algeria
[c] Department of Pharmacotherapy and Pharmaceutics, Faculty of Pharmacy, ULB, Campus de la Plaine, Blvd du Triomphe, 1050 Brussels, Belgium
[d] U-CRC, ULB Cancer Research Center, ULB, route de Lennik, 808, 1070 Brussels, Belgium
[e] Faculté de Médecine, Département de Pharmacie, Université de Batna-2, 05000, Algeria

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ABSTRACT

The secondary metabolite pattern of *Eryngium tricuspidatum* has been found to be dominated by C_{17} acetylene oxylipins, according to the chemistry reported in the literature for the genus *Eryngium*. Two new oxylipins, 11-acetoxy-falcarindiol (**4**) and 1,2-dihydro-11-acetoxy-falcarindiol (**5**) have been isolated, along with main related polyacetylenes **1–3** and the already known monoterpene aldehydes **6–10**, from the petroleum ether extract of roots. The structure and the absolute configuration of compounds **4** and **5** have been determined by spectroscopic methods as well as by comparison with related known compounds. Polyacetylenes **1–4** inhibited significantly the in vitro growth of a series of cancer cell lines, ranging from 0.3 to 29 μM, whereas **5** was inactive.

1. Introduction

The genus *Eryngium* is the largest and arguably the most taxonomically complex genus of Apiaceae family (Umbelliferae) [1]. The genus comprises approximately 300 species throughout the world including species cultivated as ornamental, vegetable, or medicinal crops for folk uses. *Eryngium tricuspidatum* L. (syn. *Eryngium zanonii*) that is native to North Africa, Malta, Sicily, and Sardinia, is commonly known as sea holly and is characterized by dark blue flowers appearing in Summer and silver foliage. The perennials reach heights of 50 to 150 cm and produce achenes whereas the leaves are arranged opposite to one another and are pinnate. The plant grows in sunny sites and can withstand sandy and drought-prone soils as well as temperatures down to −12 °C [2].

E. tricuspidatum is one of seven *Eryngium* species endemic in Algeria, mainly distributed in the northern regions of the country [3]. Analogous with other plants of this genus, *E. tricuspidatum* (vernacular name: M'ghizla) is used by local populations as folk remedy for the treatment of various disorders. In particular, the decocotion of its roots is effective against poisoning and constipation [4], and also to relieve labor pain [5].

Several studies on the chemistry and pharmacological activities of plants of *Eryngium* genus have been reported to date in the literature [6]. A large array of chemicals, that are terpenoids, polyacetylenes, steroids, triterpenoid saponins, flavonoids, and coumarins, displaying a variety of biological properties such as antitumour, antibacterial, antimicrobial, antifungal, and phototoxic activities have been described from distinct species of *Eryngium* genus [6]. The phytochemical constituents of *E. tricuspidatum* have been investigated in recent years with regards to the aerial parts whereas, to the best of our knowledge, no chemical analysis of the roots of the plant has been reported to date. In particular, two papers described the chemical composition and biological activity, including antibacterial, antioxidant, tyrosinase inhibitory, and antifungal properties, of the essential oil [7] and phenolic glycoside [8] mixtures obtained from the extracts of the aerial parts of *E. tricuspidatum* collections from northwestern and northeastern Algeria, respectively.

In continuing our phytochemical studies on Algerian plants [9–12], we have investigated the content of the petroleum ether extract of the roots of *E. tricuspidatum* collected in North Algeria. This study has revealed a secondary metabolite pattern dominated by oxygenated C_{17} polyacetylenes (compounds **1–5**) and monoterpenoid aldehydes (compounds **6–9**). Compounds **4** and **5** were new enynols structurally related to co-occurring (3*S*,8*S*)-falcarindiol (**3**). Falcarindiol-3,8-diacetate (**1**) was never reported as naturally occurring metabolite. The in vitro growth inhibitory properties of polyacelylenes **1–5** on a panel of cancer

* Corresponding author.
E-mail addresses: lciavatta@icb.cnr.it (M.L. Ciavatta), Veronique.Mathieu@ulb.ac.be (V. Mathieu), Margaux.Colin@ulb.ac.be (M. Colin), f.bitam@univ-batna2.dz (F. Bitam), mcarbone@icb.cnr.it (M. Carbone), mgavagnin@icb.cnr.it (M. Gavagnin).

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cell lines has been also evaluated and here reported.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Jasco DIP 370 digital polarimeter and a A. KRÜSS OPTRONIC P3000 polarimeter. ESIMS were performed on a Micromass Q-TOF MicroTM coupled with a HPLC Waters Alliance 2695. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation < 5 ppm RMS in the presence of a known lock mass). High resolution mass spectra (HRESIMS) were acquired on a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Scientific). NMR experiments were recorded at ICB-NMR Service Centre. Chemical shifts values are reported in ppm and referenced to internal signals of residual protons ($CDCl_3$, ^1H δ 7.26, ^{13}C 77.0 ppm; DMSO-d_6, ^1H δ 2.50, ^{13}C 40.0 ppm). 1D and 2D NMR spectra were acquired on a Bruker Avance-400 spectrometer using an inverse probe fitted with a gradient along the *Z*-axis, on a Bruker Avance III HD spectrometer equipped with a CryoProbe Prodigy, and on a Bruker DRX-600 operating at 600 MHz, using an inverse TCI CryoProbe fitted with a gradient along the *Z*-axis. HPLC separation was performed on a Shimadzu high-performance liquid chromatography using a Shimadzu liquid chromatograph LC-10 CE equipped with an UV SPD-10A wavelength detector, with a reversed-phase (RP-18) column (4.6 × 250 mm, Kromasil-Phenomenex). Silica-gel chromatography was performed using pre-coated Merck F254 plates (TLC) and Merck Kieselgel 60 powder (70–230 mesh). The spots on TLC were visualized under UV light (254 nm) and then spraying them with 10% H_2SO_4 in water followed by heating.

2.2. Plant material

The plant *Eryngium tricuspidatum* was collected in El Madher, in the northeastern region of Batna (Algeria) and identified by Prof. Bachir Oudjehih, Institute of Agronomy of University of Batna (Algeria). A voucher specimen is deposited in the herbarium of the department of the same University with code 05/ISAV/D.A.G./2015.

2.3. Extraction and isolation

Air-dried aerial parts (1.5 kg) and roots (0.8 kg) of *E. tricuspidatum* were accurately separated, powdered, and exhaustively extracted at room temperature with an hydroalcoholic solution (EtOH/H_2O, 8:2) for five times (2.5 L × 5). The extracts obtained for each part were filtered, combined and concentrated under vacuum to afford two distinct aqueous solutions which were sequentially partitioned with solvents of increasing polarity: petroleum ether (500 mL × 3), EtOAc (500 mL × 3), and *n*-BuOH (500 mL × 3). After removing the organic solvents, crude residues from petroleum ether (14.0 g for aerial parts, 7.0 g for roots), EtOAc (18.0 g for aerial parts, 6.0 g for roots), and *n*-BuOH (35.0 g for aerial parts, 20.0 g for roots) were obtained, respectively. All these extracts were analysed by TLC chromatography. A portion (4.0 g) of the petroleum ether extract of roots was fractionated by silica gel column chromatography (column diameter: 4.5 cm, height: 120 cm, silica gel: 200 g), eluting with an increasing polarity gradient of petroleum ether/Et_2O, and then $CHCl_3$/MeOH. A total of 62 fractions (volume of each tube: 50 mL) were collected starting from petroleum ether/Et_2O, 8:2 (500 mL of solvent, collected 10 tubes), petroleum ether/Et_2O, 7:3 (400 mL of solvent 8 tubes), petroleum ether/Et_2O, 6:4 (350 mL of solvent, 7 tubes), petroleum ether/Et_2O, 1:1 (300 mL of solvent, 6 tubes), petroleum ether/Et_2O, 3:7 (800 mL of solvent, 16 tubes), Et_2O (300 mL of solvent, 6 tubes), $CHCl_3$/MeOH 9:1 (350 mL of solvent, 7 tubes), and only MeOH (100 mL, 2 tubes). After TLC chromatography all these fractions were combined to give 21 final fractions (F1/1 → F1/21) some of which were subjected to subsequent purifications aided by ^1H NMR analysis. A portion of F1/2 (30 mg), eluted with petroleum ether/ Et_2O 8:2 (800 mg), was further purified on semi-preparative TLC (petroleum ether/ Et_2O 9:1) to give compounds **1** (R*f* 0.45, 6.0 mg), **6** (R*f* 0.4, 5.0 mg) and **7** (R*f* 0.6, 9.0 mg), and a mixture of fatty acid methyl esters. Also a portion (110 mg) of F1/3, eluted with petroleum ether/ Et_2O 8:2 (340 mg), was purified by semipreparative TLC (petroleum ether/ Et_2O 8:2) yielding compound **6** (R*f* 0.55, 18.0 mg), **8** (R*f* 0.5, 1.7 mg) and **2** (R*f* 0.25, 25 mg). Compound **2** was also the main component of fractions F1/4 (190 mg), F1/5 (270 mg), and F1/6 (600 mg), whereas compound **3** was present in both F1/8 (640 mg) and F1/9 (250 mg) from which it has been purified by two subsequent purifications. An aliquot (50 mg) of F1/8, eluted from the first column in petroleum ether/ Et_2O 7:3, was loaded into a SPE cartridge C18 using as eluent MeOH/H_2O 8:2. This subfraction (25 mg) was further purified by semipreparative TLC chromatography, using as solvent system CH_2Cl_2/MeOH 98:2 to obtain compound **3** (R*f* 0.55, 14.5 mg) and **9** (R*f* 0.85, 7.0 mg). Fractions F1/10 (89 mg),and F1/12 (180 mg), eluted from the first column with petroleum ether/ Et_2O from 6:4 to 1:1, were combined and subjected to a first purification on SiO_2 gel column (column diameter:1.8 cm, height:120 cm, silica gel:15 g, collected tubes: 41 tubes, each tube volume: 15 mL) using a gradient solvent system of petroleum ether/ Et_2O (100:0 to 0:100, *v*/v). Subfractions eluted with 30% to 40% of Et_2O in petroleum ether were combined (110 mg) and loaded onto a SPE cartridge C18 packed in MeOH/H_2O 3:7 and eluted first with MeOH/H_2O 3:7, then with MeOH/H_2O 1:1, MeOH/H_2O 6:4, MeOH/H_2O 8:2 and finally with only MeOH. Subfraction eluted with MeOH/H_2O 1:1 (14.5 mg) was further purified by analytical reversed phase HPLC column (Kromasil C18, eluent MeOH/H_2O 75:25, flow 1.0 mL/min, UV detector 210 and 254 nm) obtaining compounds **4** (1.5 mg, R*t* 11.9) and **5** (1.0 mg, R*t* 13.9). Finally a portion (40.0 mg) of F1/11 was loaded onto a semipreparative SiO_2 TLC plate in $CHCl_3$/MeOH 98:2 to give pure ferulol (**10**, R*f* 0.2, 10.0 mg).

2.3.1. 11-Acetoxy-falcarindiol (*4*)

Pale yellow oil; $[\alpha]_D^{25}$ + 141.1 (c 0.39, $CHCl_3$); HRESIMS: *m/z* 341.1733 $[M + Na]^+$ (calcd. 341.1723 for $C_{19}H_{26}O_4Na$); ^1H and ^{13}C NMR data, see Table 1.

2.3.2. 1,2-Dihydro-11-acetoxy-falcarindiol (*5*)

Pale yellow oil; $[\alpha]_D^{25}$ + 41.3 (c 0.06, $CHCl_3$); HRESIMS: m/z 343.1888 $[M + Na]^+$ (calcd. 343.1880 for $C_{19}H_{28}O_4Na$); ^1H and ^{13}C NMR data, see Table 1.

2.4. Biological evaluation

2.4.1. Cell lines

Human cancer cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), the European Collection of Cell Culture (ECACC, Salisbury, UK) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). B16F10 mouse melanoma (ATCC code CRL 6475) cells were cultured in RPMI culture medium (Gibco, Thermofisher, Dilbeek, Belgium) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), like human melanoma SKMEL-28 cells (ATCC HTB72), human mammary carcinoma MCF-7 (ATCC HTB22), A549 non small cell lung carcinoma cells (DSMZ ACC107), Hs683 anaplastic oligodendroglioma cells (ATCC HTB-138), and U373 glioblastoma cells (ECACC 08061901). Cell culture media were supplemented with 0.6 mg/mL L-glutamine (Gibco), 100 μg/mL gentamicin (Gibco), and penicillin-streptomycin (200 units/mL; Gibco). All cell lines were cultured in T25 flasks (Sarstedt AG & CO, Nümbrecht, Germany), maintained and grown at 37 °C, 95% humidity and 5% CO_2.

Table 1
^{13}C (150 MHz) and ^{1}H (600 MHz) NMR data[a] for compounds **4** and **5** in $CDCl_3$.

Position	**4**		**5**	
	δ_C type	δ_H (*J*, Hz)	δ_C type	δ_H (*J*, Hz)
1a	117.3, CH_2	5.49 (br d, 16.4)	9.3, CH_3	1.01 (t, 7.5)
1b		5.26 (br d, 10.2)		
2	135.7, CH	5.94 (ddd, 16.4, 10.2, 5.4)	30.6, CH_2	1.74 (m)
3	63.5, CH	4.94 (m)	64.1, CH	4.38 (app t, 6.4)
4	77.9, C	–	nd	–
5	69.6, C	–	nd	–
6	68.8, C	–	69.2, C	–
7	79.1, C	–	78.8, C	–
8	58.6, CH	5.45 (dd, 8.7, 3.6)	58.6, CH	5.45 (d, 8.6)
9	132.0, CH	5.74, (br dd, 9.8, 8.7)	132.2, CH	5.74, (br dd, 9.8, 8.6)
10	130.2, CH	5.35 (overlapped)	130.2, CH	5.36 (overlapped)
11	70.5, CH	5.35 (overlapped)	70.6, CH	5.36 (overlapped)
12	33.8, CH_2	1.69 (m)	33.8, CH_2	1.68 (m)
		1.54 (m)		1.54 (m)
13	24.9, CH_2	1.30 (m)	24.9, CH_2	1.29 (m)
14	28.9, CH_2	1.30 (m)	29.0, CH_2	1.30 (m)
15	31.6, CH_2	1.28 (m)	31.7, CH_2	1.28 (m)
16	22.5, CH_2	1.29 (m)	22.5, CH_2	1.29 (m)
17	14.0, CH_3	0.89 (t, 6.9)	14.1, CH_3	0.89 (t, 6.6)
OAc-11	171.5, *C*O		171.5, *C*O	
	21.3, *C*H_3	2.03 (s)	21.3, *C*H_3	2.03 (s)

[a] Assignments aided by COSY, edHSQC, HMBC (J = 8 Hz).

2.4.2. Antiproliferative properties

Antiproliferative properties of the compounds were evaluated by the MTT assay. All compounds were dissolved in DMSO at a concentration of 10 mM prior to cell treatment. The cells were trypsinized and seeded at various cell concentrations depending on the cell type into 96-well plates (Sarstedt). The cells were grown for 24 h, treated with compounds at concentrations ranging from 0.001 to 100 μM and incubated for 72 h in 100 μL media depending. After the treatment, culture media were replaced by 100 μL of MTT reagent (Sigma-Aldrich, St. Louis, MO) in serum free medium (0.5 mg/mL) and incubated further for 4 h. Formazan crystals formed by viable mitochondrial reduction were resolubilized in 100 μL of DMSO. Absorbance was measured at 570 nm with a spectrophotometer (680XR, Bio-Rad Laboratories, Berkeley, CA, USA; reference wavelength 610 nm). The experiments were performed once in six replicates.

2.4.3. Computer-assisted phase contrast microscopy

Computer-assisted phase contrast microscopy was performed as previously described [13]. Briefly, U373 or SKMEL-28 cells were seeded in 25 cm^2 culture flasks (Sarstedt) and left untreated or treated with the compounds at their IC_{50} concentrations determined with the MTT colorimetric assay. Pictures of one field were taken every four min during a 72 h period and further compiled into a short movie [13]. Experiments were performed once in triplicates.

3. Results and discussion

The petroleum ether extracts of *E. tricuspidatum* aerial parts and roots were compared by TLC in different eleuent systems. Significant differences in the composition and in the relative distribution of the metabolites were observed between the two extracts. In particular, the presence of a rich metabolite pattern characterized by a series of UV visible metabolites was observed almost exclusively in the roots. Thus, the petroleum ether extract of roots was taken into consideration for the chemical analysis. An aliquot (4.0 g) was fractionated as before described (Experimental) to give after sequential chromatographic steps five acetylene oxylipins, falcarindiol-3,8 diacetate (**1**) [14], falcarindiol-8-acetate (**2**) [15], falcarindiol (**3**) [16,17], new compounds 11-acetoxy-falcarindiol (**4**) and 1,2-dihydro-11-acetoxy-falcarindiol (**5**), along with four monoterpenoid aldehydes, isoferulol angelate (**6**) [18], 2,3,4-trimethyl-benzaldehyde (**7**) [19], ferulol-*O*-methyl ether (**8**) [20], isoferulol-5′-acetoxy angelate (**9**) [21], and finally ferulol (**10**) [21,22]. Known compounds were identified by ^{1}H and ^{13}C NMR and MS spectroscopic data (Supplementary Material). As NMR data of monoterpenic aldehydes were only partially reported in the literature [18–22], the complete NMR assignment was also made (Supplementary Material). In addition, stereochemical aspects of falcarindiol (**3**) and derivatives were investigated. The structures of compounds **4** and **5** were determined by spectroscopic analysis and correlation with *co*-occurring polyacetylenes.

The polyacetylene pool was predominant in the extract (ca. 60%) and compounds **2** and **3** were the main components of this fraction. Polyacetylenes of falcarindiol series are typical metabolites of several members of the Apiaceae and Arialiaceae families [23–25] including edible plants such as carrots. [26,27] (Fig. 1).

Falcarindiol-3,8-diacetate (**1**) was a minor component of the extract that has never been isolated as naturally occurring compound to date. It has been previously reported in the literature only as derived by acetylation of falcarindiol (**3**) [14]. Falcarindiol-8-acetate (**2**) has been identifed in a limited number of plants [15,28] whereas well-known falcarindiol (**3**), first isolated from *Falcaria vulgaris* [16], is a very common metabolite widely distributed in several species including important food vegetables [23–25].

The physical and chemical properties including the absolute configuration of **3** have been described in a number of papers [23–25] even though some conflicting data have been reported with regards to the stereochemical determination of C-3 and C-8 stereogenic centers [29–33]. The uncertainty in the stereochemical assignment was probably due to the difficulty of distinguishing the isomers by comparison of NMR and optical rotation data. The stereochemistry of **3** has been also investigated by a series of synthesis studies [27,34–36]. All four stereoisomers [(3*R*,8*S*), (3*S*,8*S*), (3*S*,8*R*), (3*R*,8*R*)] of falcarindiol have been synthesized [27,36] and their optical rotation have been measured showing that the sign of $[\alpha]_D$ is mainly affected by C-8 configuration (8*R* in levorotatory isomers, 8*S* in dextrorotatory isomers) [36]. Both (3*R*)- and (3*S*)- falcarindiol stereoisomers have been found in nature whereas the *S* configuration at C-8 has been assigned for all samples isolated from different sources despite the negative $[\alpha]_D$ value observed in some cases [32]. In addition, falcarindiol (**3**) isolated from an *Oplopanax* species has been found to be a mixture of C-3 epimers by careful NMR analysis of the Mosher ester derivatives [33]. Based on this, we have thus investigated the stereochemistry of falcarindiol (**3**) isolated in this work by analysis of NMR, $[\alpha]_D$ and CD data as well as by application of the Mosher method. Comparison of the ^{1}H and ^{13}C NMR spectra with those described for synthetic stereoisomers [36] along with evaluation of the $[\alpha]_D$ value [36] and CD profile [33] strongly suggested the (3*S*,8*S*) configuration. In order to further support this assignment, the modified Mosher method was applied. The NMR analysis of both *S* and *R* MTPA esters of **3** confirmed the *S* absolute configuration at both stereogenic centers (see Supplementary Material) and also revealed the presence of small amounts of (3*R*,8*S*)-falcarindiol (**3**), co-occurring with the major stereoisomer. In fact, the spectra of both *S* and *R* MTPA esters contained two sets of H_2-1 and H-2 multiplets easily distinguishable and attributable to major (3*S*)- and minor (3*R*)-falcarindiol stereoisomers. Integration of these multiplets indicated a 3*S*:3*R* ratio as approximately 10:1. The finding of C-3 epimers of falcarindiol was in accordance with the previous report on *Oplopanax elatus* [33] even though it should be evidenced that in *E. tricuspidatum* the relative ratio of 3*S*/3*R* isomers is significantly higher with respect to the literature [33]. Falcarindiol-8-acetate (**2**) was also investigated for the stereochemical aspects and the Mosher method was applied to secure the absolute configuration of C-3. Analogous with falcarindiol (**3**), the *S* and *R* MTPA esters of **2** contained small proton signals, which were readily recognizable from H_2-1 and H-2 multiplets by about 10%

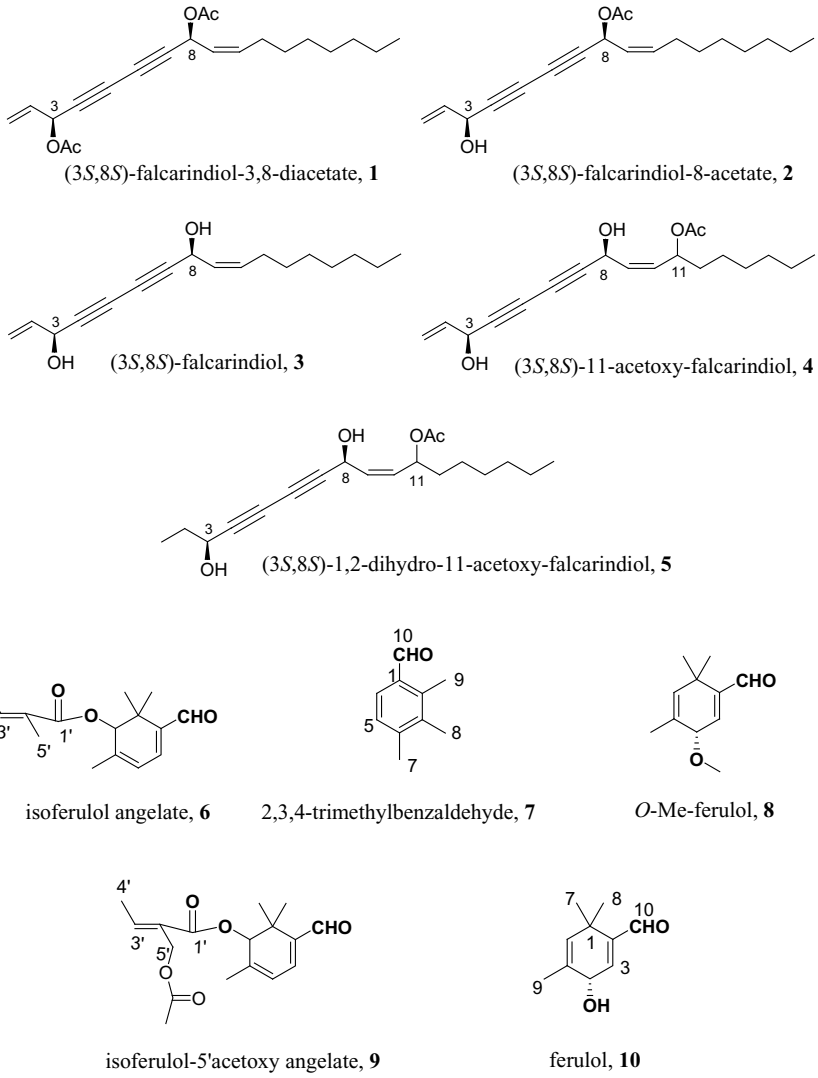

Fig. 1. Chemical structures of compounds isolated from *Eryngium tricuspidatum*.

smaller integral, confirming the expected 3*S* absolute configuration for the main stereisomer (3*S*/3*R*, 10:1 ca.) (see Supplementary Material). Based on biogenetic grounds, the absolute configuration at C-8 was assigned to be *S*, the same as falcarindiol (**3**). Similar considerations were made for falcarindiol-3,8-diacetate (**1**) for which the 3*S*,8*S* absolute configuration was also proposed. Samples of falcarindiol-8-acetate (**2**) and falcarindiol (**3**) were acetylated and the diacetyl derivatives obtained were identical (^{1}H and ^{13}C NMR, $[\alpha]_D$, and MS) with natural **1**.

The HRESIMS spectrum of 11-acetoxy-falcarindiol (**4**) contained a sodium adduct ion at *m/z* 341.17333 $[M + Na]^+$, which was consistent with the molecular formula $C_{19}H_{26}O_4$ and implying an additional acetoxy group with respect to falcarindiol (**3**). Careful analysis of ^{1}H and ^{13}C NMR data (Table 1) indicated the close similarity with **3**, in particular suggesting the presence of typical enynol partial structure C-1/C-7 with a terminal double bond, a carbinol methine and two triple bonds, sequentially. The remaining part of the C_{17} chain contained a double bond along with a hydroxy and an acetoxy group. The inspection of the ^{1}H−^{1}H COSY spectrum of **4** secured the expected C-1/C-3 spin-system [δ_H 5.94 (1H, ddd, J = 16.4, 10.2, 5.4 Hz, H-2), 5.49 (1H, br d, J = 16.4 Hz, H-1a), 5.26 (1H, br d, J = 10.2 Hz, H-1b), and 4.94 (1H, m, H-3)] and led to the definition of the subsequent carbon sequence establishing the position of the acetoxy group at C-11. The carbinol methine C-8 [δ_H 5.45 (1H, dd, J = 8.7, 3.6 Hz, H-8)] was connected to vinyl carbon C-9 [δ_H 5.74 (1H, br dd, J = 9.8, 8.7 Hz, H-9)] whereas the second vinyl carbon C-10 [δ_H 5.35 (1H, overlapped, H-10)] linked the methine bearing the acetoxy group [δ_H 5.35 (1H, overlapped, H-11) and 2.03 (3H, s, -OAc)]. Analysis of HMBC experiments showed diagnostic cross-peaks from H-8 to C-6 (δ_C 68.8) and C-7 (δ_C 79.1), from H-9 to C-6 and C-11 (δ_C 70.5), from H-11 to C-8 (δ_C 58.6) and C-12 (δ_C 33.8) confirming this assignment. The *Z*-geometry of Δ^9 double bond was inferred by the coupling constant value of olefinic protons ($J_{H9,H10}$ = 9.8 Hz) whereas the absolute configuration of C-3 and C-8 was suggested to be 3*S*,8*S*, the same as *co*-occurring main polyacetylenes **1–3**, by biogenetic considerations. The absolute configuration of C-11 remained undetermined.

1,2-Dihydro-11-acetoxy-falcarindiol (**5**) had the molecular formula $C_{19}H_{28}O_4$ as it was deduced from the sodium adduct ion at *m/z* 343.18887 $[M + Na]^+$ in the HRESIMS spectrum. Comparison of ^{1}H and ^{13}C NMR spectra with those of co-occurring compounds **1–4** revealed that **5** was lacking in the terminal double bond which was replaced by an ethyl moiety [δ_H 1.01 (3H, t, J = 7.5 Hz, H_3–1) and 1.74 (2H, m, H_2–2); δ_C 9.3 (C-1) and 30.6 (C-2)] linked to C-3 carbinol methine [δ_H 4.38 (1H, apparent t, J = 6.4 Hz, H-3)].

On the other side, the structure of **5** exhibited the fragment C-8/C-11 containing Δ^9 double bond [δ_H 5.74 (1H, br dd, J = 9.8, 8.6 Hz, H-9) and 5.36 (1H, m, overlapped, H-10); δ_C 132.2 (C-9) and 130.2 (C-10)] connected to CH(OH)- [δ_H 5.45 (1H, d, J = 8.6 Hz, H-8); δ_C 58.6 (C-8)]

Table 2
Determination of the IC50 (μM) in vitro growth inhibitory concentration by means of the MTT colorimetric assay in six cancer cell lines.

Cancer cell lines versus compounds (IC_{50}, μM)	Melanoma		Carcinoma		Glioma		Mean ± SEM
	B16F10	SKMEL28	A549	MCF-7	HS683	U373	
1	4 ± 0,3	9 ± 0,2	27 ± 0,5	18 ± 0,9	29 ± 0,9	3 ± 0,1	15 ± 5
2	0,3 ± 0,01	0,6 ± 0,01	2,5 ± 0,1	3 ± 0,7	3 ± 0,4	0,3 ± 0,02	1 ± 0,6
3	1 ± 0,04	0,5 ± 0,04	3 ± 0,09	21 ± 1	4 ± 0,07	0,7 ± 0,06	5 ± 3
4	2 ± 0,1	3 ± 0,09	3 ± 0,1	26 ± 0,6	16 ± 2,3	13 ± 4	10 ± 4
5	88 ± 3	> 100	> 100	> 100	> 100	> 100	98 ± 2
Cisplatine[a]	4 ± 0,1	9 ± 0,2	NT	NT	4 ± 0,1	2 ± 0,2	5 ± 2

Average concentration required to reduce the viability of cells by 50% after a 72 h treatment relative to a control (untreated cells) as determined by MTT assay performed once in sextuplicates,. NT: not tested.

[a] Tested in a separated experiment but with the same protocol and by the same experimentator.

and CH(OAc)- [δ_H 5.36 (1H, m, overlapped, H-11) and 2.01 (3H, s, -OAc); δ_C 70.6 (C-11), 171.5 (-OAc) and 21.3 (-OAc)] moieties, the same as **4**. The Δ^9 double bond was suggested to be *Z* by the coupling constant value of olefinic protons ($J_{H9,H10}$ = 9.8 Hz). Analogous to co-occurring metabolites, the 3*S*,8*S* absolute configuration could be proposed whereas the stereochemistry at C-11 was undefined.

Various and significant biological properties have been reported in the literature for plant acetylenic metabolites [37]. Among these, falcarindiol (**3**) is one of the most bioactive dietary polyacetylene having a vast array of biological properties including anti-inflammation, antibacterial, antifungal, antimutagenic, antiproliferative and anticancer activities [37]. Interestingly, falcarindiol (**3**) has been shown to affect cell proliferation in a biphasic manner (hormesis) thus implying that benefits were achieved at non-toxic concentrations [38]. Recent pharmacological studies confirmed the anticancer activity of **3** against some selected cancer cells such as colonrectal cancer [39], breast cancer [40], and glioblastoma cells [41]. Based on the literature data, we decided to investigate the effects on the cell proliferation of new falcarindiol-derived compounds **4** and **5** compared with falcarindiol (**3**) and acetyl derivatives **1** and **2**. A panel of six cancer cell lines containing melanoma, carcinoma and glioma models was considered and the in vitro growth inhibitory concentration for each tested compound, as determined by MTT assay, is reported in Table 2. Possible interference in terms of intrinsic absorbance and chemical reactivity with MTT salt has been ruled out for each compound (data not shown). All falcarindiol-type polyacetylenes **1**–**4** showed significant inhibition of cell proliferation against the cell lines under study with IC_{50} ranging from 0.3 to 29 μM whereas the dihydro-derivative **5** was inactive against all of them. These results were in agreement with previous reports [26,42] and supported the presence of the terminal double bond as an important structural requirement for the activity.

Importantly, falcarindiol-8-acetate (**2**) displayed significant higher

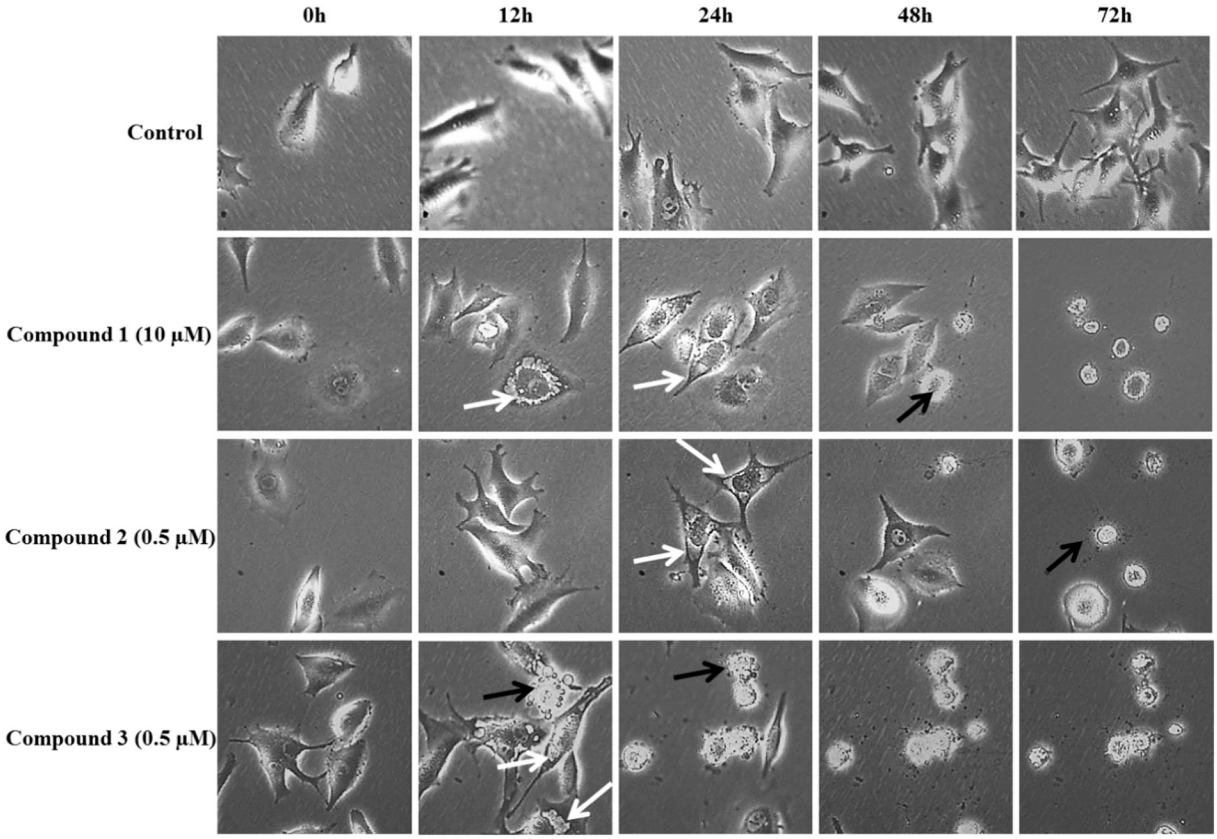

Fig. 2. Quantitative videomicroscopy analyses of compounds **1**–**3**-induced effects on SKMEL-28 melanoma (time-lapse pictures over time). Vacuoles (white arrows) appear at different timepoint according to the compound tested at its IC50. Falcarindiol (**3**) appeared to trigger cell stress earlier than compounds **1** and **2**. Cell death is consequently observed in all cases. Morphological characteritics are suggestive of apoptosis (black arrows).

inhibition on the proliferation of the cell lines than falcarindiol-3,8-diacetate (**1**) (p = .017; 2-tailed comparison conducted on the active compounds of this chemical series i.e. compounds **1–4)** but such stastistical difference could not be found with falcarindiol (**3**) suggesting a substantial structural equivalence for the activity of 3-OH and 8-OAc moieties. The ANOVA analysis of the data of compounds **1–4** between the cell lines also showed significant variation in their level of sensitivity to the different polyacetylenes. Although A549 appeared for instance less affected by compound **1** (IC_{50} ten-fold higher with respect to compound **2**) and MCF-7 less affected after treatment with **4** (IC_{50} ten-fold higher with respect to compound **2**), the 2-tailed analysis did not evidenced one cell model to be more or less sensitive to the whole chemical series.

To further confirm the anti-cancer effects observed by MTT assay, morphological evaluation of those of compounds **1–3** has been assessed by time-lapse phase contrast videomicroscopy. As illustrated in Fig. 2, all compounds **1–3** induced vacuoles in melanoma cells (SKMEL-28) at their own IC_{50} concentration but cell death occurs faster with compound **3**, i.e. within 24 h compared to 48 h for both compounds **1** and **2**. Vacuoles observed with compounds **1–3** (Fig. 2, white arrows) could derive from ER swelling due to the ER stress which may lead in turn to apoptosis in a similar manner to previously deciphered anti-cancer effects induced by falcarindiol (**3**) in colon cancer models [39].

In summary, five C_{17} polyacetylenes **1–5** of the falcarindiol series, including two new compounds **4** and **5**, and five monoterpene aldehydes **6–10** were isolated from the lipophilic extract of the roots of Algerian plant *E. tricuspidatum*. The structures of new oxylipins **4** and **5** were established by spectroscopic methods, mainly by NMR analysis, and by chemical correlation with co-occurring known compounds. Stereochemical aspects were also investigated and the absolute configuration of selected stereogenic centers was secured by the Mosher method. Full NMR assignment of aldehydes **6–10**, the spectroscopic characterization of which was not previously reported in the literature, was also made. Finally, the effects on the cell proliferation against a panel of cancer cell lines of new oxylipins **4** and **5** were investigated compared with falcarindiol (**3**) and acetyl derivatives **1** and **2**. Compound **1–4** showed significant inhibition against all cell lines under study whereas the dihydro-derivative **5** was inactive against all of them. In addition, the morphological evaluation by time-lapse phase contrast videomicroscopy of the anti-cancer effects induced by oxylipins **1–3** against SKMEL-28 melanoma cells suggested the apoptosis mechanism, analogous with that reported in the literature for falcarindiol (**3**) in colon cancer model.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. They declare don't have any conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104355.

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