Available online at www.sciencedirect.com
SCIENCE DIRECT
Phytochemistry 67 (2006) 1359–1364
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Isolation and structure elucidation of cytotoxic polyacetylenes and polyenes from *Echinacea pallida*

Federica Pellati [a,*], Samuele Calò [a], Stefania Benvenuti [a], Barbara Adinolfi [b], Paola Nieri [b], Michele Melegari [a]

[a] *Department of Pharmaceutical Sciences, University of Modena and Reggio Emilia, Via G. Campi 183, 41100 Modena, Italy*
[b] *Department of Psychiatry, Neurobiology, Pharmacology and Biotechnology, University of Pisa, Via Bonanno 6, 50126 Pisa, Italy*

Received 19 April 2006; received in revised form 3 May 2006
Available online 27 June 2006

Abstract

Bioassay-guided fractionation of *n*-hexane extracts of *Echinacea pallida* (Asteraceae) roots led to the isolation and structure elucidation of two polyacetylenes (**1**, **3**) and three polyenes (**2**, **4**, **5**). Two are known hydroxylated compounds, namely 8-hydroxy-pentadeca-(9*E*)-ene-11,13-diyn-2-one (**1**) and 8-hydroxy-pentadeca-(9*E*,13*Z*)-dien-11-yn-2-one (**2**). Two dicarbonylic constituents, namely pentadeca-(9*E*)-ene-11,13-diyne-2,8-dione (**3**) and pentadeca-(9*E*,13*Z*)-dien-11-yne-2,8-dione (**4**), were isolated and characterized for the first time. Furthermore, the structure elucidation of pentadeca-(8*Z*,13*Z*)-dien-11-yn-2-one (**5**) is described. The structure of the compounds isolated was determined on the basis of UV, IR, NMR (including 1D and 2D NMR experiments, such as ^{1}H–^{1}H gCOSY, gHSQC-DEPT, gHMBC, gNOESY) and MS spectroscopic data. The cytotoxic activity of the isolated constituents against MIA PaCa-2 human pancreatic adenocarcinoma cells was evaluated in the concentration range 1–100 μg/ml. Results show that the hydroxylated compounds (**1**, **2**) have low cytotoxicity, while the more hydrophobic polyacetylenes (**3**) and polyenes (**4**, **5**) displayed moderate activity.

Keywords: *Echinacea pallida*; Asteraceae; Structure elucidation; Cytotoxicity; Polyacetylenes; Polyenes

1. Introduction

Extracts of *Echinacea* species (Asteraceae), mainly *E. purpurea* (L.) Moench, *E. angustifolia* DC. var. *angustifolia* and *E. pallida* (Nutt.) Nutt., are traditionally used in herbal medicines and dietary supplements employed as immunostimulants in the treatment of inflammatory and viral diseases. Studies on species of the genus *Echinacea* have shown an extremely complex chemical composition, including caffeic acid derivatives (Cheminat et al., 1988; Pellati et al., 2004, 2005), alkamides, polyacetylenes and polyenes (Bauer et al., 1988a; Bauer and Remiger, 1989), polysaccharides (Wagner et al., 1988) and glycoproteins (Classen et al., 2000; Thude and Classen, 2005).

Despite of many studies that have shown the composition of alkamides in medicinally important species such as *E. purpurea* and *E. angustifolia* (Bauer et al., 1988a,b, 1989; Bauer and Remiger, 1989), there is little research on the polyines/enes of *E. pallida*. Polyacetylenes and polyenes are a class of natural products known as potent antifungal (Binns et al., 2000) and antibacterial (Parish et al., 2004) compounds. They are also known to be inhibitors of a number of enzymes, such as cholesterol acyltransferase (Rho et al., 2005). Several experiments have indicated that some polyacetylenes might exhibit antiallergenic (Wang et al., 2001) and anti-inflammatory (Chen et al., 2005) activities. In addition, polyacetylenes have proven to be cytotoxic against a number of solid and leukemic cancer cell lines (Lim et al., 2001; Youssef et al., 2003).

In a search for cytotoxic compounds from plants of the genus *Echinacea*, *n*-hexane extracts of *E. pallida* roots showed higher inhibitory activity on cell proliferation than

* Corresponding author. Tel.: +39 059 2055144; fax: +39 059 2055131.
E-mail address: pellati.federica@unimore.it (F. Pellati).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.05.006

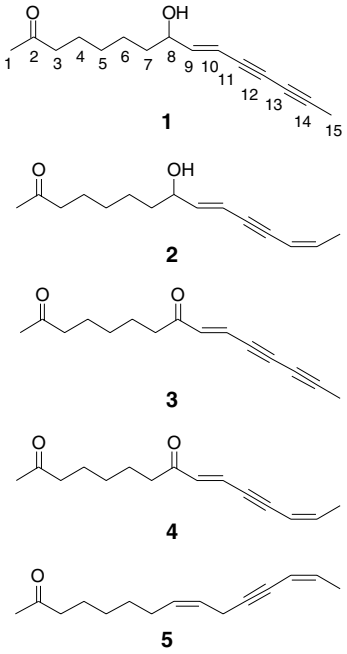

Fig. 1. Structures of polyacetylenes and polyenes (**1**–**5**) isolated from *Echinacea pallida* roots.

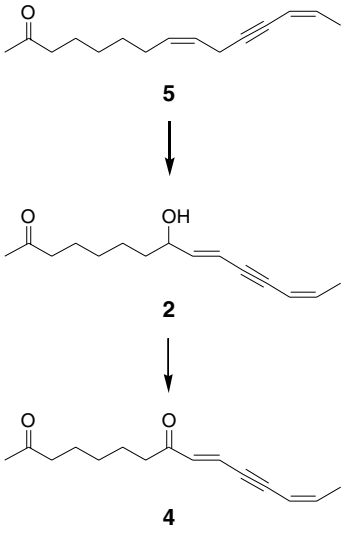

Fig. 2. Proposed oxidation process that leads to compounds **2** and **4** from the parent compound **5**.

extracts obtained from *E. purpurea* and *E. angustifolia*. Bioassay-guided fractionation of *n*-hexane extracts of *E. pallida* roots was therefore undertaken to isolate the secondary metabolites, in particular polyacetylenes and polyenes, to elucidate their structures using spectroscopic and spectrometric methods, and to determine their cytotoxic activity. Five compounds (Fig. 1) (**1**–**5**) were isolated, characterized and tested for their cytotoxic activity. For the first time, two dicarbonylic acetylenes (**3**, **4**) were isolated and fully characterized from *E. pallida* roots.

2. Results and discussion

2.1. Isolation and structural elucidation of compounds 1–5

Preliminary investigations on the cytotoxic activity of *Echinacea* species showed that *n*-hexane extracts of *E. pallida* roots had the greatest potency, with a significant concentration-dependent effect in the range 3–300 μg/ml. The fractionation of the crude *n*-hexane extract of *E. pallida* roots by a series of silica gel column chromatographic steps and prep. TLC resulted in the isolation of five acetylenes (**1**–**5**) (Fig. 1), two of which are known hydroxylated compounds (**1**, **2**) (Bauer et al., 1987). Compound **5** has been reported by Bauer et al. (1987), but in the literature its spectral data are missing. For the first time, two dicarbonylic acetylenes (**3**, **4**) were isolated from *E. pallida* roots. The structural elucidation of compound **5**, which was complicated by the substantial instability of this substance, is reported here for the first time. Compound **5**, particularly in purified form, rapidly and completely underwent allylic oxidation to compound **2** (Fig. 2). The compounds isolated were identified from their spectroscopic data, such as UV, IR, NMR, including 1D (^{1}H and ^{13}C) and 2D (^{1}H–^{1}H gCOSY, gHSQC-DEPT, gHMBC, gNOESY) techniques, and MS. The structures of compounds **1** and **2** were confirmed by comparison with the literature data (Bauer et al., 1987). In the literature cited above, the NMR data for compound **2** are incomplete; in particular, the carbonyl (C-2) and the acetylenic carbons (C-11 and C-12) were not observed. In this study, a signal was observed at 209.1 ppm (C-2) in the ^{13}C NMR spectrum, that showed a gHMBC correlation with a singlet at 2.17 ppm (H-1), and with a triplet at 2.46 ppm (H-3). Furthermore, the ^{13}C NMR spectrum indicated two typical acetylenic carbons at 91.9 (C-11) and 87.0 (C-12) ppm: the first one showed a gHMBC correlation with the signal at 6.15 ppm (H-9), while the second one showed a gHMBC correlation with the signal at 1.92 ppm (H-15). The NMR data the other part of the molecule (Tables 1 and 2) were in perfect agreement with those reported in the literature (Bauer et al., 1987).

Compound **3** had UV absorption maxima at 294 and 310 nm, that provided evidence of a highly conjugated molecule. The EI-MS data indicated a molecular ion peak $[M]^+$ at *m*/*z* 230. Elemental analysis indicated a molecular formula of $C_{15}H_{18}O_2$. The IR spectrum showed the presence of triple bonds (band at 2234 cm^{-1}) and carbonyl groups (1720 cm^{-1}), as well as olefinic double bonds (1589 and 959 cm^{-1}). gHMBC correlations of the two deshielded methyl groups at 2.16 ppm to the carbonyl carbon (C-2, 209.0 ppm) and 2.07 ppm to two acetylenic quaternary carbons (C-14 and C-13, 84.9 and 64.2 ppm) established the C-1 and C-15 positions of these methyl groups, respectively. In the ^{1}H NMR spectrum, two methylene triplets (confirmed by gHSQC-DEPT) at 2.46 and 2.56 ppm were

Table 1
^{1}H NMR spectral data [δ (ppm), *m*, *J* (Hz)] of compounds **1**–**5** (400 MHz, $CDCl_3$, TMS as reference)

Position	1	2	3	4	5
1	2.17 *s*	2.17 *s*	2.16 *s*	2.16 *s*	2.15 *s*
2					
3	2.46 *t* (7.5)	2.46 *t* (7.2)	2.46 *t* (7.3)	2.46 *t* (7.4)	2.44 *t* (7.4)
4	1.60 *m*[a]	1.62 *m*[a]	1.61 *m*[a]	1.62 *m*[a]	1.60 *m*
5	1.34 *m*[a]	1.34 *m*[a]	1.33 *m*	1.35 *m*	1.32 *m*
6	1.34 *m*[a]	1.34 *m*[a]	1.65 *m*[a]	1.64 *m*[a]	1.40 *m*
7	1.65 *m*[a]	1.63 *m*[a]	2.56 *t* (7.3)	2.58 *t* (7.5)	2.09 *m*
8	4.19 *m*	4.21 *m*			5.47 *m*[a]
9	6.28 *dd* (6.1, 15.6)	6.15 *dd* (16.0, 6.0)	6.66 *d* (16.0)	6.85 *d* (15.4)	5.47 *m*[a]
10	5.75 *d* (15.6)	5.90 *d* (16.0)	6.60 *d* (16.0)	6.54 *d* (15.4)	3.12 br. *s*
11					
12					
13		5.63 *d* (10.8)		5.69 *d* (10.2)	5.47 *d*[a] (10.6)
14		6.02 *dq* (10.8, 6.8)		6.10 *dq* (10.2, 6.6)	5.91 *dq* (10.6, 6.6)
15	2.01 *s*	1.92 *d* (6.8)	2.07 *s*	1.95 *d* (6.6)	1.87 *d* (6.6)

[a] Signals are overlapped.

Table 2
^{13}C NMR spectral data of compounds **1**–**5** (100 MHz, $CDCl_3$, TMS as reference)

Position	δ (ppm)				
	1	2	3	4	5
1	29.7	29.9	29.9	29.7	29.8
2	209.0	209.1	209.0	209.0	209.0
3	43.6	43.6	43.4	43.4	43.7
4	23.6	23.7	23.4	23.2	23.7
5	29.1	29.0	28.6	28.7	28.7
6	24.7	25.0	23.6	23.4	29.1
7	36.8	36.8	40.9	40.7	26.9
8	72.0	72.3	198.5	199.1	131.4
9	148.5	144.8	122.1	123.3	124.5
10	108.9	110.3	139.1	136.3	17.9
11	72.3[a]	91.9	73.9	92.0	92.9
12	75.1[a]	87.0	71.5	96.0	76.7
13	64.3	110.0	64.2	109.7	110.3
14	80.1	138.6	84.9	141.6	137.2
15	4.4	16.0	4.8	14.0	15.7

[a] Assignments may be interchanged.

also observed: the gHMBC spectrum indicated that the proton at 2.46 ppm (H-3) correlated with the carbon at 209.0 ppm (C-2), and that at 2.56 ppm (H-7) was close to the other carbonyl carbon at 198.5 ppm (C-8). ^{1}H–^{1}H gCOSY experiments allowed identification of the aliphatic chain between C-3 and C-7. Finally, the ^{1}H NMR spectrum showed two doublets at 6.66 (H-9) and 6.60 (H-10) ppm, with a coupling constant (^3J) of 16.0 Hz, attributable to a *E* double-bond. The gHMBC experiment placed the signal at 6.66 ppm adjacent to C-8 (198.5 ppm), and the other one close to the acetylenic carbon C-11 (73.9 ppm). The ^{13}C NMR spectrum showed the presence of another acetylenic carbon (C-12) at 71.5 ppm. All the spectroscopic data confirmed the identity of the compound as pentadeca-(9*E*)-ene-11,13-diyne-2,8-dione (**3**).

Compound **4** had a UV absorption maximum at 306 nm, that was consistent with extended conjugation of the chromophore. The EI-MS data showed $[M]^+$ at *m*/*z* 232 and, together with elemental analysis, confirmed the molecular formula as $C_{15}H_{20}O_2$. The IR spectrum of compound **4** was similar to that of compound **3**. Analysis of the aliphatic region of the ^{1}H NMR spectrum, supported by gHSQC-DEPT data, showed the presence of two methyl groups, a singlet at 2.16 ppm (H-1) and a doublet at 1.95 ppm (H-15). ^{1}H–^{1}H gCOSY indicated a scalar correlation of proton H-15 with H-14 at δ 6.10 (*dq*). The gHMBC experiment placed H-1 near a carbonyl carbon (C-2, 209.0 ppm). Carbon C-2 correlated in turn with a triplet signal (H-3, 2.46 ppm), which represents the beginning (as shown by ^{1}H–^{1}H gCOSY) of a spin system composed of five protons, ending with another triplet at 2.58 ppm (H-7). Furthermore, the gHMBC data showed that H-7 was correlated with the carbonyl carbon placed at 199.1 ppm (C-8). This carbon also correlated with an olefinic proton at 6.85 ppm (H-9). The splitting pattern of H-9, a doublet with $^3J = 15.4$ Hz, suggested the presence of a double bond located between C-9 and C-10 with a *E* configuration. The gHMBC spectrum indicated that proton H-10 is positioned close to the acetylenic carbons C-11 and C-12. Carbon C-12 also showed correlation with a doublet at 5.69 ppm (H-13). ^{1}H–^{1}H gCOSY revealed that H-13 correlated with H-14 with $^3J = 10.2$ Hz, typical of a *Z* double-bond geometry. Thus, compound **4** was identified as pentadeca-(9*E*,13*Z*)-dien-11-yne-2,8-dione.

The UV spectrum of compound **5** demonstrated an absorption maximum at 224 nm. The molecular formula was found to be $C_{15}H_{22}O$, based on EI-MS data in combination with elemental analysis. The IR spectrum was consistent with the presence of a triple bond (2238 cm^{-1}), a carbonyl group (1718 cm^{-1}) and olefinic double bonds (1654 cm^{-1}). The structure of **5** was elucidated in the same

way as for **3** and **4**. The *Z* configuration of the double bond between carbons C-8 and C-9 was determined by the presence of a strong NOESY correlation between protons H-7 and H-10. In addition, gHMBC correlations, supported by gCOSY data, revealed the presence of a spin system extended from H-3 to H-10; H-10 also showed long-range correlations with two acetylenic carbons (C-11, 92.9 ppm and C-12, 76.7 ppm). Furthermore, the gHMBC spectrum showed correlations between C-12 and two olefinic protons (H-13 and H-14, at 5.47 and 5.91 ppm, respectively), involved in a double bond with a *Z* configuration ($^3J = 10.6$ Hz). Moreover, the gCOSY spectrum showed that proton H-14 had scalar coupling with a doublet at 1.87 ppm (H-15), which was confirmed by gHSQC-DEPT as a methyl group. In conclusion, compound **5** was determined to be pentadeca-(8*Z*,13*Z*)-dien-11-yn-2-one.

The HPLC analysis of *E. pallida* fresh roots or dried roots conserved at full size did not reveal the presence of compounds **1**–**4**. Compound **5** was present in both fresh and dried roots. Bauer et al. (1988a) observed that the hydroxylated acetylenes (**1**, **2**) isolated from *E. pallida* roots are almost absent in extracts obtained from fresh roots or from root powder extracted immediately after grinding. On the other hand, **1** and **2** are present in high amounts when *E. pallida* ground plant material is stored for several days prior to extraction. Therefore, compounds **1** and **2** can be considered as artifacts that are formed during *E. pallida* root storage through an allylic oxidation reaction with molecular oxygen. The dicarbonylic acetylenes (**3**, **4**) might result from further oxidation of the parent compounds **1** and **2**, respectively (Fig. 2).

2.2. Cytotoxic activity of compounds *1*–*5*

Compounds **3**–**5** showed moderate cytotoxic activity against MIA PaCa-2 human pancreatic adenocarcinoma cells, as shown in Table 3. Compound **2** displayed low cytotoxic activity, while compound **1** was not active.

Among the constituents isolated, the hydroxylated compounds (**1**, **2**) displayed low cytotoxicity, while the more hydrophobic polyacetylene (**3**) and two polyenes (**4**, **5**) showed higher activity, which is probably owing to their higher capacity to cross the cell membrane. Further studies will be carried out to characterize the cell death mechanism. The possibility that these natural compounds work "synergistically" will also be investigated.

Table 3
Cytotoxic activity of compounds **1**–**5** against Mia PaCa-2 cells[a]

Compound	IC_{50} (μg/ml)
1	35.6 ± 0.7
2	18.8 ± 0.7
3	10.5 ± 0.3
4	14.3 ± 0.3
5	9.3 ± 0.1

[a] Data are expressed as mean ($n = 3$ replicates) ± s.e.

3. Experimental

3.1. General experimental procedures

IR spectra were obtained in CCl_4 solution with a Perkin–Elmer 1600 Series FT-IR instrument. UV spectra were recorded on-line by photodiode array detection in H_2O–ACN mixtures. NMR spectra were acquired in $CDCl_3$ (using TMS as reference) on a Bruker FT NMR AVANCE 400 spectrometer, equipped with 5-mm ^1H and ^{13}C probes operating at 400.16 and 100.61 MHz, respectively. Mass spectra were obtained on a Finnigan MAT-SSQ 710A mass spectrometer (direct inlet), in EI mode with ionization voltage of 70 eV, over the mass range 45–400 *m*/*z*. Elemental analysis was performed on an EA 1110 automatic elemental analyzer (CE instruments).

Chromatography was performed on an Agilent Technologies 1100 system, consisting of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment and a photodiode array detector. Analyses were carried out on a Lichrospher RP-18 column (125 mm × 4 mm i.d., 5 μm, Agilent Technologies) (Bauer and Remiger, 1989). Silica gel chromatography was performed with Kieselgel 60 (230–400 mesh, 0.040–0.063 mm, Merck). Preparative TLC was carried out on Kieselgel 60 F_{254} PLC plates (20 cm × 20 cm, 1 mm, Merck). Pre-coated aluminum Kieselgel 60 F_{254} plates (Merck) were used for TLC.

3.2. Plant material

Authentic dried roots (1 kg) of 3-year-old *E. pallida* (Nutt.) Nutt. were kindly donated by Dr. Federica Monti, Planta Medica s.r.l., Pistrino, Perugia, Italy, in January 2005. *E. pallida* fresh roots (178 g) were harvested in May 2005 from 3-year-old plants and were kindly donated by Dr. Sauro Biffi of the Herb Garden of Casola Valsenio, Ravenna, Italy. Voucher specimens were deposited at the Herbarium of the Botanical Garden of the University of Modena and Reggio Emilia (Italy). All the plant material was kept in the dark, protected from high temperature and humidity, until required for extraction. The roots were ground with an IKA M20 grinder immediately before extraction.

3.3. Extraction and isolation of compounds *1*–*5*

Powdered dried roots of *E. pallida* (1 kg) were extracted in a Soxhlet apparatus for 24 h using *n*-hexane (5.4 l). The extract was evaporated to dryness under vacuum to give a yellow oil (8 g). The *n*-hexane extract was dissolved in a small amount of *n*-hexane and EtOAc (2:1), subjected to silica gel chromatography and eluted with *n*-hexane/EtOAc (2:1), affording 155 fractions of 15.5 ml. Each fraction was analyzed by TLC (using *n*-hexane/EtOAc (2:1) as the mobile phase and anisealdehyde/sulfuric acid reagent for

detection) and RP–HPLC, and combined into 10 fractions (A–L) according to their chromatographic profile.

Fractions I and H, after evaporation of the solvent, yielded compound **1** (16.8 mg) and **2** (26.4 mg), respectively.

Extensive prep. TLC of fractions F and E, using *n*-hexane/EtOAc (3:1) as the mobile phase, yielded compound **3** (10.3 mg) and **4** (20 mg), respectively.

Fraction C was subjected to silica gel column chromatography and eluted with *n*-hexane/EtOAc (9:1) to afford compound **5** (33.7 mg). Purified compounds were protected from light and humidity.

3.4. 8-Hydroxy-pentadeca-(9E)-ene-11,13-diyn-2-one (*1*)

Yellow gum. The UV, IR and MS data are well matched with the literature (Bauer et al., 1987); for ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2.

3.5. 8-Hydroxy-pentadeca-(9E,13Z)-dien-11-yn-2-one (*2*)

Yellow oil. UV (H_2O/ACN) λ_{max} nm: 210, 218, 252, 264, 278. IR ν_{max} (CCl_4) cm^{-1}: 3391, 2932, 2858, 2360, 2182, 1715, 1540, 1463, 1361, 1170, 1072, 959. ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): see Tables 1 and 2. MS data are well matched with the literature (Bauer et al., 1987).

3.6. Pentadeca-(9E)-ene-11,13-diyne-2,8-dione (*3*)

Yellow-brownish gum. UV (H_2O/ACN) λ_{max} nm: 218, 230, 274, 294, 310. IR (CCl_4) ν_{max} cm^{-1}: 2956, 2927, 2856, 2234, 1720, 1589, 1461, 1408, 1363, 1274, 1164, 1124, 1073, 959. ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): see Tables 1 and 2. EI-MS *m*/*z* (rel. int.): 230 $[M]^+$ (5), 187 $[M–C_2H_3O]^+$ (3), 172 $[M–C_3H_6O]^+$ (8), 149 (12), 132 (74), 117 $[M–C_7H_{13}O]^+$ (100), 103 (7), 95 (7), 89 $[C_7H_5]^+$ (20), 71 (7), 63 $[C_5H_3]^+$ (19), 57 (8), 55 (11). Elemental analysis: found C, 78.5%; H, 7.8%. $C_{15}H_{18}O_2$ requires: C, 78.2%; H, 7.9%.

3.7. Pentadeca-(9E,13Z)-dien-11-yne-2,8-dione (*4*)

Yellow amorphous solid. UV (H_2O/ACN) λ_{max} nm: 208, 276, 306. IR (CCl_4) ν_{max} cm^{-1}: 2929, 2857, 2182, 1715, 1631, 1589, 1461, 1409, 1362, 1170, 1057, 963. ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): see Tables 1 and 2. EI-MS *m*/*z* (rel. int.): 232 $[M]^+$ (13), 180 (13), 167 $[M–C_5H_5]^+$ (9), 149 (17), 139 (25), 134 (45), 119 $[M–C_7H_{13}O]^+$ (100), 110 (45), 95 (43), 91 $[C_7H_7]^+$ (35), 81 (32), 71 (23), 65 $[C_5H_5]^+$ (27), 57 (20), 55 (56). Elemental analysis: found C, 77.4%; H, 8.8%. $C_{15}H_{20}O_2$ requires: C, 77.6%; H, 8.7%.

3.8. Pentadeca-(8Z,13Z)-dien-11-yn-2-one (*5*)

Pale yellow oil. UV (H_2O/ACN) λ_{max} nm: 224, 234. IR (CCl_4) ν_{max} cm^{-1}: 2931, 2857, 2238, 1718, 1654, 1560, 1458, 1362, 1165. ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): see Tables 1 and 2. EI-MS *m*/*z* (rel. int.): 218 $[M]^+$ (2), 203 $[M–CH_3]^+$ (2), 185 (4), 175 $[M–C_2H_3O]^+$ (4), 161 (4), 160 $[M–C_3H_6O]^+$ (10), 147 (6), 133 (16), 119 (45), 105 $[M–C_7H_{13}O]^+$ (47), 91 (100), 79 $[C_6H_7]^+$ (52), 71 (28), 65 $[C_5H_5]^+$ (14), 57 (19), 55 (31). Elemental analysis: found C, 82.3%; H, 10.1%. $C_{15}H_{22}O$ requires: C, 82.5%; H, 10.2%.

3.9. Cytotoxicity assay

Purified compounds from *E. pallida* roots were tested for their cytotoxic activity on a human pancreatic adenocarcinoma cell line (MIA PaCa-2) obtained from the American Type Culture Collection (ATCC, Rockville, MA, USA). MIA PaCa-2 cells were maintained in DMEM with L-glutamine (2 mM) supplemented with 10% fetal bovine serum, 2.5% horse serum and 1% of a 1:1 mixture of penicillin (50 IU/ml) and streptomycin (50 μg/ml). Cell viability was evaluated using a kit based on cleavage of the tetrazolium salt WST-1 to formazan by mitochondrial dehydrogenase activity (Roche). Compounds were dissolved in DMSO at 10 mg/ml and diluted to working concentrations. Cells were exposed to compounds **1**–**5** in the concentration range 1–100 μg/ml. Inhibition of cell viability was calculated by comparing the number of viable cells after treatment to cells exposed to blank DMSO. All experiments were performed in triplicate and results are expressed as mean ± s.e.

Acknowledgements

The authors thank Prof. Adele Mucci, Department of Chemistry, University of Modena and Reggio Emilia, for helpful discussions and valuable suggestions on NMR experiments during this study.

References

Bauer, R., Remiger, P., 1989. TLC and HPLC analysis of alkamides in *Echinacea* drugs. Planta Med. 55, 367–371.

Bauer, R., Khan, I.A., Wray, V., Wagner, H., 1987. Two acetylenic compounds from *Echinacea pallida* roots. Phytochemistry 26, 1198–1200.

Bauer, R., Khan, I.A., Wagner, H., 1988a. TLC and HPLC analysis of *Echinacea pallida* and *Echinacea angustifolia* roots. Planta Med. 54, 426–430.

Bauer, R., Remiger, P., Wagner, H., 1988b. Alkamides from the roots of *Echinacea purpurea*. Phytochemistry 27, 2339–2342.

Bauer, R., Remiger, P., Wagner, H., 1989. Alkamides from the roots of *Echinacea angustifolia*. Phytochemistry 28, 505–508.

Binns, S.E., Purgina, B., Bergeron, C., Smith, M.L., Ball, L., Baum, B.R., Arnason, J.T., 2000. Light-mediated antifungal activity of *Echinacea* extracts. Planta Med. 66, 241–244.

Cheminat, A., Zawatzky, R., Becker, H., Brouillard, R., 1988. Caffeoyl conjugates from *Echinacea* species: structures and biological activity. Phytochemistry 27, 2787–2794.

Chen, Y., Fu, T., Tao, T., Yang, J., Chang, Y., Wang, M., Kim, L., Qu, L., Cassady, J., Scalzo, R., Wang, X., 2005. Macrophage activating effects of new alkamides from the roots of *Echinacea* species. J. Nat. Prod. 68, 773–776.

Classen, B., Witthohn, K., Blaschek, W., 2000. Characterization of an arabinogalactan-protein isolated from pressed juice of *Echinacea purpurea* by precipitation with the β-glucosyl Yariv reagent. Carbohydr. Res. 327, 497–504.

Lim, Y.J., Lee, C.-O., Hong, J., Kim, D.-K., Im, K.S., Jung, J.H., 2001. Cytotoxic polyacetylenes alcohols from the marine sponge *Petrosia* species. J. Nat. Prod. 64, 1565–1567.

Parish, C.A., Huber, J., Baxter, J., González, A., Collado, J., Platas, G., Diez, M.T., Vicente, F., Dorso, K., Abruzzo, G., Wilson, K., 2004. A new ene-triyne antibiotic from the fungus *Baeospora myosura*. J. Nat. Prod. 67, 1900–1902.

Pellati, F., Benvenuti, S., Magro, L., Melegari, M., Soragni, F., 2004. Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. J. Pharm. Biomed. Anal. 35, 289–301.

Pellati, F., Benvenuti, S., Melegari, M., Lasseigne, T., 2005. Variability in the composition of anti-oxidant compounds in *Echinacea* species by HPLC. Phytochem. Anal. 16, 77–85.

Rho, M.-C., Lee, H.S., Lee, S.W., Chang, J.S., Kwon, O.E., Chung, M.Y., Kim, Y.K., 2005. Polyacetylenic compounds, ACAT inhibitors from the roots of *Panax ginseng*. J. Agric. Food Chem. 53, 919–922.

Thude, S., Classen, B., 2005. High molecular weight constituents from roots of *Echinacea pallida*: an arabinogalactan–protein and an arabinan. Phytochemistry 66, 1026–1032.

Wagner, H., Stuppner, H., Schäfer, W., Zenk, M., 1988. Immunologically active polysaccharides of *Echinacea purpurea* cell cultures. Phytochemistry 27, 119–126.

Wang, N., Yao, X., Ishii, R., Kitanaka, S., 2001. Structures and inhibitory effects on nitric oxide production and histamine release of five novel polyacetylene glucosides form *Bidens parviflora* Willd. Chem. Pharm. Bull. 49, 938–942.

Youssef, D.T.A., van Soest, R.W.M., Fusetani, N., 2003. Callyspongenols A–C, new cytotoxic C_{22}-polyacetylenic alcohols from a Red Sea sponge, *Callyspongia* species. J. Nat. Prod. 66, 679–681.