Polyacetylenes from the Apiaceae Vegetables Carrot, Celery, Fennel, Parsley, and Parsnip and Their Cytotoxic Activities

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A dichloromethane extract of root celery yielded falcarinol, falcarindiol, panaxydiol, and the new polyacetylene 8-*O*-methylfalcarindiol. The structure of the new compound was established by one- and two-dimensional (1D and 2D) NMR, mass spectrometry, and optical rotation data. Nonpolar extracts of roots and bulbs of carrots, celery, fennel, parsley, and parsnip were investigated for their content of polyacetylenes by high-performance liquid chromatography with diode array detection (HPLC-DAD). All five species contained polyacetylenes, although carrots and fennel only in minor amounts. Additionally, the cytotoxicity of the four polyacetylenes against five different cell lines was evaluated by the annexin V-PI assay. Falcarinol proved to be the most active compound with a pronounced toxicity against acute lymphoblastic leukemia cell line CEM-C7H2, with an IC_{50} of 3.5 μmol/L. The possible chemopreventive impact of the presented findings is discussed briefly.

KEYWORDS: Polyacetylenes; Apiaceae; 8-*O*-methylfalcarindiol; cytotoxicity

INTRODUCTION

Carrots [*Daucus carota* L. subsp. *sativus* (Hoffm.) Arcang.], celery [*Apium graveolens* L. var. *rapaceum* (Mill.) Gaud.], fennel [*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* (Mill.) Thell.], parsley [*Petroselinum crispum* (Mill.) A. W. Hill subsp. *tuberosum* (Bernh.) Soó], and parsnip (*Pastinaca sativa* L. subsp. *sativa* var. *sativa*), all belonging to the Apiaceae family, are widely cultivated vegetables in temperate regions. Despite their wide usage as foodstuff, little is known about nonpolar secondary metabolites from celery bulbs, fennel bulbs, and parsnip roots. In particular, no reports about polyacetylenes from fennel (*Foeniculum*) or parsnip (*Pastinaca*) exist in the literature, and only two records about polyacetylenes from the genus *Apium* (celery) exist (*1*, *2*), one referring to an exotic, noncultivated species (*2*). In contrast, polyacetylenes and other secondary metabolites from carrots (*3*−*5*) and parsley (*1*, *6*, *7*) have received some attention. Both species yielded falcarinol and falcarindiol (*3*, *6*), while carrots also contained the 3-*O*-acetate of falcarindiol (*3*).

Polyacetylenes not only are interesting chemosystematic marker compounds for a number of families of higher plants such as Apiaceae and Asteraceae but also are known to exhibit nematodicidal effects, which might be used in organic agriculture (*8*). Above all, their presence in food is of considerable interest, as polyacetylenes possess a great number of beneficial as well as potential detrimental bioactivities for the human consumer. Polyacetylenes are potent antifungal (*9*, *10*) and antibacterial compounds (*11*, *12*). They are also known to be inhibitors of a number of enzymes such as diacylglycerol acyltransferase (*13*), inducible nitric oxide synthase (*14*, *15*), and cholesteryl ester transfer protein (*16*) as well as microsomal and mitochondrial enzymes (*17*). In vitro experiments indicate that some polyacetylenes might exhibit antiallergenic (*18*) and antiinflammatory activities (*19*). In addition, polyacetylenes have proven to be cytotoxic against a number of solid and leukemic cancer cell lines (*20*−*23*) and to potentiate cytotoxicity of other anti-cancer drugs (*24*). For example, panaxytriol has been shown to rapidly inhibit cellular respiration in B16 melanoma cells transplanted to mice (*25*).

A medicinal usage of pure polyacetylenes is not feasible because of their pronounced chemical instability and their ability to induce allergic reactions (*26*). However, consumption of food containing polyacetylenes might have a chemopreventive benefit. A dose-dependent biphasic effect [hormesis (*27*)] of falcarinol from carrots (*Daucus carota*) on epithelial cells has

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been demonstrated recently (*28*). Low concentrations of falcarinol stimulated growth of these cells; in contrast, higher concentrations had an inhibitory effect. This is in line with the assumption that bioactive secondary metabolites contribute to the beneficial effects of a diet rich in fruits and vegetables against cancer and cardiovascular disease (*28*). Bioavailability of falcarinol from carrots in humans was demonstrated recently in biologically relevant concentrations (*5*). It was also demonstrated that dietary falcarinol intake inhibited cancerous lesions in mice treated with carcinogenic azoxymethane (*5*).

Adverse effects due to an excessive intake of polyacetylenes with the human diet are not to be expected, because polyacetylenes have a bitter off-taste in higher concentrations and are one of the main compounds contributing to the bitter taste of stored carrots (*4*).

The present study has investigated the isolation and structure elucidation of polyacetylenes from celery, the contents of these compounds in celery and other vegetables from the Apiaceae family, and the in vitro cytotoxicity of four polyacetylenes isolated from celery against different human cancer cell lines.

MATERIALS AND METHODS

Plant Material. Celery roots were commercially obtained from Südfrüchte Ploner in Innsbruck/Austria in October 2003. Specimens of freeze-dried roots are preserved in the collection of plant samples (Diettrichiana) of the Institute of Pharmacy in Innsbruck (accession number 2004-002). Samples for comparative high-performance liquid chromatography (HPLC) analyses of carrots, celery, fennel, parsnip, and parsley roots were purchased from Obst Mair-Fasolt (Innrain 23, A-6020 Innsbruck) in autumn 2004 and are also preserved in the Diettrichiana collection (accession numbers 2004-003 to 2004−007). All plants were freeze-dried prior to investigation.

General Experimental Procedures. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. NMR spectra were recorded on a Bruker AM-300 spectrometer (Karlsruhe, Germany) at 300 and 75 MHz, respectively. For thin-layer chromatography (TLC), fractions from chromatographic separations were combined by similarity based on TLC investigations onto Merck 40−63 μm silica gel 60 F$_{254}$ TLC plates (Darmstadt, Germany), with a mixture of petrol ether and EtOAc (3/1 v/v) as the mobile phase. Polyacetylenes were detected as black spots after spraying with vanillin (1% in MeOH) and sulfuric acid (5% in MeOH) and subsequent heating.

Chemicals. Organic solvents [(CH$_3$)$_2$CO, CH$_2$Cl$_2$, EtOAc, MeOH, and petrol ether] of analytical quality were purchased from Gatt-Koller (Absam, Austria) and were distilled before usage. MeCN LiChroSolv for HPLC analyses was obtained from Merck (Darmstadt, Germany); water for HPLC was demineralized and subsequently distilled before use. Other reagents (4-chlorobenzophenone, sulfuric acid, and vanillin) were also obtained from Merck (Darmstadt, Germany).

HPLC/DAD. Analyses were performed on HP-1090 and HP-1100 Chemstations (Waldbronn, Germany) equipped with autosampler and diode array detector; oven temperature, 40 °C; column, 150 × 4.6 mm i.d., particle size 3.5 μm, Zorbax Rx-C18 (Waldbronn, Germany); guard column, Merck, RP-18 (Darmstadt, Germany); detection wavelength, 205 nm; injection volume, 10 μL; mobile phase A, water, and B, CH$_3$-CN; flow rate, 1.00 mL/min; linear gradient, 0 min 80% A/20% B, 5 min 80% A/20% B, 10 min 50% A/50% B, 31 min 46.5% A/53.5% B; 55 min 5% A/95% B; stop time, 60 min; post time, 15 min; observed retention times **1**, 44.0 min, **2**, 25.5 min, **3**, 41.3 min, **4**, 27.7 min, and 4-chlorobenzophenone (internal standard), 21.2 min. All comparative analyses were performed in triplicate. An HPLC chromatogram for an extract of celery is shown in **Figure 2**.

HPLC/MS. Analyses were performed on the HPLC system described above. The HPLC was coupled to a Bruker Esquire 3000^{plus} ion trap (Bremen, Germany). The following parameters were employed: ionization, positive electrospray ionization (ESI); scanning range *m/z* 150−600; nebulizer 30 psi; dry gas 8 L/min; dry temp 300 °C. The following on-line mass spectrometry (MS) signals were observed for compounds

Table 1. NMR Data for 8-*O*-Methylfalcarindiol **3**[a]

position	^{1}H NMR, ppm (*J*, Hz)	^{13}C NMR, ppm	HMBC correlations
1	5.47, 1H, m[b]	117.4	2, 3
	5.26, 1H, br d (10.0)		
2	5.94, 1H, ddd (17.5, 10.0, 5.0)	136.1	3, 4
3	4.94, 1H, br d (5.0)	63.7	c
4		77.9	
5		70.6	
6		69.7	
7		78.6	
8	4.78, 1H, d (8.0)	67.2	6, 7, 9, 10, O*C*H$_3$
9	5.45, 1H, m[b]	126.0	7, 8, 10, 11
10	5.65, 1H, dt (10.5, 7.5)	135.3	8, 9, 11, 12
11	2.10, 2H, dt (7.5, 7.0)	28.0	9, 10, 12/13
12	1.26−1.37, 2H, m[b]	29.4	d
13	1.26−1.37, 2H, m[b]	29.3	d
14	1.26−1.37, 2H, m[b]	29.3	d
15	1.26−1.37, 2H, m[b]	22.8	d
16	1.26−1.37, 2H, m[b]	32.0	d
17	0.88, 3H, t (7.0)	14.2	15, 16
8-OCH$_3$	3.39, 3H, s	56.1	8

[a] Measured in CDCl$_3$ (^{1}H at 300 MHz, ^{13}C at 75 MHz) and referenced to solvent residual and solvent signals at 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C), respectively. [b] Overlapping signals. [c] No HMBC correlations observable. [d] HMBC correlations not assignable to a particular proton.

1, **2**, and the internal standard compound: **1**, 263 [M + H$_2$O + H]$^+$, 245 [M + H]$^+$, and 227 [M − H$_2$O + H]$^+$; **2**, 283 [M + Na]$^+$, 265 [M − H$_2$O + Na]$^+$, 247 [M − 2H$_2$O + Na]$^+$, 243 [M − H$_2$O + H]$^+$, and 225 [M − 2H$_2$O + H]$^+$; 4-chlorobenzophenone, 219 [C$_{13}$H$_9^{37}$ClO + H]$^+$ and 217 [C$_{13}$H$_9^{35}$ClO + H]$^+$. Amounts of compounds **3** and **4** in crude extracts were too low for on-line LC−MS. However, an HPLC/MS run of compound **3** yielded the following signals: 297 [M + Na]$^+$, 257 [M − H$_2$O + H]$^+$, 243 [M − HOCH$_3$ + H]$^+$, and 225 [M − HOCH$_3$ − H$_2$O + H]$^+$.

Optical Activity. The following [α]$_D^{20}$ values were obtained for compounds **1**−**4** (*c* = 0.05, CH$_2$Cl$_2$): **1**, −5°; **2**, +121°; **3**, +112°; **4**, −3°.

Isolation of Polyacetylenes. Freeze-dried bulbs of *A. graveolens* (976 g) were ground and macerated with CH$_2$Cl$_2$ for 12 h. The extract was filtered and brought to dryness in vacuo to yield 12.5 g of crude extract. The extract was redissolved in a mixture of MeOH and H$_2$O (1/1 v/v) and extracted with petrol ether. The petrol ether layer (11.7 g) was fractionated by silica gel column chromatography (CC) employing a gradient of petrol ether and ethyl acetate; finally the column was eluted with MeOH. Fractions containing compounds **1** (199 mg) and **2** (389 mg), respectively, were further purified by Sephadex LH-20 CC to yield **1** (73.1 mg) and **2** (369 mg) with a CH$_2$Cl$_2$/(CH$_3$)$_2$CO mixture (85/15 v/v) as eluant. The fraction (370 mg) containing **3** and **4** was again chromatographed on silica gel employing a gradient of petrol ether and EtOAc. Fractions containing **3** (157.0 mg) and **4** (60.7 mg), respectively, were further purified by Sephadex LH-20 CC with a CH$_2$Cl$_2$/(CH$_3$)$_2$CO mixture (85/15 v/v) as an eluant to yield **3** (14.0 mg) and **4** (25.7 mg).

8-*O*-Methylfalcarindiol (3). Colorless crystals, mp 38 °C; FTIR v_{max}^{ZnSe} (cm^{-1}) 3400 (br), 3023, 2955, 2923, 2854, 1730, 1645, 1616, 1519, 1464, 1419, 1378, 1311, 1279, 1253, 1228, 1191, 1086, 1022, 985, 958, 931, and 880. ESI-MS 297 [M + Na]$^+$; high-resolution MS experiments yielded no interpretable results; NMR data are described in **Table 1**.

Extraction Procedure for Comparative Analyses. To 1.00 g of freeze-dried and powdered plant material was added 0.5 mg of internal standard 4-chlorobenzophenone (Merck, Darmstadt, Germany) (as a stock solution in CH$_2$Cl$_2$). This compound was chosen as an internal standard because no suitable stable and chemically related compound was available and because 4-chlorobenzophenone had a retention time not interfering with any of the compounds detected in the investigated extracts, the UV absorption at the detection wavelength was pronounced, and 4-chlorobenzophenone as a synthetic compound was not expected to be contained in any of the investigated taxa.

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Table 2. Cytotoxicity of Polyacetylenes from *A. graveolens*[a]

compd	CEM-C7H 2	U937	RPMI	HRT-18	HT2912
HGH[b]	2.59 (0.23)	4.00 (0.99)	3.04 (0.22)	3.64 (0.20)	19.2 (8.86)
1	3.50 (0.42)	30.1 (0.05)	27.4 (1.35)	42.3 (6.99)	63.9 (17.8)
2	29.8 (0.26)	31.8 (2.13)	29.6 (0.25)	>100 (−)	>100 (−)
3	31.8 (1.14)	32.1 (1.50)	29.3 (0.45)	34.7 (1.65)	>100 (−)
4	29.2 (0.77)	29.5 (0.57)	27.8 (0.14)	29.8 (0.02)	32.3 (0.06)

[a] IC_{50} values are given in micromolar units. Standard deviations are indicated in parentheses; all analyses were performed in triplicate. [b] HGH, positive control 14-hydroxyhypocretenolide β-D-glucopyranoside 4′,14″-hydroxyhypocretenoate (*34*).
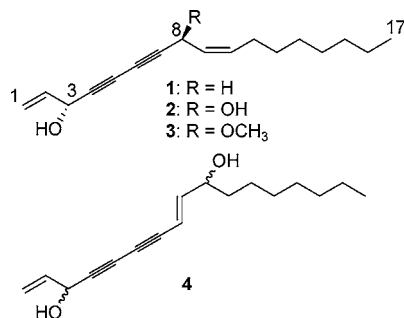
Figure 1. Structures of polyacetylenes **1**–**4** isolated from celery (*Apium graveolens*).

The mixture was then ultrasonicated three times for 30 min with 50 mL of CH_2Cl_2 and the resulting extracts were combined and brought to dryness in vacuo. The residue was redissolved in 2.00 mL of $(CH_3)_2$-CO, filtered, and used for HPLC analyses. This procedure was regarded to be an exhaustive extraction, because extracts obtained from fourth and fifth extraction from one sample of *A. graveolens* extracted with the same procedure did not contain detectable amounts of polyacetylenes.

A linear detector response for the internal standard was observed in a concentration range between 0.01 and 1.00 mg/mL (R^2 = 0.9994). The detection limit was at a concentration of 0.002 mg/mL and the accuracy (recovery rate) was 102.2%.

Bioactivity Assays. Bioactivity assays were performed according to Koopman et al. (*29*). Cells were incubated with concentrations of 1, 5, 10, 50, or 100 μM of the tested compounds for 48 h in a concentration of 0.5 × 10⁶ cells/mL before analysis. All bioassays were performed in triplicate. Mean results and standard deviations are given in **Table 2**. The following cell lines were analyzed: CEM-C7H2, a T-ALL cell line (acute lymphoblastic leukemia); U937, a human histiocytic lymphoma cell line; RPMI-8226, a human multiple myeloma cell line; and HRT-18 and HT-2912, colorectal carcinoma cell lines.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation of Polyacetylenes. Falcarinol (**1**), facarindiol (**2**), the new natural product 8-*O*-methylfalcarindiol (**3**), and panaxydiol (**4**) (**Figure 1**) were isolated from the dichloromethane extract of lyophilized and ground subaerial parts of *A. graveolens* var. *rapaceum*. Structures of known compounds **1** and **2** were identified by ESI-MS, ¹H NMR, and ¹³C NMR as well as heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments as falcarinol **1** (*30*) and falcarindiol **2** (*31*), respectively. There are conflicting assignments in the literature for the signals of the C9/C10 double bond of falcarindiol. The lower-field carbon signal was assigned by Kern and Cardellina (*32*) to C-9, while Zheng et al. (*33*) assigned this signal to C-9 and the higher-field signal to C-10. Two-dimensional (2D) NMR experiments (HSQC and HMBC) from an earlier study (*31*) and from the present study support the

assignment of Zheng et al. (*33*) by showing correlations from the proton attached to carbon with a signal in the higher field (δ_{H-9} = 5.50 ppm, δ_{C-9} = 127.9 ppm) to carbons 7, 8, 10, and 11, while the proton attached to the lower-field carbon (δ_{H-10} = 5.63 ppm, δ_{C-10} = 134.9 ppm) displays correlations with carbons 8, 9, 11, and 12.

To verify the absolute stereochemistry in position 3 and in positions 3 and 8, respectively, the optical rotations of **1** and **2** in dichloromethane were measured. The small negative optical activity measured for compound **1** ($[\alpha]_D^{20}$ = −5°) identified the substance as (3*R*)-falcarinol (*34, 35*). The high positive optical activity measured for compound **2** ($[\alpha]_D^{20}$ = +121°) proved *S*-configuration for falcarindiol **2** in position 8. However, the contribution of the asymmetric center in position 3 to the total optical activity of falcarindiol is negligible, and conflicting assignments for the stereochemistry in position 3 exist in the literature (*33, 35*−*37*). On the basis of biogenetic considerations and the fact that celery also contains (3*R*)-falcarinol, it was concluded that compound **2** was (3*R*,8*S*)-falcarindiol (*31*). Both falcarinol (**1**) and falcarindiol (**2**) are common constituents of plants from the Apiaceae family, though **1** was never previously reported for *A. graveolens*.

MS data of compound **3** in combination with ¹³C NMR data (**Table 1**) indicated a molecular formula of $C_{18}H_{26}O_2$. ¹³C NMR, distortionless enhancement by polarization transfer (DEPT), and HSQC experiments revealed the presence of two methyls, seven methylenes, five methines, and four quaternary carbons. ¹H and ¹³C NMR data (**Table 1**) closely resembled those of falcarindiol (**2**) but showed additional signals for a methoxy group (δ_H = 3.39 3H, s; δ_C = 56.1). HMBC correlations (**Table 1**) indicated that compound **3** had a C_{17} chain with a substitution pattern identical to that of falcarindiol **2**. However, ¹³C NMR shift values for the signal assignable to C-8 were shifted downfield (δ_C = 67.4) as compared to δ_C = 58.8 observed for the C-8 signal of **2**, and the ¹³C NMR signals for C-7 (from δ_C = 80.1 to 78.6) and C-9 (from δ_C = 127.9 to 126.0) were slightly shifted to the higher field. In contrast, the signal assignable to H-8 was shifted upfield (from 5.20 to 4.78). The assumption that **3** was an 8-*O*-methyl derivative of falcarindiol was verified by HMBC data, which showed a correlation between the signals assignable to the protons of the methoxy group and carbon C-8 of the polyacetylene chain. The optical rotation of **3** ($[\alpha]_D^{20}$ = + 112°) measured in dichloromethane was close to that observed for falcarindiol **2** ($[\alpha]_D^{20}$ = + 121°), thus implying the absolute stereochemistry was identical with that of falcarindiol. Conclusively, compound **3** was identified as 8-*O*-methyl-(3*R*,8*S*)-falcarindiol, which represents a new natural compound. To exclude the possibility that the new natural compound **3** was an artifact from the isolation process, in which methanol was used, an acetone extract from freeze-dried celery was prepared and analyzed by HPLC-MS. Signals assignable to compounds **1**−**4** had the same relative intensities as in the crude extract used for isolation of compounds **1**−**4**. Thus, compound **3** was proven to be a genuine natural constituent of celery roots.

MS data indicated that compound **4** was an isomer of falcarindiol (**2**). One-dimensional (1D) and 2D NMR experiments and comparison with literature values (*38, 39*) established the structure of compound **4** as panaxydiol (1,8-heptadecadiene-4,6-diyne-3,10-diol), a polyacetylene known from ginseng (*Panax ginseng*, Araliaceae) and from a number of further members of the Apiaceae and Araliaceae families. The stereochemistry of panaxydiol from ginseng (*Panax ginseng*) has been elucidated recently as 3*R*,10*S* (*40*). However, the observed optical rotation ($[\alpha]_D^{20}$ = − 3°) is neither close to this nor to

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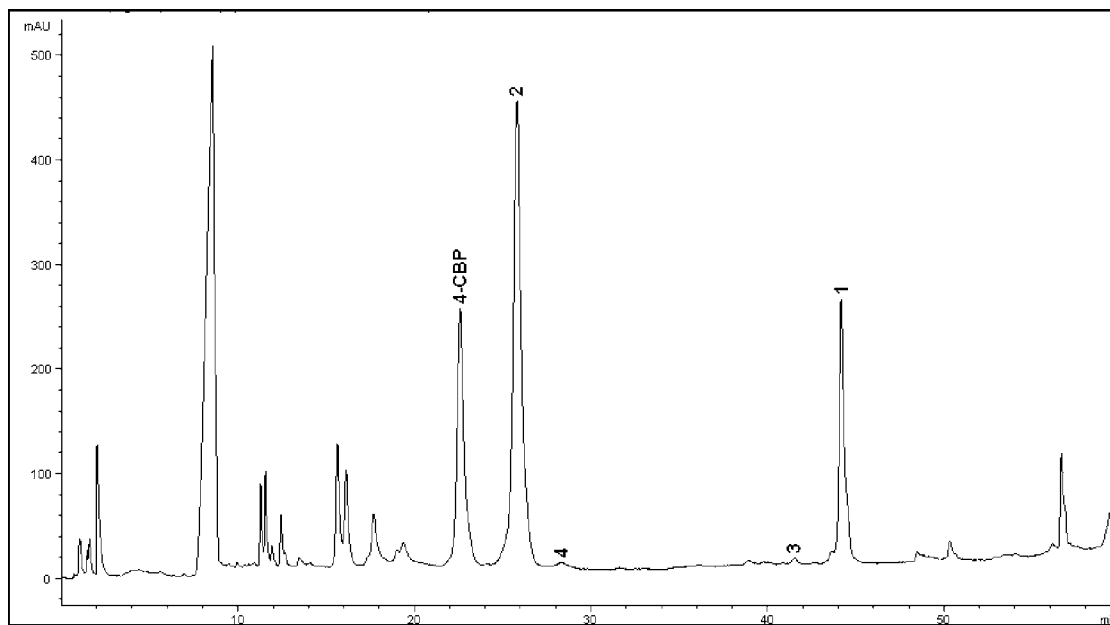*J. Agric. Food Chem.*, Vol. 53, No. 7, 2005   **2521**

Figure 2. HPLC chromatogram of an extract of celery (*Apium graveolens*). HPLC parameters: oven temperature, 40 °C; column, 150 × 4.6 mm i.d.; particle size 3.5 μm, Zorbax Rx-C18; guard column, Merck, RP-18; detection wavelength, 205 nm; injection volume, 10 μL; mobile phase A, water, and B, CH_3CN; flow rate, 1.00 mL/min; linear gradient, 0 min 80% A/20% B, 5 min 80% A/20% B, 10 min 50% A/50% B, 31 min 46.5% A/53.5% B, 55 min 5% A/95% B; stop time, 60 min; 4-CBP, internal standard 4-chlorobenzophenone; sample accession number, 2004-003.

any of the other three isomers, thus implying that **4** is a mixture of different isomers presumably arising from nonenzymatic and nonstereospecific decomposition of falcarindiol (**2**).

Cytotoxic Activity of Polyacetylenes 1−4. Cytotoxic activity of compounds **1−4** was assessed employing the annexin V-PI assay (*29*) to test the potential of polyacetylenes in a number of human cancer and leukemia cell lines. The sesquiterpenoid 14-hydroxyhypocretenolide β-D-glucopyranoside 4′,14″-hydroxyhypocretenoate (HGH), which was isolated from *Leontodon hispidus* L. in a previous study (*41*), was used as a positive control substance. This dimeric sesquiterpene derivative was identified as a potent cytotoxic agent in an earlier investigation (*42*). In the present investigation HGH also proved to be the most potent cytotoxic agent with IC_{50} values ranging from 2.59 μM (CEM-C7H2) to 19.2 μM (HT2912). The degree of sensitivity against HGH was of the same order of magnitude for CEM-C7H2, U937 (4.00 μM), RPMI (3.04 μM), and HRT-18 (3.64 μM) cells. Only HT2912 cells were much less sensitive to this agent.

All investigated polyacetylenes (**1−4**) showed medium-level cytotoxicity against the investigated leukemia, lymphoma, and myeloma cell lines with IC_{50} values of approximately 30 μM. Only falcarinol (**1**) showed much higher activity against one cell line, CEM-C7H2, with an IC_{50} value of 3.50 μM. Colorectal carcinoma cell lines were less sensitive to polyacetylenes in general with IC_{50} values above 100 μM for compound **2** against both cell lines and for compound **3** against HT2912 cells. However, compound **3** also displayed medium-level activity against HRT-18 cells, and compounds **1** and **4** displayed medium-level activities against both cell lines (IC_{50} values between 30 and 64 μM).

HPLC Analyses of Polyacetylenes 1−4 in Different Umbelliferous vegetables. For a preliminary estimation of polyacetylenes contained in different vegetables of the Apiaceae family, a comparative HPLC/DAD analysis was performed. HPLC signals were assigned to the four isolated polyacetylenes on the basis of their retention times, on-line UV spectra, and mass spectra. Falcarindiol (**2**) was detectable in all investigated

Table 3. Quantitative Estimation of Polyacetylenes **1–4** in Different Vegetables of the Apiaceae Family[a]

| taxon | amount of polyacetylene (mg/g of freeze-dried plant material) | | | |
	1	**2**	**3**	**4**
Apium graveolens I	0.23 (0.00)	2.07 (0.03)	0.17 (0.03)[b]	0.06 (0.00)[b]
Apium graveolens II	1.62 (0.06)	4.58 (0.09)	0.04 (0.00)[c]	0.02 (0.00)[c]
Daucus carota	0.29 (0.02)[c]	0.24 (0.03)[c]	nd	nd
Foeniculum vulgare	0.04 (0.00)[c]	0.24 (0.01)[c]	nd	nd
Pastinaca satica	1.60 (0.03)	5.77 (0.07)	nd	nd
Petroselinum crispum	nd	2.32 (0.02)	0.35 (0.01)[c]	0.12 (0.03)[c]

[a] Standard deviations are given in parentheses. nd, not detectable. *A. graveolens* sample I, accession number 2004-002; sample II, accession number 2004-003. [b] Compound identification based on retention time, UV spectrum, and actual isolation of compounds from this sample. [c] Compound identity based on retention time and UV spectrum only.

species and it also was the main polyacetylene in all investigated taxa except in *D. carota*, where falcarinol (**1**) was contained in slightly higher amounts (**Table 3**). Falcarinol was detectable in all investigated vegetables except parsley. Compounds **3** and **4** were detectable in minor amounts only and seem to be restricted to *A. graveolens* and *P. crispum*. The total amount of polyacetylenes was highest in our sample of *P. sativa* (>7.5 mg/g); in *A. graveolens* and *P. crispum* it was above 2.5 mg/g of dried plant material. In contrast, our samples of *D. carota* and *F. vulgare* contained only trace amounts (<0.3 mg/g) of falcarinol (**1**) and falcarindiol (**2**).

Pronounced differences in the contents of polyacetylenes between the two investigated samples of *A. graveolens* were observed (**Table 3**). These differences are not unexpected as polyacetylenes are phytoalexins, that is, compounds synthesized by the plants in response to different stresses, like microbial attack. Previous investigations of carrots proved that water stress (*43*), storage time (*44−45*), and genotype (*45*) have profound effects on the total polyacetylene content as well as on the relative contribution of each particular polyacetylene to the array of polyacetylenes contained in carrots.

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The quantitative estimates discussed above were obtained with 4-chlorobenzophenone as an internal standard by comparing peak areas of the internal standard compound with areas detected for compounds **1**−**4**. The results were calculated by use of the standard correction factor obtained for falcarinol (**1**), which was determined as 1.92 (SD = 0.02). However, the estimates based on this factor have to be treated with care, because compound purity of falcarinol was based on NMR and HPLC and "true" purity of the compound might differ considerably because of undetected impurities (i.e., compounds not absorbing UV light at 205 nm) or remaining traces of solvents from the isolation process.

Consumption of celery, parsley, and parsnip is expected to lead to an uptake of polyacetylenes in quantities where biological effects (e.g., chemopreventive effects) are occurring. The average amount of polyacetylenes contained in umbelliferous vegetables might be subject to considerable variation due to different cultural varieties present and effects of processing and storage on the content of polyacetylenes in these plants (*28*). Another point to be kept in mind is the frequency and amount of vegetables taken in by human consumers. Here carrots play a dominant role. Therefore, polyacetylenes from carrots will contribute the largest amount of polyacetylenes to the human diet, though our investigations indicate that other vegetables from the Apiaceae family may contain higher concentrations of these bioactive food constituents.

The so far unexplained paradox that high contents of natural carotenes in blood correlate with a low incidence of several types of cancer, while carotenes taken as food supplements do not have a positive effect (*46*, *47*), was linked by Brandt et al. (*5*) with the fact that carrots are the major source of carotenes in Europe and North America and that carrots are also the only known major food items that contain the bioactive polyacetylene falcarinol (**1**). These authors concluded that the content of polyacetylenes in carrots and facarinol (**1**) in particular might be responsible for the beneficial effects of carrot consumption. This implies that the observed negative correlation of low cancer risk with high intake of natural carotene is coincidental and produced by the co-occurrence of carotenes and polyacetylenes in carrots. If these findings can be verified, celery, parsley, and parsnip, which contain high amounts of bioactive polyacetylenes, will become promising ingredients of a diet aimed at cancer prevention.

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