Quantification of Polyacetylenes in Apiaceous Plants by High-Performance Liquid Chromatography Coupled with Diode Array Detection

Maike Kramer[a], Andrea Mühleis[a], Jürgen Conrad[b], Martin Leitenberger[a], Uwe Beifuss[b], Reinhold Carle[a], and Dietmar R. Kammerer[a,*]

[a] Institute of Food Science and Biotechnology, Chair of Plant Foodstuff Technology, Hohenheim University, Garbenstrasse 25, D-70599 Stuttgart, Germany. Fax: ++49-(0) 711-459-24110. E-mail: Dietmar.Kammerer@uni-hohenheim.de

[b] Institute of Chemistry, Section Bioorganic Chemistry, Hohenheim University, Garbenstrasse 30, D-70599 Stuttgart, Germany

* Author for correspondence and reprint requests

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Polyacetylenes are known for their biofunctional properties in a wide range of organisms. In the present study, the most frequently occurring polyacetylenes, *i.e.* falcarinol, falcarindiol, and falcarindiol-3-acetate, were determined in six genera of the Apiaceae family. For this purpose, a straightforward and reliable method for the screening and quantification of the polyacetylenes using high-performance liquid chromatography coupled with diode array and mass spectrometric detection without tedious sample clean-up has been developed. Peak assignment was based on retention times, UV spectra, and mass spectral data. Quantification was carried out using calibration curves of authentic standards isolated from turnip-rooted parsley and *Ligusticum mutellina*, respectively. The references were unambiguously identified by Fourier transform-IR (FT-IR) spectroscopy, GC-MS, HPLC-MS^n in the positive ionization mode, and ^{1}H NMR and ^{13}C NMR spectroscopy. To the best of our knowledge, the occurrence of falcarindiol-3-acetate in *Anthriscus sylvestris* and *Pastinaca sativa* has been reported for the first time. The data revealed great differences in the polyacetylene contents and varying proportions of individual compounds in the storage roots of Apiaceous plants. The results of the present study may be used as a suitable tool for authenticity control and applied to identify novel sources devoid or particularly rich in polyacetylenes, thus facilitating breeding programs for the selective enrichment and depletion of these plant secondary metabolites, respectively.

Key words: Apiaceae, Polyacetylenes, High-Performance Liquid Chromatography

Introduction

Plants of the Apiaceae family are divided into about 250 genera comprising approximately 2800 species, which are widely distributed all over the world (Rubatzky *et al.*, 1999). Carrots (*Daucus carota* L.), celeriac [*Apium graveolens* L. var. *rapaceum* (MILL.) GAUD.], turnip-rooted parsley [*Petroselinum crispum* (MILL.) NYM. convar. *radicosum* (ALEF.) DANERT var. *tuberosum* (BERNH.) CROV.], parsnip (*Pastinaca sativa* L.), *Ligusticum mutellina* (L.) CRANTZ, and cow parsley [*Anthriscus sylvestris* (L.) HOFFM.] are representatives of this family producing storage roots, which may be used for various purposes, such as for the production of food and pharmaceutical preparations. Apiaceous plants are not only interesting as food sources, but also from a pharmacological point of view due to their secondary metabolites. Among these compounds aliphatic acetylenes, such as falcarinol, falcarindiol, and falcarindiol-3-acetate (Fig. 1), are widely distributed also in other plant families.

Polyacetylenes of the falcarinol-type are known as highly bioactive compounds exhibiting considerable biological effects in a wide range of organisms. *Inter alia* antifungal (Garrod *et al.*, 1978; Harding and Heale, 1980), antimicrobial (Matsuura *et al.*, 1996; Rollinger *et al.*, 2003), anti-inflammatory (Metzger *et al.*, 2008), antituberculosis (Kobaisy *et al.*, 1997), anticancer, and cytotoxic (Kobæk-Larsen *et al.*, 2005; Matsunaga *et al.*, 1990) properties have been reported. Furthermore, they are notorious contact allergens (Hansen and Boll, 1986), also possessing neurotoxic properties (Crosby and Aharonson, 1967)

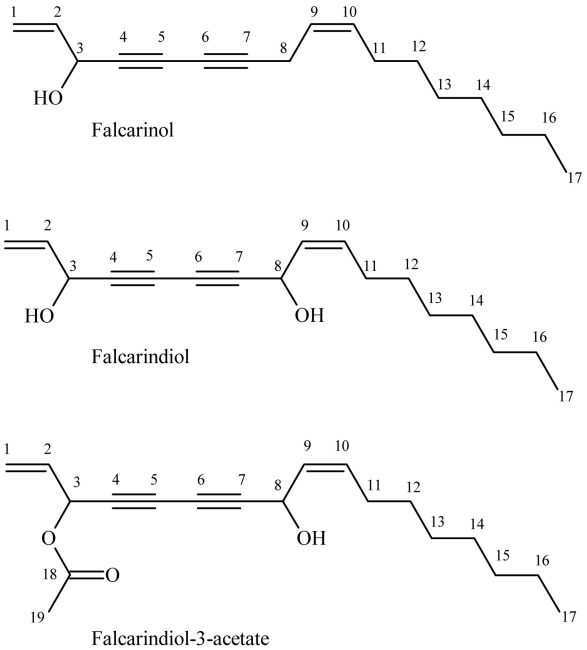

Fig. 1. Chemical structures of falcarinol, falcarindiol, and falcarindiol-3-acetate.

and causing bitter off-taste in carrots (Czepa and Hofmann, 2004). Due to these attributes and the hormetic effects of polyacetylenes, *i.e.* stimulating effects at low doses, whereas toxic properties may be observed at high concentrations (Hansen *et al.*, 2003), their isolation and quantification is of utmost importance to assess the bioactivity profile of plants and plant-derived products.

The polyacetylene accumulation in plant tissues strongly depends on biotic and abiotic factors, which are so far not fully understood. As an example, the polyacetylene contents of carrots have been shown to vary depending on growth conditions (Lund and White, 1990), type of root tissue (Baranska *et al.*, 2005), cultivar (Kidmose *et al.*, 2004), storage and processing conditions (Hansen *et al.*, 2003). Astonishingly, their contents were reported to vary between individual carrot roots grown under identical conditions (Christensen and Kreutzmann, 2007).

Polyacetylenes are of particular importance from a health promoting, sensory, and breeding point of view. Thus, the objective of the present study was the analyses of the three compounds falcarinol, falcarindiol, and falcarindiol-3-acetate in six different genera of the Apiaceae family to improve cultivation and storage conditions of plants with regard to the undesirable development of bitterness on the one hand and to assist breeding programs aiming at the production of plant material particularly rich in these putative health-beneficial compounds on the other hand. For this purpose, a straightforward, rapid, and reliable method for the unambiguous identification of the polyacetylenes falcarinol, falcarindiol, and falcarindiol-3-acetate had to be developed. The quantification of individual compounds should be based on authentic reference compounds, which are hardly commercially available. Therefore, their isolation from *Petroselinum crispum* convar. *radicosum* var. *tuberosum* and *Ligusticum mutellina*, their full characterization, and unequivocal identification using FT-IR spectroscopy, GC-MS, HPLC-MS^n, ^{1}H NMR and ^{13}C NMR spectroscopy were indispensable.

Material and Methods

Plant material

Carrot roots (*Daucus carota* L. cv. *Blanche 1/2 longue des Vosges*) were obtained from Julius Kühn-Institut (Quedlinburg, Germany), celeriac [*Apium graveolens* L. var. *rapaceum* (MILL.) GAUD. cv. *Goliath*] from Pharmaplant GmbH (Artern, Germany), turnip-rooted parsley [*Petroselinum crispum* (MILL.) NYM. convar. *radicosum* (ALEF.) DANERT var. *tuberosum* (BERNH.) CROV. cv. *Eagle*] from Clauss-Gemüse GbR (Esslingen, Germany), parsnip roots (*Pastinaca sativa* L. cv. *White King*) from the Experimental Station for Husbandry and Organic Farming of Hohenheim University (Stuttgart, Germany), and *Ligusticum mutellina* (L.) CRANTZ from Bärwurzerei Max Duschl (Lalling, Germany). Cow parsley [*Anthriscus sylvestris* (L.) HOFFM.] was collected in Werdau Forest (Saxony, Germany).

Sample preparation

The plant materials were manually cut into slices, lyophilized, ground with a knife mill GM200 (Retsch GmbH, Haan, Germany) to obtain a particle size below 0.5 μm, and stored at –20 °C until analysis. Aliquots of the freeze-dried samples (~1 g) were extracted with 30 ml of methanol in an ultrasonic bath for 15 min. The extracts were filtered, and the residues were re-extracted under continuous stirring (ambient temperature, 320 rpm) with 30 ml of methanol for 2 h. The combined filtered supernatants were dried over

anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* at 30 °C. The residues were dissolved in 5 ml (carrots), 10 ml (celeriac, turnip-rooted parsley, parsnip, and *L. mutellina*), and 30 ml of methanol (cow parsley), respectively, membrane-filtered (0.45 μm), and used for high-performance liquid chromatography with diode array detection (HPLC-DAD) analyses. To protect polyacetylenes from light-induced degradation reactions, all analyses were performed under dim light and with amber glass equipment. All analyses were carried out in duplicate.

HPLC-DAD conditions

All analyses were performed using a series 1100 HPLC system (Agilent, Waldbronn, Germany), equipped with a degasser, a binary gradient pump, a thermoautosampler, a column oven, and a diode array detector system controlled by Agilent ChemStation software (ver. A.09.03). Polyacetylene separation was performed using a Luna C_{18} column (250 mm x 3.0 mm i.d., 5 μm particle size; Phenomenex, Torrance, CA, USA) equipped with a C_{18} ODS guard column (4.0 mm x 2.0 mm i.d.), operated at a temperature of 40 °C and a flow rate of 0.5 ml/min. The mobile phase consisted of water (eluent A) and methanol (eluent B) using a gradient program as follows: 70% B to 90% B (29 min), 90% B to 100% B (4 min), 100% B isocratic (5 min), 100% B to 70% B (1 min), 70% B isocratic (5 min). Total run time was 44 min. The injection volume for all samples was 20 μl. Polyacetylenes were monitored at 205 nm and quantified using calibration curves of authentic standards.

Polyacetylene standard isolation

Polyacetylenes were isolated according to the procedure described by Hansen *et al.* (2003) and Kidmose *et al.* (2004) with a few modifications. All extraction and fractionation steps were performed under dim light to protect the polyacetylenes from light-induced degradation reactions. For the isolation of falcarinol and falcarindiol 6 kg of turnip-rooted parsley were cut into pieces (1 x 1 x 1 cm^3) and extracted twice with 8 l of ethyl acetate each for 24 h at 10 °C under continuous stirring. The combined ethyl acetate phases were dried with Na_2SO_4, filtered and concentrated *in vacuo* at 30 °C. The residue was dissolved in 100 ml of *n*-hexane and subsequently applied to a silica gel column (873 ml bed volume), eluting 1-l fractions with *n*-hexane, *n*-hexane/ethyl acetate mixtures (9:1, 4:1, 7:3, 1:1, 3:7, 1:4, v/v), ethyl acetate, and methanol, respectively. For the isolation of falcarindiol-3-acetate 600 g of *L. mutellina* roots were ground and extracted twice with 500 ml of ethyl acetate each. The combined extracts were concentrated *in vacuo*, dissolved in 45 ml of *n*-hexane and 5 ml of ethyl acetate, and fractionated as described for the parsley extract. The fractions obtained from both plant extracts were concentrated *in vacuo* to a volume of 200 ml each and subsequently analysed by GC-MS to identify and discard fractions being devoid of polyacetylenes. The fractions containing falcarinol, falcarindiol, and falcarindiol-3-acetate were further purified using silica cartridges (500 mg). Therefore aliquots of 5 ml of the concentrated fractions were evaporated to dryness, dissolved in 2 ml of *n*-pentane, and centrifuged at 1720 x *g* for 10 min. After applying the solutions to the cartridge the sorbent was washed with 5 ml of *n*-pentane/diethyl ether (60:40, v/v). The compounds were subsequently eluted with 5 ml of *n*-pentane/diethyl ether (95:5, v/v). After concentrating the eluates *in vacuo* the residues were dissolved in 2 ml methanol, membrane-filtered, and used for preparative HPLC (Bischoff, Leonberg, Germany). Separation and preparative isolation of the polyacetylenes were performed with a Phenomenex Aqua C_{18} column, 125 Å (250 mm x 21.2 mm i.d., 5 μm particle size), equipped with a C_{18} guard column (20 mm x 18 mm i.d.), operated at 20 °C. The mobile phase consisted of water (eluent A) and acetonitrile (eluent B) using a gradient program as follows: 65% B isocratic (5 min), 65% B to 75% B (20 min), 75% B isocratic (2 min), 75% B to 95% B (18 min), 95% B isocratic (5 min), 95% B to 20% B (5 min), 20% B to 65% B (10 min). Total run time was 65 min. The injection volume was 0.4 ml. Polyacetylenes were monitored at 205 nm at a flow rate of 7 ml/min. Falcarindiol, falcarindiol-3-acetate, and falcarinol eluted after 32, 44, and 51 min, respectively. The isolated compounds were concentrated *in vacuo* and dissolved in methanol for characterization by GC-MS, UV spectroscopy, and HPLC-MS^n, and in deutero-chloroform for identification by NMR spectroscopy, respectively.

Polyacetylene characterization

GC-MS analyses were carried out using an Agilent 6890 N GC system equipped with a mass

selective detector model MSD Inert 5975. The column employed was an HP-5 (Agilent) MS 5% phenyl-methyl-siloxane (30 m x 0.25 mm i.d.). Helium was used as carrier gas at a constant flow rate of 0.9 ml/min. The injector and interphase temperature were set at 280 °C. Samples were injected in the splitless mode. The column temperature was kept at 60 °C for 2 min, subsequently increased to 260 °C at a rate of 10 °C/min, and held at 260 °C for 15 min. The mass spectrometer was operated in a scan range of *m/z* 40 to 320.

FT-IR analyses were performed using a Fourier transform middle infrared Spectrum 1000 spectrometer (Perkin-Elmer, Norwalk, CT, USA) equipped with Perkin-Elmer Spectrum software (version 3.02.00). Using sodium chloride plates, samples were scanned from 4000 to 600 cm^{-1} in 2.0-cm^{-1} intervals with a resolution of 4.0 cm^{-1}. Each sample was scanned 16 times to obtain average MIR spectra. Background correction of the spectra was performed using a nujol blank sample.

HPLC-DAD-MS^n analyses were performed using an Agilent HPLC series 1100 system coupled on-line with a Bruker (Bremen, Germany) Esquire 3000+ ion trap mass spectrometer fitted with an APcI source. Data acquisition and processing were performed using Esquire Control software. The separation was performed with a Phenomenex Luna C_{18} column (250 mm x 3.0 mm i.d., 5 μm particle size) equipped with a C_{18} ODS guard column (4.0 mm x 2.0 mm i.d.), operated at 40 °C. The mobile phase consisted of water (eluent A) and methanol (eluent B) using a gradient program as follows: 55% B to 60% B (3.3 min), 60% B to 90% B (33.3 min), 90% B to 95% B (3.3 min), 95% B isocratic (5 min), 95% B to 55% B (1 min), 55% B isocratic (5 min). Total run time was 51 min. The injection volume for all samples was 5 μl. Polyacetylenes were monitored at 205 nm at a flow rate of 0.5 ml/min. UV/Vis spectra were obtained using a model G1315B diode array detector and recorded in a range of 200–450 nm at a spectral acquisition rate of 1.25 scans/s (peak width 0.2 min). Positive ion mass spectra of the column effluent were recorded in the range *m/z* 50–1000 at a scan speed of 13000 Th/s (peak width 0.6 Th, FWHM). Nitrogen was used both as drying gas at a flow rate of 5.0 l/min and as nebulizing gas at a pressure of 50.0 psi. The nebulizer and vaporizer temperatures were set at 350 and 475 °C, respectively. Helium was used as collision gas for collision-induced dissociation (CID). The fragmentation amplitude was 1.5 V. Polyacetylene purity was calculated based on the peak areas of the chromatograms at 205 nm.

NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer (Darmstadt, Germany). ^1H and ^{13}C chemical shifts were referenced to residual solvent signals at δ 7.27 ppm (^1H) and 77.0 ppm (^{13}C) relative to TMS. ^1H, $^{13}C\{^1H\}$, ROESY, LR-COSY NMR spectra were measured with standard Varian pulse sequences. Adiabatic gH2BAD, adiabatic broadband gHSQC and gHMBC spectra were recorded using CHEMPACK 4.0 pulse sequences (implemented in Varian Vnmrj 2.1B software).

Falcarinol [(9*Z*)-heptadeca-1,9-dien-4,6-diyn-3-ol, $C_{17}H_{24}O$]: UV: λ_{max} = 231, 243, 257 nm. – IR: ν_{max} = 3370 (-OH), 3088 (-CH=CH_2), 3022 (-CH=CH-), 2956 (-CH_3), 2927 [-CH_2- (C-H ν_{as})], 2856 [-CH_2- (C-H ν_s)], 2255 (-C≡C-), 1867 (-CH=CH_2), 1644 (-CH=CH-), 1465 [-C-H (δ)], 1417 (-CH_2-), 1378 [-CH_3 (δ_s)], 1285 [-OH (C-O-stretching)], 1226 (-C-O), 1117 (-C-OH), 1016 (-C-O), 984 (-CH=CH_2), 931 (-CH=CH_2, -C-H), 904 (-CH=CH-), 876 (-CH=CH-), 759 (-CH=CH-), 701 (-CH=CH_2, -CH=CH-) cm^{-1}. – GC-MS: *m/z* (%) = 91 (100), 55 (93), 115 (88), 41 (80), 43 (69), 117 (68), 129 (51), 77 (49), 131 (47), 128 (41), 159 (41), 103 (38), 141 (36), 78 (32), 116 (32), 65 (30), 79 (29), 105 (29), 81 (27), 145 (26). – ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.89 [3H, *t*, *J* = 6.7 Hz, H-C(17)], 1.24–1.30 [8H, ov, H-C(13–16)], 1.34–1.40 [2H, *m*, H-C(12)], 2.03 [2H, *dt*, *J* = 7.2 Hz, H-C(11)], 3.04 [2H, *d*, *J* = 7.1 Hz, H-C(8)], 4.92 [1H, *bd*, *J* = 5.5 Hz, H-C(3)], 5.25 [1H, *bdd*, *J* = 1.3, 11.0 Hz, Ha-C(1)], 5.35–5.41 [1H, *m*, H-C(9)], 5.48 [1H, *bdd*, *J* = 1.5, 17.1 Hz, Hb-C(1)], 5.49–5.55 [1H, *m*, H-C(10)], 5.95 [1H, *ddd*, *J* = 5.5, 10.3, 17.0 Hz, H-C(2)]. – ^{13}C NMR ($CDCl_3$, 125 MHz) (a, b, assignments may be interchanged): δ = 14.09 [CH_3, C(17)], 17.68 [CH_2, C(8)], 22.63b [CH_2, C(16)], 27.19 [CH_2, C(11)], 29.14a [CH_2, C(12)], 29.17a [CH_2, C(13)], 29.22a [CH_2, C(14)], 31.81b [CH_2, C(15)], 63.55 [CH, C(3)], 63.99 [C, C(6)], 71.31 [C, C(5)], 74.20 [C, C(4)], 80.30 [C, C(7)], 117.04 [CH_2, C(1)], 121.89 [CH, C(9)], 133.11 [CH, C(10)], 136.15 [CH, C(2)].

Falcarindiol [(9*Z*)-heptadeca-1,9-dien-4,6-diyn-3,8-diol, $C_{17}H_{24}O_2$]: UV: λ_{max} = 233, 246, 259 nm. – IR: ν_{max} = 3338 (-OH), 3090 (-CH=CH_2), 3022 (-CH=CH-), 2956 (-CH_3), 2927 [-CH_2- (C-H ν_{as})],

2856 [-CH_2- (C-H ν_s)], 2252 (-C≡C-), 2151 (-C≡C), 1868 (-CH=CH_2), 1653 (-CH=CH-), 1458 [-C-H (δ)], 1407 (-CH_2-), 1378 [-CH_3 (δ_s)], 1303 (-OH in plane), 1268 [-OH (C-O-stretching)], 1118 (-C-OH), 1088 (-C-O), 1015 (-C-O), 986 (-CH=CH_2), 933 (-CH=CH_2, -C-H), 879 (-CH=CH-), 782 (-CH=CH-), 722 [-CH_2- (δ)], 668 (*cis*-CH=CH-) cm^{-1}. – GC-MS: *m/z* (%) = 55 (100), 41 (96), 129 (92), 91 (89), 128 (81), 77 (79), 43 (77), 115 (77), 79 (41), 105 (33), 157 (31), 127 (30), 65 (28), 78 (28), 103 (27), 117 (27), 51 (26), 53 (26), 57 (26), 63 (22). – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.9 [3H, *t*, *J* = 7.2 Hz, H-C(17)], 1.27–1.34 [8H, ov, H-C(13–16)], 1.36–1.43 [2H, *m*, H-C(12)], 2.12 [2H, *bq*, *J* = 7.5 Hz, H-C(11)], 4.96 [1H, *bd*, *J* = 5.5 Hz, H-C(3)], 5.22 [1H, *d*, *J* = 8.4 Hz, H-C(8)], 5.28 [1H, *bd*, *J* = 10.0 Hz, Ha-C(1)], 5.49 [1H, *d*, *J* = 17.4 Hz, Hb-C(1)], 5.53 [1H, *bdd*, *J* = 8.1, 9.9 Hz, H-C(9)], 5.63 [1H, *ddt*, *J* = 1.1, 7.7, 10.5 Hz, H-C(10)], 5.96 [1H, *ddd*, *J* = 4.9, 10.1, 17.0 Hz, H-C(2)]. – ^{13}C NMR ($CDCl_3$, 125 MHz) (a, b, assignments may be interchanged): δ = 14.08 [CH_3, C(17)], 22.62b [CH_2, C(16)], 27.68 [CH_2, C(11)], 29.09a [CH_2, C(13)], 29.14a [CH_2, C(14)], 29.26a [CH_2, C(12)], 31.78b [CH_2, C(15)], 58.61 [CH, C(8)], 63.50 [CH, C(3)], 68.73 [C, C(6)], 70.44 [C, C(5)], 78.30 [C, C(4)], 80.10 [C, C(7)], 117.34 [CH_2, C(1)], 127.64 [CH, C(9)], 134.72 [CH, C(10)], 135.78 [CH, C(2)].

Falcarindiol-3-acetate [(9*Z*)-3-acetoxyheptadeca-1,9-dien-4,6-diyn-8-ol, $C_{19}H_{26}O_3$]: UV: λ_{max} = 234, 247, 261 nm. – IR: ν_{max} = 3446 (-OH), 3093 (-CH=CH_2), 3022 (-CH=CH-), 2956 (-CH_3), 2927 [-CH_2- (C-H ν_{as})], 2856 [-CH_2- (C-H ν_s)], 2255 (-C≡C-), 2157 (-C≡C-), 1748 (-O-acetate), 1733 (-O-acetate), 1653 (-CH=CH-), 1457 [-C-H (δ)], 1371 [-CH_3 (δ_s)], 1221 (-C-O), 1096 (-C-O), 1015 (-C-O), 975 (-CH=CH_2), 940 (-CH=CH_2, -C-H), 909 (-CH=CH-), 720 [-CH_2- (δ)], 668 (*cis*-CH=CH-) cm^{-1}. – GC-MS: *m/z* (%) = 43 (100), 57 (87), 41 (76), 55 (75), 98 (60), 129 (55), 116 (54), 69 (34), 84 (32), 83 (25), 56 (24), 42 (23), 71 (20), 97 (19), 112 (19). – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.89 [3H, *t*, *J* = 6.7 Hz, H-C(17)], 1.23–1.35 [8H, ov, H-C(13–16)], 1.36 – 1.43 [2H, *m*, H-C(12)], 1.84 [1H, *d*, *J* = 5.1 Hz, HO-C(8)], 2.11 [3H, *s*, H-C(19)], 2.12 [2H, *bq*, *J* = 7.5 Hz, H-C(11)], 5.21 [1H, *dd*, *J* = 4.9, 8.4 Hz, H-C(8)], 5.35 [1H, *d*, *J* = 9.7 Hz, Ha-C(1)], 5.52 [1H, *bdd*, *J* = ~10.0 Hz, H-C(9)], 5.55 [1H, *d*, *J* = 17.2 Hz, Hb-C(1)], 5.62 [1H, *ddt*, *J* = 0.8, 7.4, 10.3 Hz, H-C(10)], 5.87 [1H, *ddd*, *J* = 5.9, 9.7, 16.9 Hz, H-C(2)], 5.93 [1H, *d*, *J* = 5.9 Hz, H-C(3)]. – ^{13}C NMR ($CDCl_3$, 125 MHz) (a, b, c, assignments may be interchanged): δ = 14.07 [CH_3, C(17)], 20.84 [CH_3, C(19)], 22.61a [CH_2, C(16)], 27.68 [CH_2, C(11)], 29.07b [CH_2, C(14)], 29.13b [CH_2, C(13)], 29.24 [CH_2, C(12)], 31.76a [CH_2, C(15)], 58.58 [CH, C(8)], 64.41 [CH, C(3)], 68.59c [C, C(6)], 70.81c [C, C(5)], 74.34 [C, C(4)], 79.99 [C, C(7)], 119.70 [CH_2, C(1)], 127.53 [CH, C(9)], 131.91 [CH, C(2)], 134.78 [CH, C(10)], 169.43 [C, C(18)].

Results and Discussion

Isolation and identification of polyacetylene standards

Falcarinol and falcarindiol were isolated from the ethyl acetate extract of turnip-rooted parsley and falcarindiol-3-acetate from a *L. mutellina* extract. The isolated compounds were obtained as colourless oils showing an increasing yellowish tint upon storage at ambient temperature. The purity of all isolated compounds as deduced from the peak areas at 205 nm exceeded 98%. The structures of the three polyacetylenes were characterized by their UV spectra, fragment ions obtained in HPLC-MS^n and GC-MS experiments, by FT-IR, and unambiguously confirmed by 1D (^{1}H, ^{13}C) and 2D NMR (ROESY, LR-COSY, gH2BAD, gHSQC, gHMBC) spectra. The results and complete interpretation of the analytical data are specified in the Material and Methods section and in Table I. The data are in close agreement with literature reports (Czepa and Hofmann, 2003; Schulte and Pötter, 1977; Spitaler *et al.*, 2002).

In contrast to previous investigations of polyacetylenes, the mass spectrometric behaviour of the compounds (Table I) was thoroughly studied. Upon ionization using an APcI interface in the positive ionization mode the polyacetylenes readily produced in-source fragments by the loss of a water molecule and, thus, were even detected as base peaks in the MS^1 experiments (Pferschy-Wenzig *et al.*, 2009; Zschocke *et al.*, 1998). The quasi-molecular ion $[M+H]^+$ was only observed for falcarinol. The fragmentation behaviour of falcarindiol, *i.e.* all signals of the MS^2 experiments except for *m/z* 95, indicated the preferred cleavage of the OH function at C-8.

Additionally, all compounds revealed the formation of adducts with acetonitrile which is at-

Table I. LC-MS data of falcarinol, falcarindiol, and falcarindiol-3-acetate and ion assignments for the MS^1 and MS^2 experiments.

Falcarinol ($C_{17}H_{24}O$, molecular weight 244.377 Da)					
MS^1			MS^2 (*m/z* 227)		
m/z	Rel. int. (%)	Tentative assignment	*m/z*	Rel. int. (%)	Tentative assignment
227	100	$[M+H-H_2O]^+$	157	100	$[M+H-H_2O+MeOH-C_8H_6]^+$
245	46	$[M+H]^+$	199	49	$[M+H-H_2O-C_2H_4]^{+\,b}$
268	46	$[M+H-H_2O+MeCN]^+$	143	41	$[M+H-H_2O-C_6H_{12}]^{+\,b}$
228	21	$[M+H-H_2O]^{+\,a}$	145	35	$[M+H-H_2O+MeOH-C_9H_6]^{+\,b}$
243	13	$[M-1]^+$	91	29	$[M+H-H_2O+2MeOH-C_{15}H_{20}]^+$
			79	29	$[M+H-H_2O+MeOH+Na-C_9H_{17}]^{2+}$
			119	21	$[M+H-H_2O+MeOH+Na-C_3H_7]^{2+}$
			185	20	$[M+H-H_2O\text{-}C_3H_6]^{+\,b}$
			131	19	$[M+H-H_2O+MeOH-C_{10}H_8]^+$
			117	15	$[M+H-H_2O+MeOH-C_{11}H_{10}]^+$
Falcarindiol ($C_{17}H_{24}O_2$, molecular weight 260.376 Da)					
MS^1			MS^2 (*m/z* 243)		
m/z	Rel. int. (%)	Tentative assignment	*m/z*	Rel. int. (%)	Tentative assignment
243	100	$[M+H-H_2O]^+$	173	100	$[M+H-H_2O-C_5H_{10}]^{+\,b}$
225	40	$[M+H-2H_2O]^+$	159	86	$[M+H-H_2O-C_6H_{12}]^{+\,b}$
284	24	$[M+H-H_2O+MeCN]^+$	133	74	$[M+H-H_2O+2MeOH-C_{13}H_{18}]^+$
244	18	$[M+H-H_2O]^{+\,a}$	145	64	$[M+H-H_2O+2MeOH-C_{12}H_{18}]^+$
467	15	$[(M-H_2O)_2-H_2O+H]^+$	169	58	$[M+H-H_2O+2MeOH-C_{10}H_{18}]^+$
			117	50	$[M+H-H_2O+MeOH-C_{11}H_9OH]^+$
			93	48	$[M+H-H_2O-C_{11}H_{18}]^+$
			162	44	$[M+H-H_2O-C_5H_4OH]^+$
			95	39	$[M+H-H_2O+Na-C_6H_4]^{2+}$
			160	39	$[M+H-H_2O-C_6H_{11}]^{+\,b}$
Falcarindiol-3-acetate ($C_{19}H_{26}O_3$, molecular weight 302.414 Da)					
MS^1			MS^2 (*m/z* 285)		
m/z	Rel. int. (%)	Tentative assignment	*m/z*	Rel. int. (%)	Tentative assignment
285	100	$[M+H-H_2O]^+$	145	100	$[M+H-H_2O+MeOH-C_9H_5CH_3COO]^{+\,b}$
243	87	$[M+H-CH_3COOH]^+$	243	76	$[M+H-H_2O-C_3H_6]^{+\,b}$
225	36	$[M+H-H_2O-CH_3COOH]^+$	141	45	$[M+H-H_2O+Na-C_2H_3]^{2+}$
244	24	$[M+H-CH_3COOH]^{+\,a}$	155	40	$[M+H-H_2O+MeOH-C_{12}H_{18}]^+$
284	18	$[M+H-CH_3COOH+MeCN]^+$	173	34	$[M+H-H_2O-C_8H_{16}]^+$
			256	32	$[M+H-H_2O-C_2H_5]^+$
			131	28	$[M+H-H_2O+MeOH-C_{10}H_7CH_3COO]^+$
			117	26	$[M+H-H_2O+MeOH-C_{11}H_9CH_3COO]^+$
			170	26	$[M+H-H_2O+MeOH-C_7H_4CH_3COO]^+$
			207	24	not assigned

[a] Isotopologue containing a ^{13}C isotope.
[b] Rearrangement of the C(9,10) double bond.

tributed to residual solvent originating from the previous purification step. Astonishingly, falcarinol generated an ion at *m/z* 243. Kite *et al.* (2006) also observed such $[M-1]^+$ species when analysing the polyacetylenes 2,3-dihydro-oenanthotoxin and oenanthotoxin from *Oenanthe crocata* L., suggesting either hydride abstraction $[M-H]^+$ or dehydrogenation of the protonated molecule $[(M+H)-H_2]^+$. The formation of adducts with methanol, sodium ions or both as reported by

Kite *et al.* (2006), Pferschy-Wenzig *et al.* (2009), and Zschocke *et al.* (1998) was confirmed for all three compounds. In addition, the fragmentation patterns of the compounds indicate that the localization of the C(9,10) double bond is not fixed under the conditions of ionization and fragmentation applied. It rather appears that rearrangement of the unsaturation may occur, which has also been observed for unsaturated carbon chains of other compound classes (Knödler *et al.*, 2008).

Identification and quantification of polyacetylenes in Apiaceous plants

To assess the contents of the polyacetylenes falcarinol, falcarindiol, and falcarindiol-3-acetate contained in different storage roots of Apiaceous plants comparative HPLC-DAD analyses were performed with the root extracts. Perfect chromatographic separation of the three polyacetylenes was achieved within 44 min (Fig. 2). Peak assignment was corroborated by HPLC-MS^n analyses, and quantification was carried out by external calibration with authentic standards.

Table II summarizes the amounts of falcarinol, falcarindiol, and falcarindiol-3-acetate found in the investigated roots. Total amounts of the quantified polyacetylenes excelled in *Anthriscus sylvestris* [3.8 g/kg dry matter (DM)], while, celeriac only contained trace amounts (<0.1 g/kg DM) of polyacetylenes.

Comparison of these data revealed marked differences in the contents and composition of polyacetylenes among the genera of the Apiaceae. As an example, falcarindiol was the predominant compound in cow parsley amounting to 94% of the quantified polyacetylene contents. In carrot roots the falcarindiol content was also approx. four times exceeding the falcarinol contents, whereas in celeriac, falcarinol dominated with 63% of the quantified polyacetylenes, and its proportion in *Ligusticum mutellina* only amounted to 10%. Maximum falcarinol amounts were observed in turnip-rooted parsley. Furthermore, falcarindiol-3-acetate was only detected in minor amounts in carrots and parsnips, while turnip-rooted parsley and celeriac were devoid of the acylated compound. In contrast, *L. mutellina* and cow parsley were particularly rich sources of falcarindiol-3-acetate, representing 58% and only 6% of the quantified polyacetylenes, respectively, despite comparatively equal absolute amounts of this compound in both roots. Most interestingly, to the best of our knowledge, this is the first report of falcarindiol-3-acetate occurrence in the genera *Anthriscus* and *Pastinaca*, respectively.

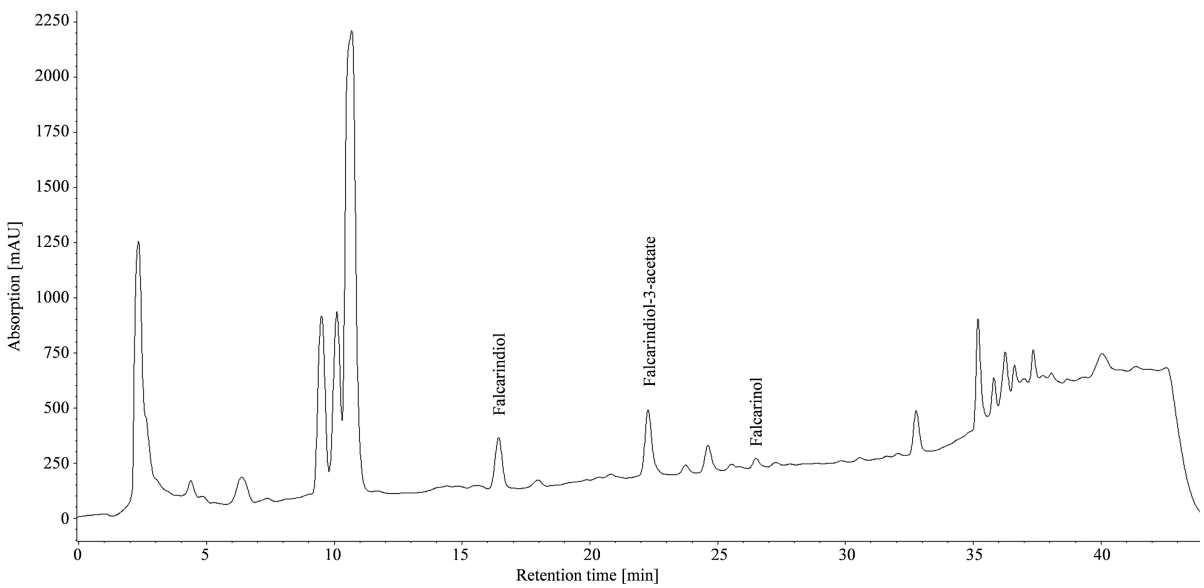

Fig. 2. HPLC chromatogram of *Ligusticum mutellina* extract (205 nm; retention times are 16.5 min, 22.3 min, and 26.5 min for falcarindiol, falcarindiol-3-acetate, and falcarinol, respectively).

Table II. Polyacetylene contents in roots of Apiaceous plants.

Plant material	Polyacetylene content (mg/kg DM)[a]		
	Falcarinol	Falcarindiol	Falcarindiol-3-acetate
Daucus carota L. cv. *Blanche ½ longue des Vosges*	82.23 ± 0.48	315.10 ± 2.23	32.33 ± 1.40
Apium graveolens L. var. *rapaceum* (MILL.) GAUD. cv. *Goliath*	50.14 ± 0.88	29.80 ± 2.20	n.d.[b]
Anthriscus sylvestris (L.) HOFFM.	28.28 ± 0.04	3555.00 ± 4.40	207.31 ± 4.18
Ligusticum mutellina (L.) CRANTZ	50.95 ± 1.45	161.33 ± 2.48	297.35 ± 0.36
Petroselinum crispum (MILL.) NYM. convar. *radicosum* (ALEF.) DANERT var. *tuberosum* (BERNH.) CROV. cv. *Eagle*	629.35 ± 0.30	402.74 ± 1.16	n.d.[b]
Pastinaca sativa L. cv. *White King*	164.74 ± 0.06	240.18 ± 0.29	2.88 ± 0.01

[a] Mean ± standard error of the mean; $n = 2$.
[b] n.d., not detected.

Previous studies have shown falcarinol contents in celeriac ranging from 21.5–253.4 mg/kg DM and falcarindiol contents from 0–125.5 mg/kg DM (Jabłońska-Ryś, 2007). For carrots, various data have been reported within the range of 45.7–290 mg/kg DM and 3.1–67.0 mg/kg fresh weight (FW) for falcarinol, 4.8–69.5 mg/kg FW for falcarindiol, and 4.8–40.4 mg/kg FW for falcarindiol-3-acetate (Hansen *et al.*, 2003; Kidmose *et al.*, 2004; Kreutzmann *et al.*, 2008; Lund and White, 1990; Pferschy-Wenzig *et al.*, 2009; Zidorn *et al.*, 2005). These obvious variations in polyacetylene contents are likely to be cultivar-related and due to pre- and postharvest treatments as well as to methodological differences, respectively. According to the findings presented in Table II polyacetylene proportions may be a suitable tool for authentication of Apiaceous plant materials, which may easily be mixed up due to morphological similarities and identical habitats. Thus, the method presented may also be applied to the authenticity control of pharmaceutical and food preparations or spices based on their polyacetylene profile.

Conclusions

The contents of the three polyacetylenes varied within a wide range when six different genera of Apiaceous plants were compared. The polyacetylenes falcarinol and falcarindiol were detected in different ratios in all investigated storage roots. Falcarindiol-3-acetate was found in high amounts in *Ligusticum mutellina* and *Anthriscus sylvestris*, and its occurrence in the latter and in *Pastinaca sativa*, respectively, was described for the first time.

Since polyacetylenes display health-promoting and hormetic effects but are also associated with bitter taste, their quantification is of utmost relevance for the food and pharmaceutical industry. For exploiting the potential of polyacetylenes high yield and reliable sources for their recovery can be identified, and breeding programs to foster or suppress polyacetylene accumulation may be monitored using the method presented here, which may further enhance bioavailability and bioactivity studies and the production of foods devoid or even enriched in such compounds. Furthermore, more detailed knowledge of the polyacetylene profiles of different Apiaceous plants can be used for classification purposes and for authenticity control.

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