Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 683–693
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Review

Bioactive polyacetylenes in food plants of the Apiaceae family: Occurrence, bioactivity and analysis

Lars P. Christensen [a,*], Kirsten Brandt [b]

[a] *Department of Food Science, Danish Institute of Agricultural Sciences, Research Centre Aarslev, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark*
[b] *School of Agriculture, Food and Rural Development, University of Newcastle upon Tyne, Agriculture Building, Newcastle upon Tyne NE1 7RU, UK*

Received 10 December 2005; received in revised form 29 January 2006; accepted 30 January 2006
Available online 7 March 2006

Abstract

Many bioactive compounds with known effects on human physiology and disease have been identified through studies of plants used in traditional medicine. Some of these substances occur also in common food plants, and hence could play a significant role in relation to human health. Food plants of the Apiaceae plant family such as carrots, celery and parsley, contain a group of bioactive aliphatic C_{17}-polyacetylenes. These polyacetylenes have shown to be highly toxic towards fungi, bacteria, and mammalian cells, and to display neurotoxic, anti-inflammatory and anti-platelet-aggregatory effects and to be responsible for allergic skin reactions. The effect of these polyacetylenes towards human cancer cells, their human bioavailability and their ability to reduce tumour formation in a mammalian in vivo model indicates that they may also provide benefits for health. The present state of knowledge on the occurrence of polyacetylenes in Apiaceae food plants, their biochemistry and bioactivity is presented in this review as well as relatively new methods for the isolation and quantification of these compounds from plants, plant products and biological fluids.

Keywords: Apiaceae food plants; Bioactive polyacetylenes; Analysis; HPLC; LC–MS; GC

Contents

* Corresponding author. Tel.: +45 89 99 33 67; fax: +45 89 99 34 95.
E-mail address: larsp.christensen@agrsci.dk (L.P. Christensen).

0731-7085/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.jpba.2006.01.057

1. Introduction

Through epidemiological studies it is well known that a high consumption of vegetables and fruits protect against certain types of cancer and other important diseases [1–5]. In order to explain the health promoting effects of fruit and vegetables focus has primarily been on vitamins, minerals, fibres and antioxidants, but still we do not know which components are responsible for these effects of food plants. A possible explanation could be that plants contain other bioactive compounds, i.e. compounds having a direct or indirect effect on living tissue in vitro and/or in vivo, which provide benefits for health, even though they are not essential nutrients [6].

Plants contain a great number of different secondary metabolites, many of which display biological activity as "natural pesticides" with a role in plant defence against, e.g. insects, fungi and other microorganisms. Many bioactive substances with known effects on human physiology and disease have been identified through studies of plants used in traditional medicine. Some of these plants are also food plants, or the same compounds also occur in food plants, although many of these bioactive compounds are normally considered undesirable in human food due to their potentially toxic effects in high concentrations (toxicant properties). However, a low daily intake of these "toxins" may be an important factor in the search for an explanation of the beneficial effects of fruit and vegetables on human health [6].

Polyacetylenes are examples of bioactive secondary metabolites that have been considered undesirable in plant foods due to their toxicant properties. Some polyacetylenes are known to be potent skin sensitizers, and to be neurotoxic in high concentrations, but have also been shown to have a pronounced selective cytotoxic activity against cancer cells.

Some of the most bioactive polyacetylenes are found in the Apiaceae family, which in addition to some well-known medicinal and toxic plants also includes common food plants such as carrot, celery, and parsley. This review highlights the present state of knowledge on the occurrence of naturally occurring polyacetylenes in the edible parts of food plants of the Apiaceae plant family, including their biochemistry, bioactivity, analysis and possible relevance for human health.

2. Distribution and biosynthesis of polyacetylenes in Apiaceae food plants

Polyacetylenes form a distinct group of relatively chemically reactive natural products, and more than 1400 different polyacetylenes and related compounds have been isolated from higher plants. They are widely distributed in the families Apiaceae (formerly Umbelliferae), Araliaceae, and Asteraceae (formerly Compositae) and have been found sporadically in 21 other families [7–11]. Aliphatic C_{17}-polyacetylenes of the falcarinol-type such as falcarinol (**1**) and falcarindiol (**2**) (Fig. 1), are widely distributed in the Apiaceae and Araliaceae [7,8], and consequently nearly all polyacetylenes found in the utilized/edible parts of food plants of the Apiaceae, such as carrot, celeriac, parsnip and parsley are of the falcarinol-type (Fig. 1, Table 1). Falcarinol-type polyacetylenes are less common in food plants of other plant families, although they have been found in tomatoes and aubergines of the Solanaceae [12–14].

The structures of most polyacetylenes indicate biosynthesis from unsaturated fatty acids. Feeding experiments with ^{14}C- and ^{3}H-labelled precursors have confirmed this assumption and shown that they are built up from acetate and malonate units [7–11,15–19]. Polyacetylenes of the falcarinol-type are formed from oleic acid by dehydrogenation leading to the C_{18}-acetylenes crepenynic acid and dehydrocrepenynic acid, which is then transformed to C_{17}-acetylenes by β-oxidation. Further oxidation and dehydrogenation leads to falcarinol and related C_{17}-acetylenes of the falcarinol-type [7,8].

3. Bioactivity of polyacetylenes in Apiaceae food plants

3.1. Antifungal activity

Falcarinol (**1**) and falcarindiol (**2**) have been identified as antifungal compounds in many Apiaceae plant species inhibiting spore germination of different fungi in concentrations ranging from 20 to 200 μg/ml [8,20–25]. Polyacetylenes of the falcarinol-type tend to be present constitutively, indicating that they act primarily as pre-infectional compounds in the species producing them, although some increase can be observed in response to infections [20–23]. Polyacetylenes of the falcarinol-type has also been shown to be phytoalexins in common food plants of the Solanaceae, such as tomatoes [12,13] and aubergines [14].

3.2. Neurotoxicity

The acetylenes oenanthotoxin (**15**) and cicutoxin (**16**) (Fig. 2) isolated from water-hemlock (*Cicuta virosa* L.), spotted water-hemlock (*C. maculata* L.), and from the hemlock water-dropwort (*Oenanthe crocata* L.), of the Apiaceae family [26–29] are extremely poisonous causing violent convulsions and death [26]. The toxicity of the closely related poison hemlock (*Conium maculatum* L.) is ascribed to alkaloids such as coniine [30]. The acetylenes **15** and **16** are closely related to the polyacetylenes **10–13** found in dill and/or ajowan (Table 1, Fig. 1). However, a comparative study on the mechanism of the convulsive action [31] indicated that the effect requires a quite specific distance between two OH-groups of the molecule, indicating that acetylenes **10–13** do not have similar toxic effects as the acetylenes **15** and **16**.

The neurotoxicity of falcarinol (**1**) has been demonstrated upon injection into mice (LD_{50} = 100 mg/kg) whereas no acute

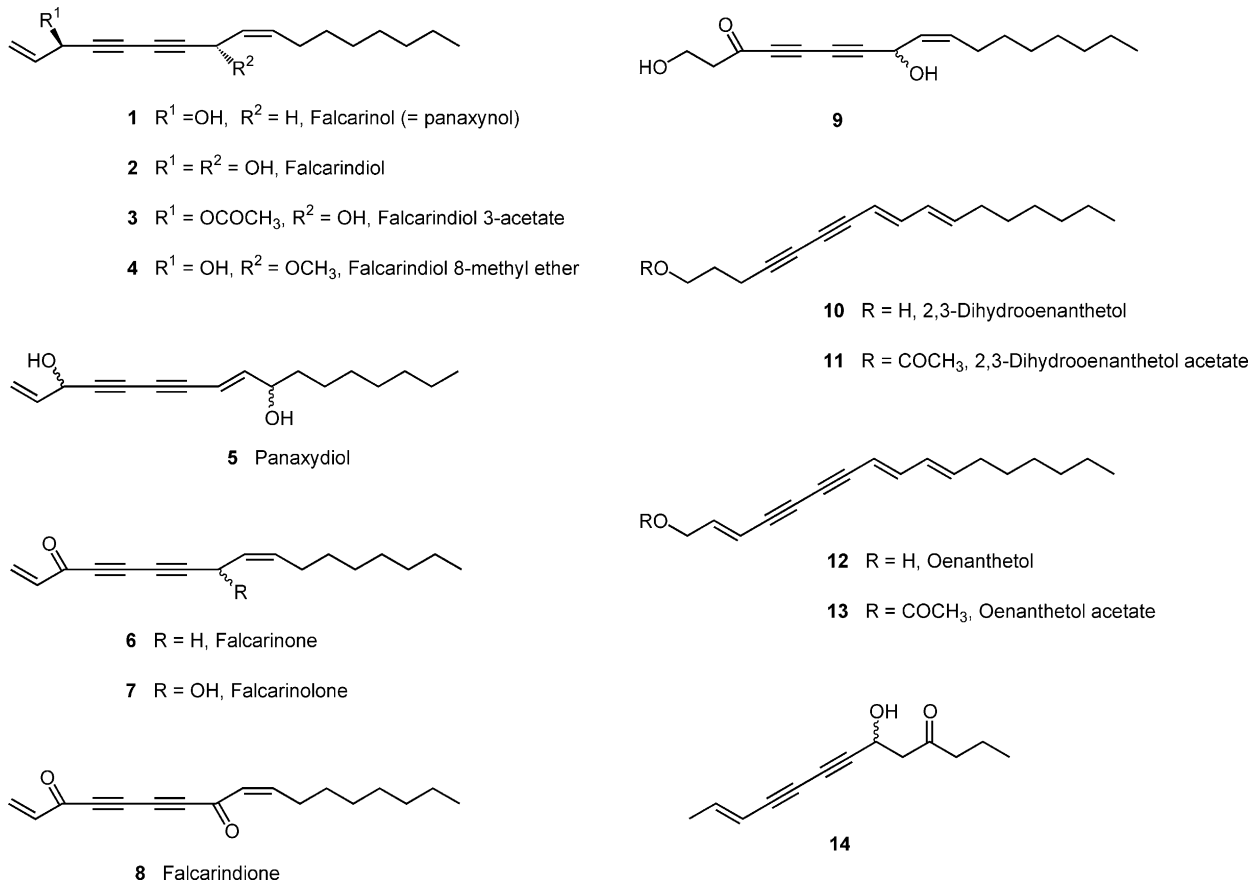

Fig. 1. Aliphatic acetylenes isolated from the utilized parts of Apiaceae food plants.

effects have been demonstrated for the related falcarindiol (**2**) [32]. The type of neurotoxic symptoms produced by falcarinol is similar to those of oenanthotoxin and cicutoxin, although a much higher dose is required, and no poisoning of mammals has been reported from voluntary ingestion of natural sources.

3.3. Allergenicity

Many plants containing aliphatic C_{17}-polyacetylenes have been reported to cause allergic contact dermatitis (ACD, Type IV allergy) and irritant skin reactions [33]. It has been shown that falcarinol (**1**) is responsible for most of the allergic skin reactions caused by plants of the Apiaceae and Araliaceae [33–37], whereas related polyacetylenes such as falcarindiol (**2**) and falcarinone (**6**) (Fig. 1) do not seem to be allergenic [34]. The allergenic properties of falcarinol indicate that it can form a hapten–protein complex (antigen). This is probably due to its hydrophobicity and its ability to form an extremely stable carbocation with the loss of water, thereby acting as a very reactive alkylating agent towards mercapto and amino groups in proteins and other biomolecules. This mechanism may also explain its anti-inflammatory and antibacterial effect (Section 3.4), its cytotoxicity (Section 3.5) and perhaps its bioactivity in general.

ACD from common vegetables of the Apiaceae is known but rare [36,37], probably due to their relatively low concentrations of allergenic polyacetylenes compared to ornamental and wild plant species [33], or possibly a desensitising effect of oral intake.

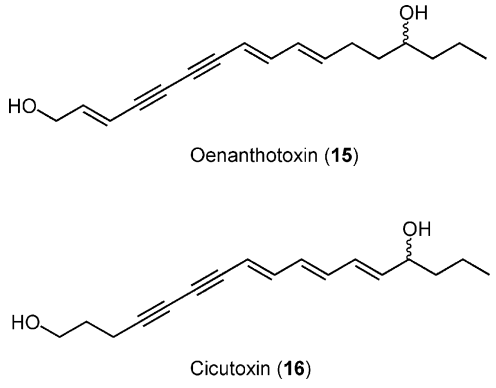

Fig. 2. Highly neurotoxic polyacetylenes isolated from non-food Apiaceae plants.

3.4. Anti-inflammatory, anti-platelet-aggregatory and antibacterial effects

Falcarinol (**1**) and falcarindiol (**2**) have shown anti-inflammatory and anti-platelet-aggregatory effects [38–40]. For falcarinol it has been suggested that this is related to an ability to inhibit lipoxygenases and to modulate prostaglandin

Table 1
Polyacetylenes in major and minor food plants of the Apiaceae, their primary uses and plant part utilized

Family/species	Common name	Plant part used for foods[a]	Uses[b]	Acetylenes in used plant parts	References
Apiaceae (=Umbelliferae)					
Aegopodium podagraria L.	Bishop's weed, ground elder	L, St	V	**1**, **2**, **9**	[7,25,85]
Anethum graveolens L.	Dill	L, S	C, V	**1**, **2**, **13**	[7,85]
Anthriscus cerefolium (L.) Hoffm.	Chervil, salad chervil, French parsley	L, S	C, V	**1**, **2**	[85][c]
A. sylvestris Hoffm.	Cow parsley	L	V	**2**	[86][c]
Apium graveolens L. var. *dulce*	Celery	L, S	C, V	**1**, **2**	[68][c]
A. graveolens L. var. *rapaceum*	Celeriac, knob celery, celery root	R	V	**1**, **2**, **4**–**7**	[7,51,87]
Bunium bulbocastanum L.	Great earthnut	T, L, F	C, V	**1**, **6**, **7**	[7]
Carum carvi L.	Caraway	R, L, S	C	**1**, **2**, **7**, **8**	[7,85,88]
Centella asiatica L.	Asiatic or Indian pennywort	L	V	[d]	[86]
Chaerophyllum bulbosum L.	Turnip-rooted chervil	R, L	V	**1**, **6**	[c]
Coriandrum sativum L.	Coriander, cilantro	L, S	C, V	[d]	[86]
Crithmum maritimum L.	Samphire, marine fennel	L	V	**1**, **2**	[48]
Cryptotaenia canadensis (L.) DC.	Hornwort, white or wild chervil	R, L, St, F	V	**1**, **2**	[89]
C. japonica Hassk.	Japanese hornwort, Mitsuba	R, L, St	V	[d]	[86]
Daucus carota L.	Carrot	R, L	V	**1**–**3**, **7**	[22,32,51,61,69, 72,73,85,90–92]
Ferula assa-foetida L.	Asafoetida, giant fennel	R, S, Sh	C	**7**	[7]
F. communis L.	Common giant fennel	L, S	C, V	**2**	[38]
Foeniculum vulgare Mill.	Fennel	L, S	C, V	**1**, **2**	[51,85,86]
Heracleum sphondylium L.	Common cow parsnip, hogweed	L, Sh	V	**1**, **2**	[85][c]
Levisticum officinale Koch.	Lovage, garden lovage	L, S	C, V	**2**, **7**	[c]
Myrrhis odorata (L.) Scop.	Sweet cicely, sweet chervil	R, L, S	C	[d]	[7]
Oenanthe javanica (Blume) DC.	Water-dropwort, water celery	L, St, Sh	V	**1**, **2**	[93,94]
Pastinaca sativa L.	Parsnip	R, L	V	**1**, **2**, **6**, **7**	[7,51,85]
Petroselinum crispum (Mill.) Nyman ex A. W Hill. (=*P. sativum* Hoffm.)	Parsley	L	C, V	**1**, **2**, **6**, **7**	[7,85][c]
P. crispum (Mill.) Nyman ex A.W. Hill. var. *tuberosum*	Hamburg parsley, turnip-rooted parsley	R, L	C, V	**1**, **2**, **4**, **5**	[51,75]
Pimpinella major (L.) Hud.	Greater burnet saxifrage	R, L, S	C	**1**, **2**	[85]
P. saxifraga L.	Burnet saxifrage	R, L, S	C	**14**	[7,95]
Sium sisarum L.	Skirret, chervil	R	V	**7**	[7,88]
Trachyspermum ammi (L.) Spr.	Ajowan, ajwain	L, S	C	**10**–**13**	[7]

[a] R, roots; T, tubers; L, leaves; St, stems; Sh, shoots; F, flowers; S, seeds.
[b] V, vegetable; C, condiment or flavouring [96,97].
[c] L.P. Christensen, unpublished results.
[d] Polyacetylenes detected but not identified.

catabolism by inhibiting the prostaglandin-catabolizing enzyme 15-hydroxy-prostaglandin dehydrogenase [41]. Falcarinol and related C_{17}-acetylenes have also shown activity against bacteria and mycoplasma [42]. These beneficial effects occur at non-toxic concentrations and thus represent pharmacologically useful properties.

3.5. Cytotoxicity

Roots of *Panax ginseng* C.A. Meyer (Araliaceae) is an herbal drug widely used in Asia for a range of ailments, including cancer. In the beginning of 1980s anticancer activity was demonstrated for petrol extracts of the roots of *P. ginseng* [43], and since then the lipophilic portion of this plant has been intensively investigated. This had led to the isolation and identification of several cytotoxic polyacetylenes [24,44–47], including falcarinol (**1**), panaxydol (**17**) and panaxytriol (**18**) (Figs. 1 and 3). The polyacetylenes **1**, **17** and **18** have been found to be highly cytotoxic against numerous cancer cell lines [45–47] showing the strongest cytotoxic activity towards human gastric adenocarcinoma (MK-1) cancer cells with an ED_{50} of 0.108, 0.059, and 0.605 μM, respectively [47]. However, the ED_{50} against normal human fibroblasts cells (MRC-5) was around 20 times higher than for cancer cells [47], indicating that these compounds may be useful in the treatment of cancer. Falcarindiol (**2**) has also been shown to possess cytotoxic [48,49] and anti-mutagenic

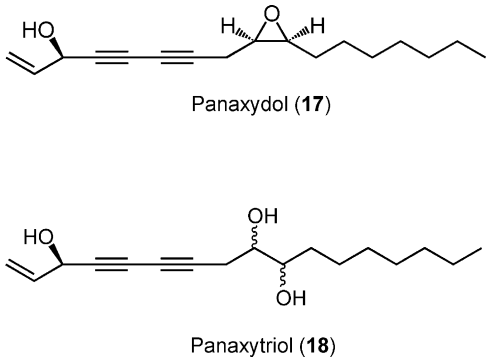

Fig. 3. Examples of highly cytotoxic polyacetylenes of the falcarinol-type isolated from ginseng species (Araliaceae) that may contribute to the anti-cancer effect of ginseng roots.

[50] activity in vitro, although it appears to be less bioactive than falcarinol, and in a recent study also the closely related polyacetylenes falcarindiol 8-methyl ether (**4**) and panaxydiol (**5**) (Fig. 1) isolated from celeriac and parsley (Table 1) exhibited cytotoxic effect in five human cancer and leukaemia cell lines [51]. However, only a few in vivo studies on the effect of falcarinol and related polyacetylenes have been conducted. Preliminary in vivo evaluation of the cytotoxic activity of falcarinol and related polyacetylenes using a LOX melanoma mouse xenograft model indicated that falcarinol had some potential for in vivo anti-tumour activity [49], although the therapeutic effect was not significant. Recently falcarinol has shown significant inhibitory effect on the development of preneoplastic lesions in the rat colon in physiologically relevant concentrations [52], and hence a possible anti-cancer effect in vivo (see also Section 3.6).

The mechanism for the inhibitory activity of falcarinol and related C_{17}-acetylenes is still not known but may be related to their reactivity and hence their ability to interact with various biomolecules (Section 3.3). This is in accordance with a recent in vitro study, which showed that the suppressive effect of falcarinol on cell proliferation of various tumour cells probably was due to its ability to arrest the cell cycle progression of the tumour cells into various phases of their cell cycle [53].

As falcarinol and related aliphatic C_{17}-acetylenes are common in the Araliaceae and Apiaceae one might expect that more species within these families exhibit cytotoxic activity. The selective cytotoxic activity of polyacetylenes of the falcarinol-type towards cancer cells indicates that they may be valuable in the treatment and/or prevention of different types of cancer, and could contribute to the health promoting properties of Apiaceae food plants.

3.6. Falcarinol and the health promoting properties of carrots and related vegetables

Many studies have shown that a high content of natural β-carotene in blood is correlated with a low incidence of several types of cancer, while intervention studies have shown that supplementation with β-carotene does not protect against development of this disease [54,55]. In most European countries and North America carrot consumption appears to be better correlated with the intake of α-carotene [56]. Several studies have also found stronger negative correlations of lung cancer with intake of α-carotene rather than β-carotene [57–59], confirming the central role of carrots as a protecting vegetable against development of cancer. Until recently this was seen as a strong indication of a cancer-preventing effect of β-carotene or α-carotene or a combination of these [60]. However, a beneficial effect of any compound primarily found in carrots, not only carotenoids, would give the same correlations. As shown in Table 1, carrots contain a group of bioactive polyacetylenes, of which falcarinol (**1**) clearly is the most bioactive of these, as described in Section 3.5.

In the human diet carrots are the major dietary source of falcarinol, although falcarinol may also be supplied by many other plant food sources (Table 1). A recent in vitro study aiming to screen for potentially health promoting compounds from vegetables, showed that falcarinol, but not β-carotene, could stimulate differentiation of primary mammalian cells in concentrations between 0.004 and 0.4 μM falcarinol. Toxic effects were found above >4 μM falcarinol (Fig. 4), while β-carotene had no effect even at 400 μM [61]. This biphasic effect (hormesis) of falcarinol on cell proliferation is fully in accordance with the hypothesis that toxic compounds have beneficial effects at certain lower concentrations [62,63]. Therefore falcarinol appears to be one of the bioactive components in carrots and related vegetables that could explain their health promoting properties, rather than carotenoids or other types of primary and/or secondary metabolites. This hypothesis is further supported by recent studies on the bioavailability of falcarinol in humans [6,64–66]. When falcarinol was administered orally via carrot juice (13.3 mg falcarinol/l carrot juice) in amounts of 300, 600 and 900 ml, respectively, it was rapidly absorbed, reaching a

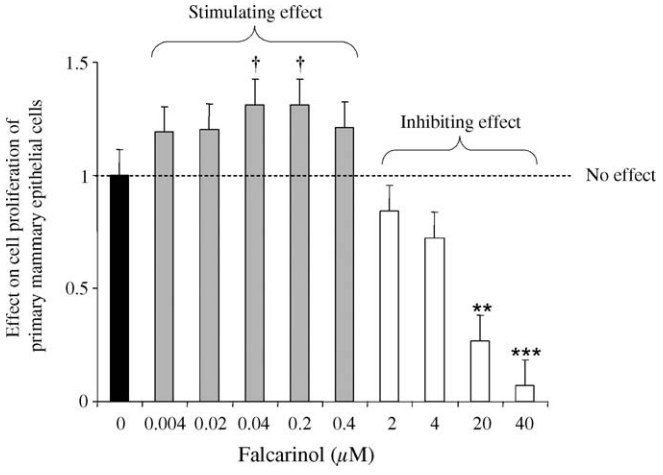

Fig. 4. Effects of increasing concentrations of falcarinol (**1**) on proliferation, measured by incorporation of [methyl-^{3}H]thymidine into mammary epithelial cells prepared from prepubertal Frisian heifers and grown in 3D collagen gels (redrawn from Hansen et al. [61]). Stimulating as well as inhibitory effects on cell proliferation were significantly different from those obtained in basal medium (~no effect). No effect on proliferation was observed for β-carotene when tested in the same bioassay [61]. $^{\dagger}P < 0.09$, $^{*}P < 0.01$, $^{**}P < 0.001$.

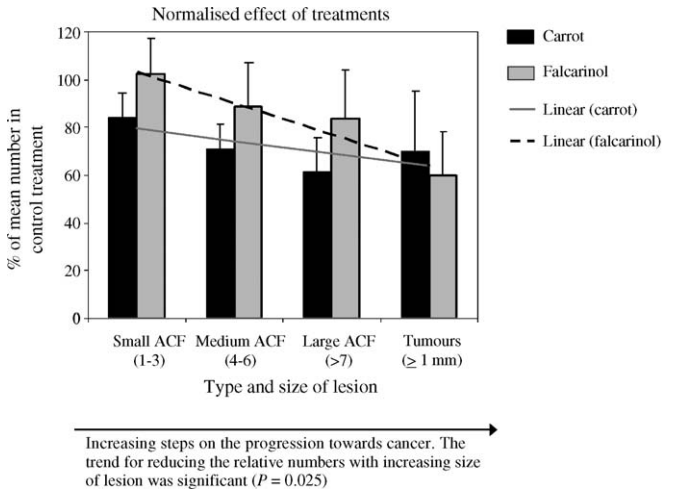

Fig. 5. Effect of treatments with carrot or falcarinol (**1**) on the average numbers per animal of four types of (pre)cancerous lesions in rat colons. Smallest tumours correspond to aberrant crypt foci (ACF) size of approximately 20 (redrawn from Kobæk-Larsen et al. [52]).

maximum concentration in serum between 0.004 and 0.01 μM at 2 h after dosing [6,65]. This is within the range where the in vitro data indicate a potentially beneficial physiological effect (Fig. 4), and a possible inhibitive effect on the proliferation of cancer cells (see Section 3.5).

This effect has been studied in an established rat model for colon cancer by injections of the carcinogen azoxymethane (AOM) in the inbred rat strain BDXI by feeding with carrot or purified falcarinol (with the same, physiologically relevant, intake of falcarinol) [52]. Eighteen weeks after the first AOM injection, the rats were killed and the colon examined for tumours and their microscopic precursors, aberrant crypt foci (ACF) [67]. The carrot and falcarinol treatments showed a significant tendency to reduced numbers of (pre)cancerous lesions with increasing size of lesion as shown in Fig. 5. Although other polyacetylenes such as falcarindiol (**2**) and falcarindiol 3-acetate (**3**) (Fig. 1) present in carrots (Table 1) may have a similar mode of action as falcarinol they are expected to have less effect than those of falcarinol. The possibility to generate two active centers for nucleophilic attack in falcarindiol and falcarindiol 3-acetate reduces the lipophilic character of these compounds and hence their reactivity, in accordance with the observed non-allergenic properties of falcarindiol (Section 3.3) and the less cytotoxic activity observed for falcarindiol compared to falcarinol (Section 3.5). So the physiological effects of falcarindiol and falcarindiol 3-acetate are expected to be qualitative similar but quantitatively less than those of falcarinol, and furthermore, they may even interact with falcarinol in an antagonistic manner thereby affecting its effectiveness. This may also explain the possible, although not significant, differences in the effect and trend observed between the carrot diet and the falcarinol diet shown in Fig. 5 [52].

The results clearly suggest that the protective effect of carrot can be explained to a high degree by its content of falcarinol, and that the traditional view of polyacetylenes in food as generally undesirable toxicants may need to be revised, indicating a need to re-investigate the significance also of all the other bioactive polyacetylenes described in the present review.

4. Analysis of polyacetylenes of the falcarinol-type in Apiaceae food plants

4.1. Introduction

Determining the content of potential bioactive polyacetylenes of the falcarinol-type in Apiaceae food plants and/or plant food products and to evaluate their bioactivity in vitro and in vivo, requires chromatographic methods for isolation and quantification of these compounds. Chromatographic methods for the isolation of polyacetylenes of the falcarinol-type from plant extracts by a combination of column chromatography (CC) and preparative and semi-preparative high-performance liquid chromatography (HPLC) have been developed allowing isolation of relative large amounts of these polyacetylenes for the use in for example preclinical and in clinical trials. Only relative few qualitative and quantitative chromatographic methods have been described for these secondary metabolites, including analytical HPLC combined with UV-detection [28,51,60,68–71] and capillary gas chromatographic techniques (GC–FID and/or GC–MS) [72–75] as well as one method for the quantification of falcarinol and related polyacetylenes in plasma samples by liquid chromatography combined with mass spectrometry (LC–MS/MS) [6,64–66,76].

4.2. High-performance liquid chromatography (HPLC)

4.2.1. Preparative and semi-preparative HPLC

Most polyacetylenes, including polyacetylenes of the falcarinol-type, are thermally unstable and may undergo photodecomposition if exposed to daylight. This instability is most marked in condensed phases where the stability depends on the distance between polyacetylene molecules that are oriented in parallel [7]. A consequence of these properties of polyacetylenes is that only gentle methods of isolation can be used for these compounds.

Polyacetylenes of the falcarinol-type are usually extracted from fresh or dried plant material by an organic solvent such as ethyl acetate, diethyl ether, methylene chloride or methanol. Sometimes, the extracts are first subjected to bulk chromatography to obtain fractions with different polarity but this is normally not necessary when focusing on lipophilic polyacetylenes. Falcarinol and related polyacetylenes have been separated in extracts by repeated CC and/or thin-layer chromatography (TLC) on silica gel [28,50,61,69,77] or by using a combination of silica gel CC and gel permeation CC on Sephadex LH-20 [49,51]. For silica gel CC a gradient consisting of different proportions of *n*-hexane or petrol ether and ethyl acetate or diethyl ether has been used to separate polyacetylenes of the falcarinol-type [28,50,61,69,72] whereas a mixture of CH_2Cl_2/acetone [85:15 (v/v)] has been used to separate these compounds by Sephadex LH-20 CC [51]. Also more special techniques such as multilayer coil countercurrent chromatography (MLCCC) have been used for the isolation of polyacetylenes

of the falcarinol-type [75]. However, isolation techniques such as CC, TLC and MLCCC may be very time-consuming especially if large scale isolation of these compounds is required from plant material and in some cases it may be very difficult to separate the polyacetylenes from other compounds in the extracts using these isolation techniques. These problems can be overcome using preparative and/or semi-preparative HPLC as a final purification step. After separation of the polyacetylenes from the extracts by silica gel and/or Sephadex LH-20 CC the polyacetylenes can be directly isolated from the crude fractions by preparative HPLC techniques [49,52,61,69,72,78–80]. The most used HPLC techniques for the isolation of polyacetylenes of the falcarinol-type are preparative and/or semi-preparative reversed phase (RP)-HPLC using a simple stepwise gradient of aqueous methanol or acetonitrile containing increasing proportions of methanol or acetonitrile [52,61,69,72], although the use of silica and aminopropyl-bonded preparative HPLC columns for large scale isolation of falcarinol and related polyacetylenes have been described [49,78,79]. Preparative or semi-preparative reversed phase columns such as ODS-Hypersil [71], Ultracarb-ODS [80] and ODS-HG-5 [52,61,69] has been shown to be very efficient in separating falcarinol and related polyacetylenes from crude polyacetylene fractions. Polyacetylenes of the falcarinol-type are detected by UV and even though they have characteristic UV-maxima above 230 nm, detection are normally performed at 205 nm [51,61,69] to be able to detect other types of compounds in the sample. This normally ensures an optimal separation and purification of the polyacetylenes. For example, the isolation of pure falcarinol (**1**) and panaxydol (**17**) from ginseng root extracts can easily be achieved by preparative RP-HPLC even from very crude fractions as illustrated in Fig. 6. However, fractionation of extracts by silica gel CC normally results in a relative good separation of the individual polyacetylenes that only require a single purification step by preparative HPLC to be obtained in a purity >98%. To obtain the compounds in a very high purity is important in order (1) to ensure a proper characterisation of the isolated polyacetylenes, which are identified by their characteristic UV-spectra combined with mass spectrometry and 1D- and 2D-NMR techniques [51,72,75,78] and (2) to ensure reliable bioactivity data if the isolated polyacetylenes is to be tested in bioassays and preclinical trials. Fig. 7 shows the separation by preparative RP-HPLC of minute amounts of impurities from a fraction containing relative pure falcarindiol (**2**). This falcarindiol fraction was a result of the separation of an ethyl acetate extract of carrot root by silica gel CC using a solvent gradient of *n*-hexane and ethyl acetate [61,69].

4.2.2. Analytical HPLC

The number of publications describing the qualitative and quantitative measurement of polyacetylenes of the falcarinol-type by analytical HPLC is limited [28,51,61,68–71]. Separations of polyacetylenes by analytical HPLC have been performed on various types of reversed phase C18 columns such as LiChrospher 100 RP-18 [61,69], Zorbax Rx-C18 [51,71], Sperisorb 5S ODS [28,70], Econosphere C18 [68], LiChrosorb RP-18 [81] and Luna 3 μ C18(2) 100A (Fig. 8) normally using a gradient consisting of methanol/water or acetonitrile/water. Polyacetylenes of the falcarinol-type have very characteristic UV-spectra due to their conjugated triple bonds and hence they are easily identified in extracts by diode array detection (DAD) [28,70]. However, due to the low number of conjugated unsaturated bonds in their structures the excitation coefficients ($\varepsilon < 6000$ for two triple bonds in conjugation) of these compounds at their characteristic UV-maxima and hence the sensitivity above 230 nm is generally very low [7,28,70]. By instead detecting the compounds at 205 nm the UV sensitivity is improved approximately 10 times and hence the detection of the polyacetylenes. This is especially important in many Apiaceae vegetables where the concentration can be relatively low (down to 5 mg/kg fresh weight) [51,69]. However, depending on

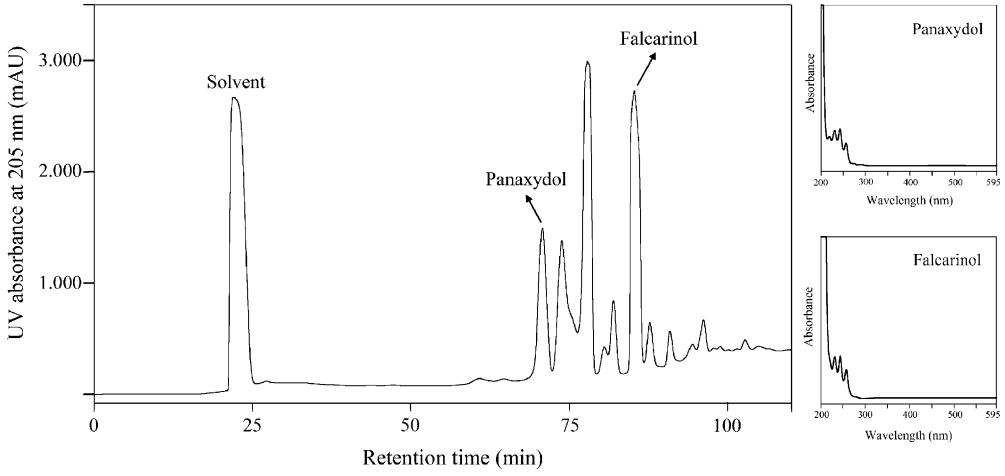

Fig. 6. Preparative RP-HPLC chromatogram showing the separation of the polyacetylenes panaxydol (**17**) and falcarinol (**1**) from of a crude polyacetylene fraction resulting from the separation of a ginseng extract by silica gel column chromatography (L.P. Christensen, unpublished work). Solvent = ethyl acetate. Polyacetylenes were separated on a reversed-phase Develosil ODS-HG-5 HPLC column (RP-18, 250 mm × 20 mm i.d.) at 25 °C using the following solvent gradient: CH_3OH–H_2O [0 min (40:60), 10–40 min (75:25), 80–110 min (100:0), 115 min (40:60)]. All increases/decreases in the gradient were programmed as linear. Flow rate: 5 ml/min. Injection volume: 25 ml. Detection: diode array detector (DAD) operating from 200 to 595 nm and acquisition off at 110 min.

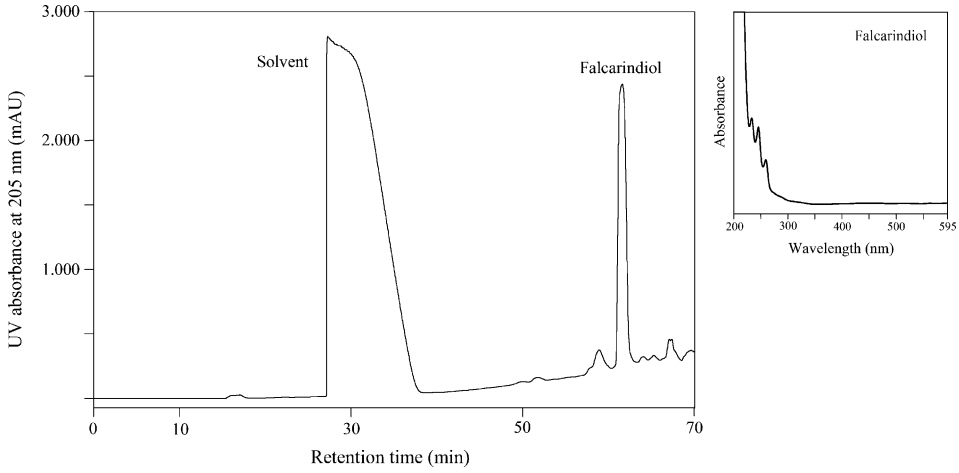

Fig. 7. Preparative RP-HPLC chromatogram showing the separation of falcarindiol (**2**) from minor impurities in a crude polyacetylene fraction, which originate from the separation of a carrot root extract by silica gel column chromatography (L.P. Christensen, unpublished work). Solvent = ethyl acetate. Separations performed on a reversed-phase Develosil ODS-HG-5 HPLC column (RP-18, 250 mm × 20 mm i.d.) at 25 °C using the following solvent gradient: CH_3OH–H_2O [0 min (20:80), 50–60 min (100:0), 70–80 min (20:80)]. All increases/decreases in the gradient were programmed as linear. Flow rate: 5 ml/min. Injection volume: 25 ml. Detection: diode array detector (DAD) operating from 200 to 595 nm and acquisition off at 70 min.

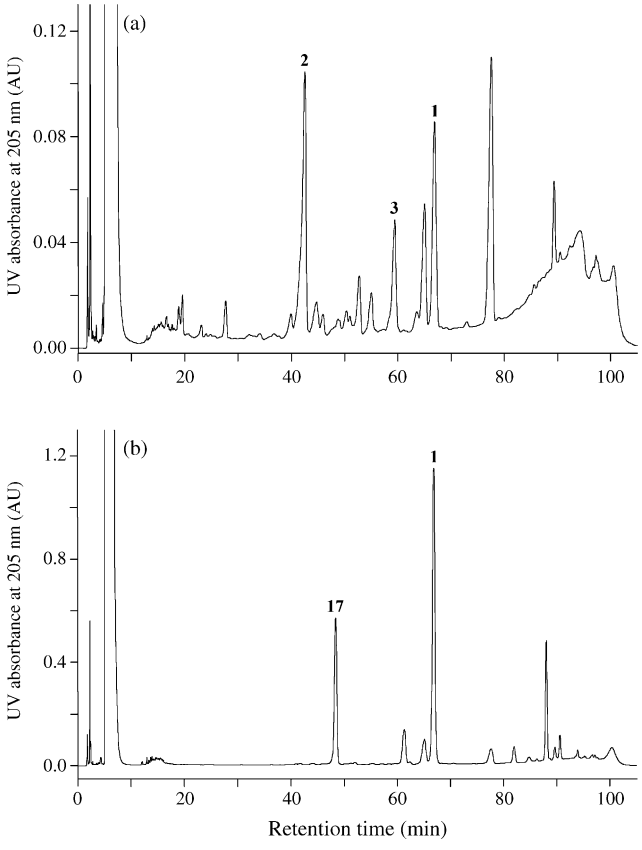

Fig. 8. Analytical RP-HPLC chromatogram of (a) an ethyl acetate extract of carrot roots and (b) an ethyl acetate extract of an American ginseng root (L.P. Christensen, unpublished work). Falcarinol (**1**), falcarindiol (**2**), falcarindiol 3-acetate (**3**) and panaxydol (**17**). Polyacetylenes were separated on a reversed-phase Luna 3 μ C18(2) 100A column (3 μm; 150 mm × 4.6 mm i.d.) at 40 °C using the following solvent gradient: CH_3CN–H_2O [0–5 min (20:80), 10 min (50:50), 30 min (53:47), 45–50 min (65:35), 70–72 min (75:25), 90–95 min (95:5), 100–110 min (20:80)]. All increases/decreases in the gradient were programmed as linear. Flow rate: 1 ml/min. Injection volume: 20 μl. Detection: diode array detector (DAD) operating from 200 to 600 nm and acquisition off at 105 min.

the extracts investigated many other compounds also absorb UV light at 205 nm. A typical analytical RP-HPLC chromatogram at 205 nm of a carrot root extract (ethyl acetate) is shown in Fig. 8a. Despite a good separation of the polyacetylenes many additional peaks are observed that are not related to polyacetylenes (revealed by UV-DAD). In contrast the number of additional peaks not related to polyacetylenes are limited in ethyl acetate extracts of American ginseng root (Fig. 8b) where the concentration of polyacetylenes is relatively high compared to carrots and related vegetables. In spite of the additional peaks in the RP-HPLC chromatograms of carrots the polyacetylenes are easily separated in the extracts as demonstrated in Fig. 8a. Quantification of polyacetylenes by analytical RP-HPLC is performed by using an appropriate internal standard [51] or using a calibration curve of authentic polyacetylene standards [61,69].

4.3. Liquid chromatography mass spectrometry (LC–MS)

Liquid chromatography mass spectrometry (LC–MS) methodologies have been used to characterize purified polyacetylenes and to determine the polyacetylene profile in Apiaceae food plants [51,71]. To obtain a high degree of sensitivity by LC–MS require much effort and do not seem to be suitable for characterization and quantification of small amounts of polyacetylenes, which is often relevant for samples prepared from Apiaceae food plants or products and in biological fluid samples. On the other hand using liquid chromatography–tandem mass spectrometry (LC–MS/MS) or similar LC–MS/MS techniques it is possible to examine selectively the fragmentation of particular ions, and the substantially higher selectivity and sensitivity of these methods make them very suitable for examination of specific compounds or classes of compounds in complex matrices appearing in very low concentrations such as in biological fluid samples [64–66,76,82]. LC–MS/MS techniques have been widely applied for the determination of for example bioavilability by quantification of carotenoids [83] and citrus

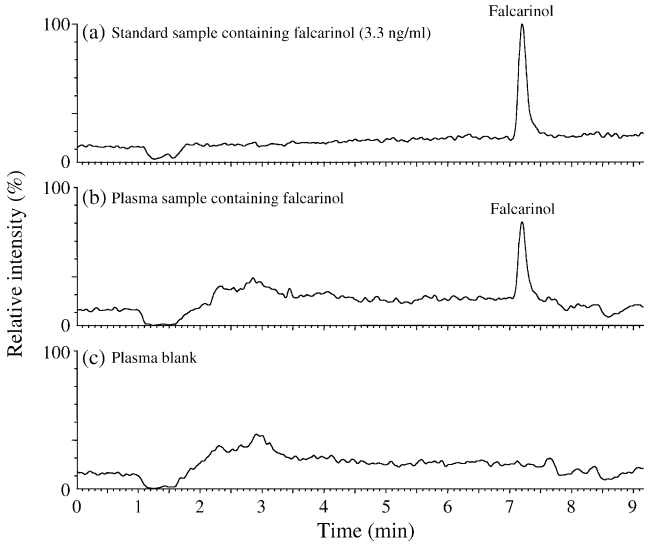

Fig. 9. MRM chromatogram obtained for (a) standard sample of falcarinol (**1**) [concentration 3.3 ng/ml], (b) plasma sample containing falcarinol and (c) blank plasma sample (reference) [64,66,76]. ESI+ MS data were acquired by LC–MS/MS on a Quattro LC (Micromass) using N_2 as desolvation gas at a flow rate of 650 l/h and a temperature of 400 °C. Potentials applied on the electrospray capillary and on the cone were 3.5 kV and 20 V, respectively. Detection: MRM method using argon as collision gas and the mass transition *m/z* 227 → 91. Separations were performed on a Chromolite RP-C18 column (50 mm × 4.6 mm i.d.) using a binary gradient consisting of 0.5% formic acid in water and CH_3CN. Flow rate: 0.3 ml/min. Extraction recoveries were in the range 79–100% depending on the analyte concentration [64,66,76].

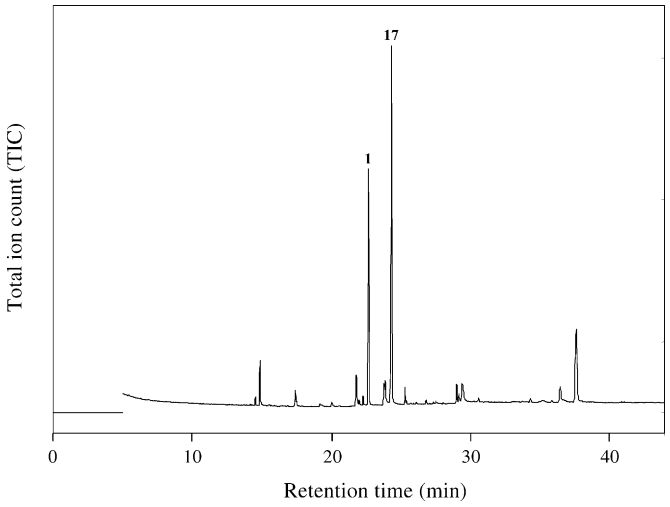

Fig. 10. High resolution GC–MS chromatogram of an ethyl acetate extract of American ginseng roots (*Panax quinquefolium*). The polyacetylenes falcarinol (**1**) and panaxydol (**17**) quantified by the use of external calibration curves (L.P. Christensen, unpublished work). GC was performed on a Varian Star 3400 CX gas chromatograph using a SE-54 fused silica capillary column (30 m × 0.25 mm i.d.) by on-column injection (1 μl). Polyacetylenes were separated by the following oven temperature gradient: 40 °C for 2 min, linear increase to 280 °C at 8.5 °C/min and held 15 min isothermally. The flow of the carrier gas (helium) was 1.4 ml/min. MS analysis was performed with a Varian ion trap MS in the electron impact mode (MS/EI) at an ionisation potential of 70 eV. The MS was operated in scan mode over a mass range from 39 to 450 amu (1 scan/s).

limonoids [82] in human plasma samples and phytoestrogens in milk samples [84]. However, to the best of our knowledge only one investigation has been reported where LC–MS/MS was used for the detection and quantification of polyacetylenes of the falcarinol-type in biological fluid samples [6,64–66,76]. In connection with the bioavailability studies described in Section 3.6, a method based on liquid chromatography combined with electrospray tandem mass spectrometry (LC–ESP–MS/MS) was developed for the quantification of the polyacetylenes falcarinol (**1**) and falcarindiol (**2**) in plasma samples [6,64–66,76]. The polyacetylenes were extracted from plasma samples with acetonitrile and analysed directly by LC–ESP–MS/MS using falcarinol and falcarindiol as external standards. Fragment ions of falcarinol and falcarindiol were generated in the electrospray positive ion mode (ESI+) with argon as collision gas using the mass transitions *m/z* 247 → 91 and 227 → 91 for detection of falcarindiol and falcarinol, respectively [64–66,76]. The number of ions produced permitted the development of an efficient analytical method based on multiple reaction monitoring (MRM) acquisition mode as illustrated in Fig. 9 for falcarinol [64,66,76]. The mass traces from the MRM method clearly illustrate its selectivity. The quantification limits of both polyacetylenes were 0.001 μM [64,66,76] illustrating the sensitivity of the method and hence its usefulness for the determination of polyacetylenes of the falcarinol-type in plasma samples and in other sample types where the concentration of these compounds are very low.

4.4. Capillary gas chromatography (GC) and mass spectrometry (GC–MS)

Only a few publications dealing with the qualitative and quantitative analysis of polyacetylenes of the falcarinol-type by capillary GC–FID and GC–MS have appeared [72–75]. Separation of polyacetylenes of the falcarinol-type have been achieved on various types of fused silica gel capillary GC-columns such as DB-5, Rtx-5 and SE-54 and similar phases (see Fig. 10 and [73–75]). As demonstrated in Fig. 10 the separation of these compounds by capillary GC is very good. Quantification of the compounds in extracts is made from an appropriate internal standard or by the use of calibration curves made from authentic polyacetylene standards (see Fig. 10 and [72–75]).

5. Conclusions

The present review clearly indicates that despite the existence of highly toxic polyacetylenes in non-food plants, the polyacetylenes of the falcarinol-type comprise important health promoting compounds that seem to be able to explain some of the health promoting properties of Apiaceae food plants and products. The traditional view of polyacetylenes in food as generally undesirable toxicants may therefore need to be revised and perhaps these compounds may instead be regarded as important nutraceuticals. Consequently, it is important to have good and reliable analytical methods for the qualitative and quantitative analysis of these compounds in different matrices and the present review has clearly demonstrated that they are available, although the literature on this subject is limited. The major

challenge with regard to bioactive polyacetylenes is to test their health promoting effects in vivo in clinical as well as in further preclinical studies, which will require large amounts of purified polyacetylenes. The present review has also demonstrated that methods for large scale isolation of these compounds are available, so it should be only a matter of time before more conclusive evidence is obtained for or against the health promoting effects of polyacetylenes of the falcarinol-type.

Acknowledgements

The support of the Danish Ministry of Science Technology and Innovation (grant no. 2011-00-0040), Fyn Region and of the Development Centre Aarslev is gratefully acknowledged.

References

[1] P. Greenvald, C.K. Clifford, J.A. Milner, Eur. J. Cancer 37 (2001) 948–965.
[2] P.M. Kris-Etherton, T.D. Etherton, J. Carlson, C. Gardner, Curr. Opin. Lipid 13 (2002) 397–407.
[3] M. Maynard, D. Gunnell, P. Emmett, S. Frankel, G.D. Smith, J. Epidemiol. Community Health 57 (2003) 218–225.
[4] J. Gundgaard, J.N. Nielsen, J. Olsen, J. Sørensen, Public Health Nutr. 6 (2003) 25–30.
[5] A. Trichopoulou, A. Naska, A. Antoniou, S. Friel, K. Trygg, A. Turrini, Int. J. Vitamin Nutr. Res. 73 (2003) 63–69.
[6] K. Brandt, L.P. Christensen, J. Hansen-Møller, S.L. Hansen, J. Haraldsdóttir, L. Jespersen, S. Purup, A. Kharazmi, V. Barkholt, H. Frøkiær, M. Kobæk-Larsen, Trends Food Sci. Technol. 15 (2004) 384–393.
[7] F. Bohlmann, T. Burkhardt, C. Zdero, Naturally Occurring Acetylenes, Academic Press, London, 1973.
[8] L. Hansen, P.M. Boll, Phytochemistry 25 (1986) 285–293.
[9] L.P. Christensen, J. Lam, Phytochemistry 29 (1990) 2753–2785.
[10] L.P. Christensen, J. Lam, Phytochemistry 30 (1991) 11–49.
[11] L.P. Christensen, Phytochemistry 31 (1992) 7–49.
[12] P.J.G.M. De Wit, E. Kodde, Physiol. Plant Pathol. 18 (1981) 143–148.
[13] D.M. Elgersma, A.C.M. Weijman, H.J. Roeymans, G.W. van Eijk, Phytopathol. Z. 109 (1984) 237–240.
[14] S. Imoto, Y. Ohta, Plant Physiol. 86 (1988) 176–181.
[15] J.D. Bu'Lock, H.M. Smalley, J. Chem. Soc. (1962) 4662–4664.
[16] J.D. Bu'Lock, G.N. Smith, J. Chem. Soc. (C) (1967) 332–336.
[17] E.R.H. Jones, V. Thaller, J.L. Turner, J. Chem. Soc. Perkin Trans. I (1975) 424–428.
[18] G.C. Barley, E.R.H. Jones, V. Thaller, in: J. Lam, H. Breteler, T. Arnason, L. Hansen (Eds.), Chemistry and Biology of Naturally-Occurring Acetylenes and Related Compounds (NOARC), Elsevier, Amsterdam, 1988, pp. 85–91.
[19] E.B. Cahoon, J.A. Schnurr, E.A. Huffman, R.E. Minto, Plant J. 34 (2003) 671–683.
[20] V.K. Harding, J.B. Heale, Physiol. Plant Pathol. 18 (1981) 7–15.
[21] V.K. Harding, J.B. Heale, Physiol. Plant Pathol. 17 (1980) 277–289.
[22] B. Garrod, B.G. Lewis, D.T. Coxon, Physiol. Plant Pathol. 13 (1978) 241–246.
[23] K. Olsson, R. Svensson, J. Phytopathol. 144 (1996) 441–447.
[24] L.P. Christensen, Recent Res. Dev. Phytochem. 2 (1998) 227–257.
[25] M.S. Kemp, Phytochemistry 17 (1978) 1002.
[26] E.F.L.J. Anet, B. Lythgoe, M.H. Silk, S. Trippett, J. Chem. Soc. (1953) 309–322.
[27] T. Konoshima, K.-H. Lee, J. Nat. Prod. 49 (1986) 1117–1121.
[28] U. Wittstock, F. Hadacek, G. Wurz, E. Teuscher, H. Greger, Planta Med. 61 (1995) 439–445.
[29] K. Uwai, K. Ohashi, Y. Takaya, T. Ohta, T. Tadano, K. Kisara, K. Shibusawa, R. Sakakibara, Y. Oshima, J. Med. Chem. 43 (2000) 4508–4515.
[30] J. Vetter, Food Chem. Toxicol. 42 (2004) 1373–1382.
[31] U. Wittstock, K.H. Lichtnow, E.R. Teuscher, Planta Med. 62 (1997) 120–124.
[32] D.G. Crosby, N. Aharonson, Tetrahedron 23 (1967) 465–472.
[33] B.M. Hausen, I.K. Vieluf, Allergiepflanzen—Pflanzenallergene: Handbuch und Atlas der allergie-induzierenden Wild- und Kulturpflanzen, 2. erweit, Auflage, Ecomed Verlagsgesellschaft mbH, Landsberg/München, 2001.
[34] L. Hansen, O. Hammershøy, P.M. Boll, Contact Dermat. 14 (1986) 91–93.
[35] B.M. Hausen, J. Bröhan, W.A. König, H. Faasch, H. Hahn, G. Bruhn, Contact Dermat. 17 (1987) 1–9.
[36] S. Machado, E. Silva, A. Massa, Contact Dermat. 47 (2002) 113–114.
[37] S.R. Murdoch, J. Dempster, Contact Dermat. 42 (2000) 236.
[38] G. Appendino, S. Tagliapietra, G.M. Nano, Fitoterapia 64 (1993) 179.
[39] C.-M. Teng, S.-C. Kuo, F.-N. Ko, J.C. Lee, L.-G. Lee, S.-C. Chen, T.-F. Huang, Biochim. Biophys. Acta 990 (1989) 315–320.
[40] J. Alanko, Y. Kurahashi, T. Yoshimoto, S. Yamamoto, K. Baba, Biochem. Pharmacol. 48 (1994) 1979–1981.
[41] Y. Fujimoto, S. Sakuma, S. Komatsu, D. Sato, H. Nishida, Y.-Q. Xiao, K. Baba, T. Fujita, J. Pharm. Pharmacol. 50 (1988) 1075–1078.
[42] M. Kobaisy, Z. Abramowski, L. Lermer, G. Saxena, R.E.W. Hancock, G.H.N. Towers, J. Nat. Prod. 60 (1997) 1210–1213.
[43] S.C. Shim, H.Y. Koh, B.H. Han, Bull. Korean Chem. Soc. 4 (1983) 183–188.
[44] Y. Fujimoto, M. Satoh, Chem. Pharm. Bull. 36 (1988) 4206–4208.
[45] B.-Z. Ahn, S.-I. Kim, Arch. Pharm. 321 (1988) 61–63.
[46] H. Matsunaga, M. Katano, H. Yamamoto, M. Mori, K. Takata, Chem. Pharm. Bull. 37 (1989) 1279–1281.
[47] H. Matsunaga, M. Katano, H. Yamamoto, H. Fujito, M. Mori, K. Takata, Chem. Pharm. Bull. 38 (1990) 3480–3482.
[48] F. Cunsolo, G. Ruberto, V. Amico, M. Piattelli, J. Nat. Prod. 56 (1993) 1598–1600.
[49] M.W. Bernart, J.H. Cardellina II, M.S. Balaschak, M. Alexander, R.H. Shoemaker, M.R. Boyd, J. Nat. Prod. 59 (1996) 748–753.
[50] M. Miyazawa, H. Shimamura, R.C. Bhuva, S. Nakamura, H. Kameoka, J. Agric. Food Chem. 44 (1996) 3444–3448.
[51] C. Zidorn, K. Johrer, M. Ganzera, B. Schubert, E.M. Sigmund, J. Mader, R. Greil, E.P. Ellmerer, H. Stuppner, J. Agric. Food Chem. 53 (2005) 2518–2523.
[52] M. Kobæk-Larsen, L.P. Christensen, W. Vach, J. Ritskes-Hoitinga, K. Brandt, J. Agric. Food Chem. 53 (2005) 1823–1827.
[53] Y.-C. Kuo, Y.L. Lin, C.-P. Huang, J.-W. Shu, W.-J. Tsai, Cancer Invest. 20 (2002) 955–964.
[54] E.R. Greenberg, J.A. Baron, M.R. Karagas, T.A. Stukel, D.W. Nierenberg, M.M. Stevens, J.S. Mandel, R.W. Haile, J. Am. Med. Assoc. 275 (1996) 699–703.
[55] G.S. Omenn, G.E. Goodmann, M.D. Thornquist, J. Balmes, M.R. Cullen, A. Glass, J.P. Keogh, F.L. Meyskens Jr., B. Valanis, J.H. Williams Jr., N. Engl. J. Med. 334 (1996) 1150–1155.
[56] M.E. O'Neil, Y. Carroll, B. Corridan, B. Olmedilla, F. Granado, I. Blanco, H. Van der Berg, I. Hininger, A.M. Rousell, M. Chopra, S. Southon, D.I. Turnham, Br. J. Nutr. 85 (2001) 499–507.
[57] R.G. Ziegler, E.A. Colavito, P. Hartge, M.J. McAdams, J.B. Schoenberg, T.J. Mason, J.F. Fraumeni Jr., J. Natl. Cancer Inst. 88 (1996) 612–615.
[58] D.S. Michaud, D. Feskanich, E.B. Rimm, G.A. Colditz, F.E. Speizer, W.C. Willett, E. Giovannucci, Am. J. Clin. Nutr. 72 (2000) 990–997.
[59] M.E. Wright, S.T. Mayne, C.A. Swanson, R. Sinha, M.C.R. Alavanja, Cancer Causes Control 14 (2003) 85–96.
[60] W. Stahl, H. Sies, Biochim. Biophys. Acta 1740 (2005) 101–107.
[61] S.L. Hansen, S. Purup, L.P. Christensen, J. Sci. Food Agric. 83 (2003) 1010–1017.
[62] E.J. Calabrese, L.A. Baldwin, Environ. Health Perspect. 106 (Suppl. 1) (1998) 357–362.
[63] E.J. Calabrese, L.A. Baldwin, Toxicol. Sci. 71 (2003) 246–250.
[64] J. Hansen-Møller, S.L. Hansen, L.P. Christensen, L. Jespersen, K. Brandt, J. Haraldsdóttir, Proceedings of the International Symposium on "Dietary Phytochemicals and Human Health", Salamanca, Spain, 18–20 April, 2002, pp. 203–204.

[65] J. Haraldsdóttir, L. Jespersen, J. Hansen-Møller, S.L. Hansen, L.P. Christensen, K. Brandt, in: K. Brandt, B. Åkesson (Eds.), Health Promoting Compounds in Vegetables and Fruit, Proceedings of Workshop in Karrebæksminde, Denmark, 6–8 November, vol. 29, DIAS Report-Horticulture, 2002, pp. 24–28.
[66] J. Hansen-Møller, S.L. Hansen, L.P. Christensen, L. Jespersen, K. Brandt, J. Haraldsdóttir, in: K. Brandt, B. Åkesson (Eds.), Health Promoting Compounds in Vegetables and Fruit, Proceedings of Workshop in Karrebæksminde, vol. 29, Denmark, 6–8 November, DIAS Report-Horticulture, 2002, pp. 137–138.
[67] E.A. McLellan, R.P. Bird, Cancer Res. 48 (1988) 6187–6192.
[68] J. Avalos, G.P. Fontan, E. Rodriguez, J. Liq. Chromatogr. 18 (1995) 2069–2076.
[69] U. Kidmose, S.L. Hansen, L.P. Christensen, M. Edelenbos, E. Larsen, R. Nørbæk, J. Food Sci. 69 (2004) S388–S394.
[70] F. Hadaček, A. Werner, H. Greger, in: J. Lam, H. Breteler, T. Arnason, L. Hansen (Eds.), Chemistry and Biology of Naturally Occurring Acetylenes and Related Compounds (NOARC), Elsevier, Amsterdam, 1988, pp. 107–114.
[71] C. Zidorn, S. Sturm, J.W. Dawson, J.W. van Klink, H. Stuppner, N.B. Perry, Phytochemistry 59 (2002) 293–304.
[72] A. Czepa, T. Hofmann, J. Agric. Food Chem. 51 (2003) 3865–3873.
[73] A. Czepa, T. Hofmann, J. Agric. Food Chem. 52 (2004) 4508–4514.
[74] P.A.G. Santos, A.C. Figueiredo, M.M. Oliveira, J.G. Barroso, L.G. Pedro, S.G. Deans, J.J.C. Scheffer, Plant Sci. 168 (2005) 1089–1096.
[75] S. Nitz, M.H. Spraul, F. Drawert, J. Agric. Food Chem. 38 (1990) 1445–1447.
[76] J. Hansen-Møller, S.L. Hansen, L.P. Christensen, L. Jespersen, H. Frøkiær, V. Barkholt, J. Haraldsdóttir, K. Brandt, Proceedings of the International Symposium on "Dietary Phytochemicals and Human Health", Salamanca, Spain, 18–20 April, 2002, pp. 58–59.
[77] J. Lam, L. Hansen, in: J.L. Harwood, J.R. Bowyer (Eds.), Methods in Plant Biochemistry, vol. 4: Lipids, Membranes and Aspects for Photobiology, Academic Press, London, 1990, pp. 159–183.
[78] Y. Fujimoto, M. Satoh, N. Takeuchi, M. Kirisawa, Chem. Pharm. Bull. 39 (1991) 521–523.
[79] K. Hirakura, M. Morita, K. Nakajima, Y. Ikeya, H. Mitsuhashi, Phytochemistry 30 (1991) 3327–3333.
[80] B.-M. Kwon, S.-H. Ro, M.-K. Kim, J.Y. Nam, H.-J. Jung, I.-R. Lee, Y.-K. Kim, S.-H. Bok, Planta Med. 63 (1997) 552–553.
[81] D. Washida, S. Kitanaka, Chem. Pharm. Bull. 51 (2003) 1314–1317.
[82] G.D. Manners, R.A. Jakob, A.P. Breksa III, T.K. Schoch, S. Hasegawa, J. Agric. Food Chem. 51 (2003) 4156–4161.
[83] Y. Wang, X. Xu, M. van Lieshout, C.E. West, J. Lugtenburg, M.A. Verhoeven, A.F.L. Creemers, Muhilal, R.B. van Breemen, Anal. Chem. 71 (2000) 4999–5003.
[84] J.-P. Antignac, R. Cariou, B. Le Bizec, J.-P. Cravedi, F. Andre, Rapid Commun. Mass Spectrom. 17 (2003) 1256–1264.
[85] T. Degen, H.-R. Buser, E. Städler, J. Chem. Ecol. 25 (1999) 67–87.
[86] Y. Nakano, H. Matsunaga, T. Saita, M. Mori, M. Katano, H. Okabe, Biol. Pharm. Bull. 21 (1998) 257–261.
[87] F. Bohlmann, Chem. Ber. 100 (1967) 3454–3456.
[88] F. Bohlmann, C. Arndt, H. Bornowski, K.-M. Kleine, Chem. Ber. 94 (1961) 958–967.
[89] U. Eckenbach, R.L. Lampman, D.S. Seigler, J. Ebinger, R.J. Novak, J. Chem. Ecol. 25 (1999) 1885–1893.
[90] E.D. Lund, Phytochemistry 31 (1992) 3621–3623.
[91] E.D. Lund, J.M. White, J. Sci. Food Agric. 51 (1990) 507–516.
[92] S.G. Yates, R.E. England, J. Agric. Food Chem. 30 (1982) 317–320.
[93] S.G. Yates, J.C. Fenster, Abstr. Pap. Am. Chem. Soc. 186 (1983) 149-AGFD.
[94] T. Fujita, Y. Kadoya, H. Aota, M. Nakayama, Biosci. Biotech. Biochem. 59 (1995) 526–528.
[95] K.E. Schulte, G. Rücker, W. Backe, Arch. Pharm. 303 (1970) 912–919.
[96] M. Yamaguchi, World Vegetables: Principles, Production and Nutritive Values, AVI Publishing company Inc., Westport, Connecticut, 1983.
[97] V.E. Rubatzky, C.F. Quiros, P.W. Simon, Carrots and Related Vegetable Umbelliferae, CABI Publishing, New York, 1999.