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Structural Changes of Polyacetylenes in American Ginseng Root Can Be Observed in Situ by Using Raman Spectroscopy

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The presented results show the special advantage of Raman spectroscopy in the investigation of polyacetylenes in American ginseng (*Panax quinquefolium* L.) roots. The compounds are measured directly in the plant tissue without any preliminary sample preparation. The polyacetylene signal is strong and well-separated from other bands so the spectral impact of the surrounding biological matrix can be clearly distinguished. The Raman spectrum taken in situ from the fresh ginseng root revealed a characteristic polyacetylene key band at 2237 cm^{-1} whereas in the spectrum obtained from dried root this band was shifted to about 2258 cm^{-1}. The latter is in good agreement with signals obtained from isolated standards, falcarinol (2258 cm^{-1}) and panaxydol (2260 cm^{-1}), occurring as predominant polyacetylenes in this species. The shift of the polyacetylene band observed in root extracts or at a certain stage of root drying indicates the molecular modification of polyacetylenes resulting from the loss of water. Furthermore, it was found that the process upon root hydration is reversible as the shift of polyacetylene band from 2258 to 2237 cm^{-1} is observed. An explanation of this phenomenon can be an interaction of polyacetylene molecules with plant components in the presence of water molecules forming a stable entity in situ that is broken after dehydration (loss of water) of the fresh ginseng root. Application of the Raman mapping technique to ginseng roots of different size showed that the content of both main polyacetylenes decreases with increasing root size in accordance with quantitative high-performance liquid chromatography data.

KEYWORDS: ***Panax quinquefolium*; FT-Raman; mapping; falcarinol; panaxydol; nondestructive analysis; in situ**

INTRODUCTION

For the past 2000 years, *Panax ginseng* C. A. Meyer (Korean ginseng) has been used as a medicinal herb in traditional Asian medicine (*1*). Another *Panax* species, *Panax quinquefolium* L. (American ginseng), was valued by American Indians long before the arrival of Europeans in the New World and since the 18th century it has been cultivated in North America for medicinal purposes (*2*).

P. quinquefolium is indicated for fatigue and as an immunostimulant in times of stress. Intensive studies have shown this herb to have estrogenic, antimutagenic, and hypoglycemic effects and also to improve impaired memory and learning (*3*, *4*). Some of these pharmacological properties of ginseng roots are attributed to the presence of dammarane saponins, commonly referred to as ginsenosides (*5*, *6*); however, much of the bioactivity is associated also with polyacetylenes, of which some exhibit anti-platelet-aggregatory and anti-inflammatory effects (*7*−*11*) and anti-carcinogenic activity (*12*−*18*).

More than 10 polyacetylenes have been isolated from *P. quinquefolium*, but two of them, falcarinol (synonymous with panaxynol) and panaxydol, were found to be the dominant compounds. Significant discrepancy can be noticed in several papers reporting quantitative analysis of ginseng roots, but generally falcarinol has been found in amounts between 200 and 560 mg kg^{-1} dry weight whereas panaxydol occurs at concentrations of 180−950 mg kg^{-1} dry weight (*17*, *18*).

Falcarinol and panaxydol are among the most bioactive polyacetylenes isolated from ginseng and hence are very important in relation to the anti-cancer effect and other pharmacological properties of ginseng roots. In particular, the cytotoxic properties of falcarinol have recently been extensively investigated in relation to its health-promoting properties of Apiaceae food vegetables as it is also a major polyacetylene constituent in carrot roots and related vegetables. It has for example been demonstrated that falcarinol has an effect on the development of colon cancer in vivo in physiological relevant concentrations in contrast to other bioactive compounds in carrot

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roots, such as β-carotene, and therefore it has been hypothesized that falcarinol may be responsible for the ability of carrots to prevent cancer (*19*−*22*). On the other hand, falcarinol contributes strongly to the bitter taste of carrot (*23*, *24*) and may have some negative effects due to its allergenic properties (*25*) and its toxicity in relatively high concentrations (*26*).

Until now, nothing is known about the distribution of polyacetylenes in ginseng roots and their interactions with other plant constituents, which could be important in relation to the pharmacological properties of polyacetylenes in for example ginseng preparations. However, the investigation of polyacetylenes is in part hampered by the fact that these compounds are unstable, being sensitive to heat and light (*23*). The most popular method used for their analysis is high-performance liquid chromatography (HPLC), which requires solvent extraction (*21*−*23*, *27*, *28*) and hence is a procedure that does not allow the determination of the distribution of polyacetylenes in cellular dimensions. These limitations can be overcome using Raman spectroscopy, which has already been successfully applied to the analysis of polyacetylenes in several plants (*29*, *30*). In particular, the Raman mapping technique gives a deeper knowledge of the localization of secondary metabolites in various intact plant tissues (*31*, *32*).

In this paper, FT-Raman spectroscopy is used for investigation of polyacetylenes occurring in *P. quinquefolium* roots of different size, i.e., hairy, lateral, and main roots. The measurements are performed in situ as well as on isolated pure compounds. Raman spectroscopy is successfully applied to the study of molecular changes of polyacetylenes occurring through their extraction or at certain stages of root drying. Furthermore, the mapping technique is used to evaluate spatial distribution of polyacetylenes directly in the plant tissue.

MATERIALS AND METHODS

Plant Material. Roots of 5-year-old American ginseng (*P. quinquefolium* L.) were collected for measurements from a small ginseng field at the Research Centre Aarslev, Denmark. The ginseng plants were grown organically without use of any pesticides. Root disks of about 3 mm thickness were transversely cut and used for analyses without any further preparation.

Chemicals. Ethyl acetate (EtOAc), methanol (MeOH), *n*-hexane, and acetonitrile (MeCN), obtained from Aldrich-Chemie, Steinheim, Germany, were of HPLC grade. The water used for HPLC analysis was ultrapure generated by an Elgastat Maxima Analytica Water Purification System (Elga Ltd., United Kingdom). All eluents for HPLC were filtered through a 0.45 μm Minisart SRP 25 filter (Bie & Berntsen, Rødovre, Denmark) and degassed with ultrasound for 20 min before use.

Polyacetylene Standards. Five-year old roots (650 g fresh weight) of *P. quinquefolium* were ground and extracted twice with 1.5 L of EtOAc for 24 h at 8 °C. The combined extracts were filtered, dried over anhydrous Na_2SO_4, and concentrated in vacuo (30 °C) to 15 mL. The concentrated extract was subjected to silica gel column chromatography (CC) [silica gel, 60 230-400 mesh, Merck, Darmstadt, Germany], eluting with *n*-hexane−EtOAc (v/v) [20:1, 10:1, 20:3, 5:1, 4:1, 7:3, 6:4, 11:9, 1:1, 4:6, 3:7; 200 mL at each step], and finally with MeOH (250 mL). Fractions containing crude falcarinol and panaxydol, respectively, were further purified by preparative HPLC on a Dionex P680 HPG SemiPrep system (Dionex Denmark A/S, Denmark) equipped with a diode array detector (DAD) [UVD 340U] operating from 200 to 595 nm. The DAD was employed at 205 nm. Separations were performed on a reversed-phase (RP) Develosil ODS-HG-5 HPLC column (RP-18, 250 × 20 mm i.d., Nomura Chemical Co., Seto, Japan), at 22 °C using the following solvent gradient: MeOH−H_2O [0 min (40:60), 10−40 min (75:25), 80−110 min (100:0), 115 min (40:60)]. All increases or decreases in the gradient were programmed as linear. Flow rate: 5 mL min^{-1}. Injection volume: 20 mL. Panaxydol (125 mg) and falcarinol (115 mg) were finally obtained as colorless oils in a purity >98%, as shown by analytical HPLC. Falcarinol ([α]$_D$ −36.6°, c 0.9, $CHCl_3$; lit. (*33*) [α]$_D$ −36.93°, c 0.77, $CHCl_3$) and panaxydol ([α]$_D$ −26.8°, c 1.0, $CHCl_3$; lit. (*34*) [α]$_D$ −22.3°, c 1.7, $CHCl_3$) were identified by UV, mass spectrometry (MS) [gas chromatography (GC)-MS (EI, 70 eV)], and one- and two-dimensional NMR (^{1}H and ^{13}C NMR and ^{1}H−^{1}H and ^{1}H−^{13}C correlation spectroscopy recorded in $CDCl_3$ with TMS as internal standard) and the complete spectral data set corresponded fully with literature values for falcarinol (*23*, *35*, *36*) and panaxydol (*17*, *36*, *37*), respectively. Falcarinol and panaxydol dissolved in ethanol (5 mg mL^{-1}) were used as standards.

Table 1. Major Polyacetylenes Occurring in *Panax quinquefolium* of Different Root Sizes or Types[a]

root type	root diameter (mm)	panaxydol (mg kg^{-1} fresh roots)	falcarinol (mg kg^{-1} fresh roots)
1	0.5–3.0	780 ± 21	2070 ± 18
2	10.0–16.0	270 ± 16	320 ± 17
3	24.0–30.0	210 ± 12	190 ± 10
4	38.0–43.0	230 ± 18	230 ± 19

[a] Root type 1 corresponds to hairy roots, root type 2 corresponds to lateral roots, and root types 3 and 4 correspond to main roots. Analysis was performed in triplicates (mean ± standard deviation). Panaxydol and falcarinol were quantified in extracts by analytical HPLC using calibration curves of panaxydol and falcarinol (external standards), respectively (see Materials and Methods).

Quantification of Polyacetylenes by Analytical HPLC. The concentration of falcarinol and panaxydol were determined in the following root types: 0.5−3 mm (root hairs), 10.0−16.0 mm (lateral roots), and 24.0−30.0 mm and 38.0−43.0 mm (main roots), which were obtained by cutting off the different root parts (according to size) of fresh 5-year old ginseng roots (**Table 1**). The different root types were cut into small pieces and mixed carefully in order to obtain homogeneous and representative samples. Fresh cut root material (8 g) was weighed in a 100 mL flask with screw cap and homogenized with an Ultra-Turrax T25 for 60 s with 25 mL of EtOAc and re-extracted with 25 mL of EtOAc. The combined EtOAc extracts were dried with anhydrous Na_2SO_4 and transferred into a 50-mL volumetric flask and adjusted to a final volume of 50 mL with EtOAc and then carefully shaken. The extract was filtered through a 0.45 μm Minisart SRP 25 filter into a 2 mL brown vial for analysis of polyacetylenes by analytical HPLC on a SUMMIT/Dionex HPLC system (Dionex Denmark A/S, Denmark) equipped with a DAD. The DAD was employed at 205 nm and absorption spectra were recorded between 200 and 450 nm. Polyacetylenes were separated on a reversed-phase Luna 3μ C18(2) 100A column (3 μm; 150 × 4.6 mm i.d., Phenomenex, Aschaffenburg, Germany) at 40 °C using the following solvent gradient: MeCN−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 or decreases in the gradient were programmed as linear. Flow rate: 1 mL min^{-1}. Injection volume: 20 μL. Quantification of falcarinol and panaxydol was made by external calibration curves of authentic standards. Mean recovery rates for the individual polyacetylenes were 96 ± 2% (falcarinol) and 98 ± 3% (panaxydol) and were determined by spiking a known amount of authentic standard (from a stock solution) of each polyacetylene to a ginseng root extract sample.

Raman Spectroscopy. Raman spectra were recorded using a Bruker NIR-FT-Raman Spectrometer (model RFS 100) equipped with a Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. The instrument was equipped with an *xy* stage, a mirror objective, and a prism slide for redirection of the laser beam. Compared with the standard vertical sampling arrangement, the samples were mounted horizontally. Ginseng root disks were mounted between two glass slides to avoid their movement and deformation during the measurement.

Single measurements from ginseng roots were obtained with 128 scans and an unfocused laser beam of 100 mW, whereas for falcarinol and panaxydol solutions, 256 scans and a laser power of 100 mW were used for spectroscopic analysis. All spectra were obtained with a spectral

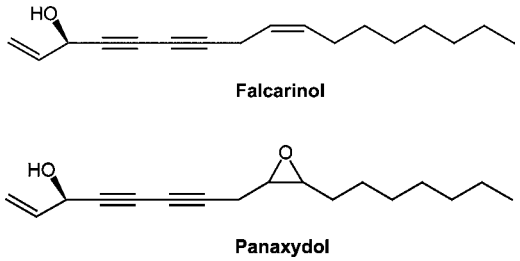

Figure 1. Chemical structure of falcarinol and panaxydol.

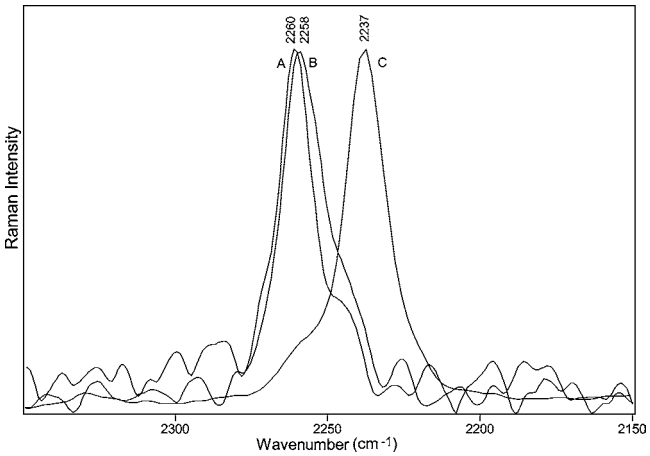

Figure 2. FT-Raman spectra of panaxydol (**A**) and falcarinol (**B**) isolated from ginseng root (dissolved in ethanolic solution) and measured directly in fresh plant (**C**).

resolution of 4 cm^{-1} in the wavenumber range from 100 to 4000 cm^{-1}. 2-D Raman maps of flat samples of the transversely cut *P. quinquefolium* roots were obtained point by point moving the *xy* stage; *x* and *y* directions of the accessory were automatically controlled by the spectrometer software. Slices of fresh and freeze-dried ginseng roots were mapped with a focused laser beam of 100 mW with a diameter of about 0.1 mm; four scans were collected at each measured point. Other parameters used for micro-Raman measurements, such as mapping area and step size (increment), are listed in the legend to the figures as footnotes. The spectra collected from the mapped areas were baseline-corrected and processed by the Bruker Opus/map software package v. 4.3. The maps were obtained by integration of specific signal characteristics for the individual analyte and colored according to the different Raman intensity.

Quantum-Chemical Calculations. Density functional theory (DFT) calculations of falcarinol, panaxydol, and their complexes with ginsenoside were performed with the B3LYP functional and the 6-31G** basis set using Gaussian 03 (*38*).

RESULTS AND DISCUSSION

Raman Identification of Main Polyacetylenes Occurring in *P. quinquefolium* Root. The molecular structure of the two major polyacetylenes occurring in *P. quinquefolium* root, falcarinol and panaxydol, is presented in **Figure 1**. Both compounds were found in the roots of *P. quinquefolium* investigated in this study, isolated by silica gel CC and preparative RP-HPLC, and identified by UV, mass spectrometry, and 1D- and 2D-NMR spectroscopy (see Materials and Methods). FT-Raman spectra taken from pure panaxydol and falcarinol, dissolved in ethanolic solution, are demonstrated in **Figures 2A and 2B**, respectively. Falcarinol can be identified by a distinctive key band at 2258 cm^{-1}, observed also earlier when measurements were taken from carrot roots (*29*), where this compound occurs as a dominant polyacetylene (*24*). The Raman spectrum of panaxydol shows a characteristic, strong,

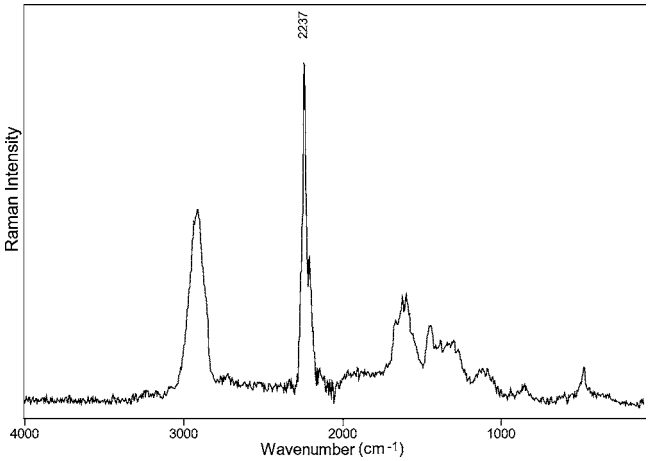

Figure 3. FT-Raman spectrum obtained from fresh ginseng root demonstrated in the whole wavenumber range from 100 to 4000 cm^{-1}.

and polarized band of $-C\equiv C-C\equiv C-$ symmetric stretching vibration at 2260 cm^{-1}. Although both polyacetylene key bands are very intense and comparatively narrow, the wavelength difference of 2 cm^{-1} does not allow discrimination between both compounds when they occur together. The asymmetric stretch of the $-C\equiv C-C\equiv C-$ group is not seen in the Raman spectrum of polyacetylenes because it is only IR active (*39*).

The presence of polyacetylenes in *P. quinquefolium* root can be visually detected by the occurrence of brownish spots located centrically in the phloem parenchyma tissue. Raman spectrum taken directly from these brownish areas in fresh ginseng root is demonstrated in **Figure 2C**, in the wavenumber range from 2150 to 2350 cm^{-1}. Additionally, a whole spectrum is presented in **Figure 3**, where a strong and well-separated polyacetylene band is clearly seen. Generally, in the spectra obtained from fresh root, a strong band can be noticed with a maximum at about 2237 cm^{-1} that is about 20 cm^{-1} less than has been observed for isolated polyacetylenes. Such a discrepancy in the wavenumber position indicates structural changes of these compounds occurring during the extraction process.

It has been reported by Zhang et al. (*40*) that polyacetylenes in Asian ginseng root can be bonded to ginsenosides and usually such complexes are broken when they are extracted, and as a result both compounds are isolated as separate entities. However, Zhang et al. managed to isolate the whole complex, polyacetyleneginsenoside-Ro, from *P. ginseng* root, composed of two subunits: an oleanolic acid-derived triterpene saponin and polyacetylene moiety. Furthermore, they found that polyacetyleneginsenoside-Ro inhibits the in vitro replication of HIV-1 and they suggested that the polyacetylene moiety might be responsible for the higher antiviral potency of this compound relative to that of the other HIV-1 inhibitory saponins. The structure of the isolated polyacetyleneginsenoside-Ro was confirmed by NMR spectroscopy and indicates that the saponin part is linked to the polyacetylene in the distance of three bonds from the $-C\equiv C-$ group. To investigate the influence of such faraway group attachment onto the wavenumber position of the polyacetylene triple bond group, we have performed density functional theory (DFT) frequency calculation for free polyacetylene molecules as well as for their complexes with ginsenosides. The obtained results have shown that change of the wavenumber position of $-C\equiv C-C\equiv C-$ symmetric stretching upon complexation is only 1−2 cm^{-1}. So the observed shift (about 20 cm^{-1}) of the Raman signal of polyacetylenes measured in fresh ginseng compared to the ones obtained from isolated

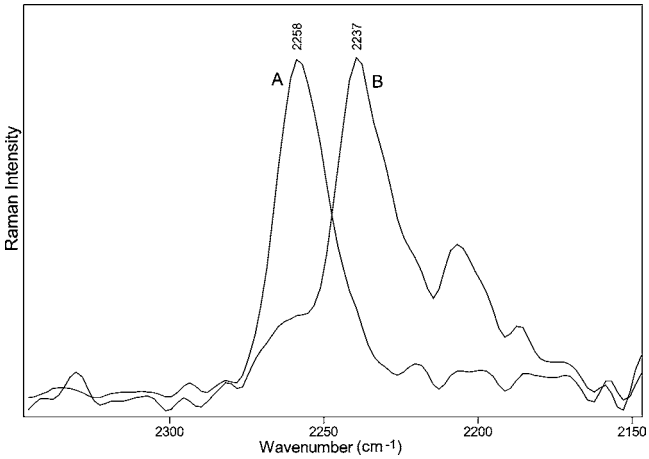

Figure 4. FT-Raman spectra obtained from freeze-dried ginseng root before (**A**) and after (**B**) hydration.

compounds cannot be explained by the possibility of forming polyacetylene glycosides, e.g., polyacetylenes linked to saponins.

Interaction of Polyacetylenes with Plant Constituents. In addition to measurements on fresh *P. quinquefolium* roots, Raman spectra were also taken from freeze-dried roots. Characteristic key bands of polyacetylenes in situ were found at about 2258 cm^{-1}, which is in the same wavenumber region as registered for the isolated standards (see **Figures 2A and 2B**). Furthermore, the same spectrum as for freeze-dried root was obtained after 4-months storage of *P. quinquefolium* root at room temperature. These results indicate that the molecular structure of polyacetylenes occurring in dried roots as well as from extracts of fresh ginseng is the same. Moreover, it is clear that water plays a key role in the observed structural changes caused by solvent extraction or drying of the root.

To investigate this topic closely, a slice of freeze-dried ginseng root was put into water for 24 h. Raman spectra taken from this root, before (**A**) and after hydration (**B**), are demonstrated in **Figure 4**. The position of the polyacetylene band at 2237 cm^{-1} obtained from the "hydrated" root is exactly the same as observed before in the spectrum of fresh root and indicates therefore that the process of hydration results in structural changes of polyacetylenes occurring in the root. So the molecular change of polyacetylenes due to loss of water can be reversed under the access of water; however, this phenomenon has been observed only in situ. During the described process of ginseng root hydration, a fraction of polyacetylenes was isolated from the root and found to be spread on the surface of water; polyacetylenes have lipophilic properties and are not miscible with water. A Raman spectrum obtained from this fraction showed an intense peak at 2258 cm^{-1}, indicating that water did not influence the structure of isolated polyacetylenes. This finding shows that besides water also other plant constituents are responsible for the observed molecular changes of polyacetylenes. The explanation for this phenomenon could be an interaction of polyacetylene molecules with plant components in the presence of water molecules, forming a stable entity in situ that is broken after dehydration (loss of water) of fresh ginseng root. This is very plausible as the π-electrons of the acetylene bonds are likely to be donated to form a complex, as is the case for the lone pairs in water (water is a common ligand in complexes).

The results published by Okninska and Starowieyski, describing a shift of polyacetylene signal of 18 cm^{-1} due to coordination with metal ions, are in some accordance with our data (*41*, *42*). They have described the presence of a relatively strong intramolecular ground-state interaction between the zinc atom and π orbitals of the carbon−carbon triple bond in dihexyn-4-ylzinc. In the Raman spectrum of dihexyn-4-ylzinc, the ν(C≡C) vibration occurs at a lower position (2220 cm^{-1}) in comparison to the one obtained from a parent alkyne (2238 cm^{-1}) and is attributed to the attraction of π-electrons by the zinc atom. Furthermore, the described complex is stable in the presence of solvents such as Et_2O and Et_3N and is broken only by strong electron donors, such as pyridine.

According to Nakamoto (*43*), such complexes consisting of alkyne with a transition metal should be named "σ-bonded" in contrast to "π-bonded" ones. A σ-type bond is formed by the overlap of the filled 2pπ bonding orbital of the −C≡C− with the vacant bonding orbital of the metal whereas a π-type bond could be formed by the overlap of the 2pπ* antibonding orbital of the −C≡C− with a filled orbital of the metal. In the case of σ-bonded complexes the −C≡C− stretching band shifts to lower frequency of about a few tens of wavenumbers whereas π-bonded complexes show a marked shift to lower frequencies (a few hundreds of wavenumbers), relative to those of free ligand. The reason for such a significant shift of an acetylene signal in the case of π-bonded complexes is a considerable lengthening of the −C≡C− bond and partial reduction of the triple bond to a double bond.

The signal shift of the polyacetylene −C≡C− group after hydration of a ginseng root of about 20 cm^{-1} to lower frequencies with respect to the signal obtained from the polyacetylenes occurring in dried root indicates similar engagement of π orbitals of acetylene groups as described above for dihexyn-4-ylzinc complex. So we can expect that σ-type coordination of polyacetylene molecules to some plant components with addition of water molecules forms a stable complex in fresh ginseng root. The loss of water results in breaking of this stable structure and releasing "free" polyacetylene molecules. The same phenomenon of destroying the polyacetylene complexes is observed when they are extracted from the plant.

The obtained results do not indicate unequivocally which plant components are involved in the assumed polyacetylene complexation: it is feasible that proteins or other macromolecules as well as metal ions may play a key role in this process, either in a direct manner or in enhancing the miscibility of polyacetylenes and water. We performed Raman measurements on aqueous solutions containing isolated natural polyacetylenes and metal ions (e.g., $ZnSO_4 \cdot 7H_2O$) and found no band shifts in comparison to the spectra obtained from pure polyacetylenes. So direct proof of the formation of the above-described polyacetylene complex in vitro cannot be presented here; further research and more detailed investigations are necessary in this context. Nevertheless, we suppose that the only way that such a complex can be formed is in situ.

It should be mentioned that we have not observed polyacetylene complexes in carrot roots investigated before (*29*, *32*); Raman spectra of polyacetylenes measured in situ in fresh root and isolated as pure compounds have shown −C≡C− signal exactly at the same wavenumber position (for falcarinol it was 2258 cm^{-1}). The reason we are unable to detect polyacetylene complexes in carrot roots may be their different distribution in comparison to ginseng or some other genetic reasons.

Distribution of Polyacetylenes in *P. quinquefolium* Root. The intense and well-separated polyacetylene band occurring in Raman spectra obtained from ginseng root give good precondition to apply Raman mapping to investigate the distribution of these compounds in situ. The advantage of this technique is the possibility to perform measurements directly

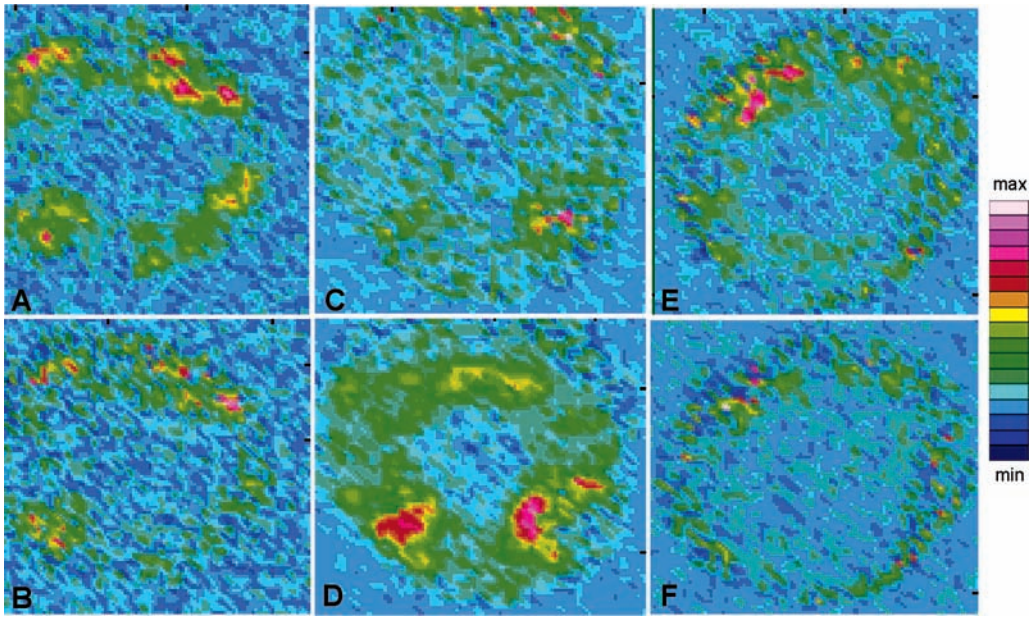

Figure 5. Raman maps obtained from fresh (**A**, **B**), freeze-dried (**C**, **D**) root tissue and after hydration of freeze-dried ginseng root (**E**, **F**) colored according to the individual band intensity at 2237 cm^{-1} (**A**, **C**, **E**) and 2258 cm^{-1} (**B**, **D**, **F**) (mapping parameters: (**A**, **B**) area = 7600 × 7900 μm, increment = 150 μm; (**C**, **D**) area = 6700 × 7450 μm, increment = 150 μm; (**E**, **F**) area = 9300 × 9400 μm, increment = 200 μm).

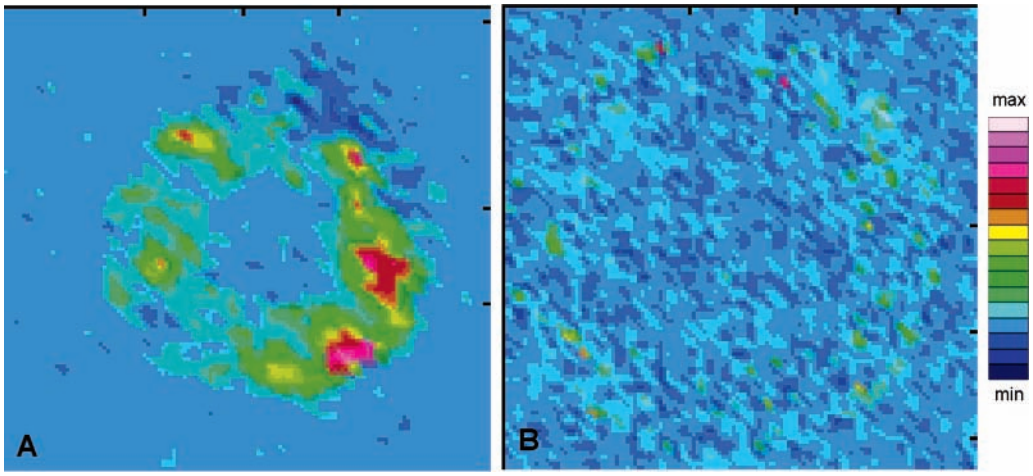

Figure 6. Raman maps obtained from small (8 mm of diameter) and medium (15 mm) root size colored according to the individual band intensity at 2237 cm^{-1}, respectively, **A** and **B** (mapping parameters: (**A**) area = 10000 × 9900 μm, increment = 250 μm; (**B**) area = 23400 × 22300 μm, increment = 400 μm).

in plant tissue, without the necessity of isolating the investigated analyte. Results of Raman mapping obtained at slices of transversely cut ginseng roots are presented in **Figure 5** (**A**, **B**: fresh root; **C**, **D**: freeze-dried root; **E**, **F**: after hydration of freeze-dried root). All spectra were baseline-corrected and integrated in two wavenumber ranges, in the vicinity of signals at 2237 and 2258 cm^{-1}, which corresponds to the presence of complexed and unbounded polyacetylenes, respectively. Raman maps demonstrated in **Figure 5** are colored according to the band intensity at 2237 (**A**, **C**, **E**) and 2258 cm^{-1} (**B**, **D**, **F**), which show the relative content of both compounds in the measured section.

Raman maps obtained from fresh ginseng root (**Figures 5A and 5B**) show the presence of polyacetylenes mainly in a complex form whereas in freeze-dried roots (**Figures 5C and 5D**) principally the unbounded type of polyacetylenes can be seen. After root hydration (**Figures 5E and 5F**) a general decrease in polyacetylene concentration is observed probably due to the fact that a fraction of these compounds is released into the water. Confirmation of this fact is a visible layer on the surface formed by polyacetylenes which have lipophilic properties and are not miscible with water. The Raman spectrum obtained from this layer shows an intense band at 2258 cm^{-1} and therefore confirms that polyacetylenes are present mainly in the unbounded form. This finding also indicates that the observed decrease in polyacetylene concentration after root hydration is not related to a chemical degradation but to an increased release of polyacetylenes into the aqueous environment. Moreover, the described experiment confirms that polyacetylenes are stored in the root channels, which can be penetrated by water. The polyacetylenes remaining in the root after its hydration show mainly complex structure (**Figure 5E**), and only small amounts are still present in the unbounded form (**Figure 5F**).

Generally, it can be observed that polyacetylenes are concentrated in specific areas of the ginseng root. Their high accumulation is located in the outer section of the root, but even there they are not distributed uniformly. Contrary to that, the inner part contains only small amounts of polyacetylenes. Red spots seen in **Figure 5** demonstrate polyacetylene aggregations

whereas in blue areas the level of these compounds is below the detection limit.

These results correspond to the previous reports on polyacetylenes measured in carrot roots. During microscopic observations performed at sections of carrot roots, several conglomerations of extracellular oily droplets containing polyacetylenes were noticed in periderm/pericyclic parenchyma tissue and it seemed that a channel system is formed, extending down the length of the root. Traces of polyacetylenes were also found in oil droplets of phloem parenchyma tissue, but they were smaller and less frequent than those found in periderm/pericyclic parenchyma (*44*, *45*).

It seems that the localization of the polyacetylenes in ginseng root can also be related to the presence of vascular bundles; a higher density of them can be observed in the pericyclic part. These channels could be responsible for transport and accumulation of polyacetylenes.

Effect of Root Size on Polyacetylene Accumulation. We have examined the total content of polyacetylenes in ginseng roots of different sizes corresponding to hairy roots, lateral roots, and main roots, respectively (Table 1). The concentration of polyacetylenes has been found to be significantly higher in small roots (up to 8 mm in diameter) than in large roots, as presented in **Figure 6**, respectively, **A** and **B**. The obtained data correlate very well with the analytical HPLC results (see **Table 1**), which gives precise information about an independent concentration of falcarinol and panaxydol. From the quantitative HPLC data a marked decrease of falcarinol and a less distinctive decrease of the panaxydol content was observed with increasing root size.

An investigation correlating the polyacetylene content and the root size has already been performed by Kidmose et al. (*27*) for carrot root. They found that falcarindiol and falcarindiol 3-acetate decrease significantly with increasing root size whereas the content of falcarinol content was almost the same regardless of size. Increasing of root diameter had only a minor effect on falcarinol because this component is mainly located in the phloem tissue in the carrot root (*21*, *46*). Contrary to that, the concentration of falcarindiol is the highest in the periderm tissue, so during the increase of root size this compound is "diluted" (*27*).

From the results obtained for ginseng root it is clear that the content of both polyacetylenes decreases with increasing root size but the ratio between falcarinol and panaxydol changes markedly at each stage of this process. It may indicate a different distribution of these compounds with falcarinol located in more external tissues with respect to panaxydol. However, further research and more detailed investigations are necessary.

Conclusion. The presented results illustrate that FT-Raman spectroscopy is a powerful tool that in addition to other analytical techniques can provide very informative data of polyacetylene distribution in ginseng root. The opportunity to perform Raman measurements in situ provides a unique chance to investigate the structure of polyacetylenes directly in the plant tissue. The obtained results indicate that a loss of water occurring during the drying process of the root or solvent extraction results in changes of the molecular structure of polyacetylenes, which most likely form complexes with the addition of water in fresh ginseng roots. A dominant role of water in the formation of polyacetylene complexes is not doubted, but it seems that other substances also may be involved in this reaction: a polyacetylene fraction released to water after root hydration did not show any complex formation. The only way to observe the assumed polyacetylene complex was by in situ measurements of ginseng roots containing enough water and other plant components that may participate in this process. However, such a complexation of polyacetylenes may influence their bioactivity and hence the health-promoting properties of this medicinal plant. On the other hand, if the interaction of polyacetylenes with metals takes place, then it should also be considered in the context of toxic properties of the latter.

As polyacetylenes belong to the most important health-promoting compounds in ginseng root, the decrease of their content with increasing root size indicates that especially the smaller roots are of special interest. The Raman technique can thus be used for fast screening of the polyacetylene content in ginseng roots and applied to perform quality checks of incoming raw materials or to select high-quality single plants for selection and breeding of new ginseng genotypes with a significant higher content of polyacetylenes for use in the phytopharmaceutical industry.

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