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Quantification of Two Polyacetylenes in Radix Ginseng and Roots of Related *Panax* Species Using a Gas Chromatography–Mass Spectrometric Method

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A sensitive method for quantitating the pharmacologically active polyacetylenes panaxynol and panaxydol in Radix Ginseng was developed using a capillary gas chromatography–mass spectrometric (GC–MS) method. The detection mode of selected ion monitoring (SIM) allowed sensitive and selective quantitation of the two compounds in ginseng. Method validation showed that the GC–MS method has much lower detection and quantitation limits than the high-performance liquid chromatography (HPLC)-UV method. This indicates that GC–MS is particularly useful for the analysis of polyacetylene compounds, which have relatively low abundances compared with ginsenosides in ginseng. Based on the quantitative results of different types of ginseng herbs, it was found that the panaxydol and panaxynol contents were higher in forest ginseng than in cultivated ginseng. This method was further applied to the quantitative analyses of panaxynol and panaxydol in Radix Notoginseng and American ginseng. The ratio of panaxydol to panaxynol can be utilized as a marker for differentiating ginseng, notoginseng, and American ginseng. This study introduces the first GC–MS method for the quantitative analysis of polyacetylenes in herbs of the *Panax* genus.

KEYWORDS: Ginseng; *Panax*; GC–MS; polyacetylene; panaxydol; panaxynol

INTRODUCTION

For over 1000 years, Radix Ginseng has been considered one of the most valued herbs in traditional Chinese medicine. The petroleum ether fraction extracted from Radix Ginseng has been reported to inhibit the growth of murine leukemia L5178Y and murine sarcoma 180 cells *in vitro* and to inhibit DNA, RNA, and protein synthesis in murine ascetic sarcoma 180 cells *in vitro* (*1*, *2*). Panaxynol (**Figure 1**), a polyacetylene compound, was isolated from the petroleum ether fraction of ginseng roots in 1964 (*3*, *4*). Panaxydol (**Figure 1**), another C_{17} polyacetylene compound, was isolated from ginseng in 1980. Other plants of the *Panax* genus, including *Panax quinquefolius* and *Panax notoginseng*, also contain polyacetylene compounds. Panaxynol and panaxydol have been reported to have antiproliferative effects on various cancer cell lines including murine sarcoma, murine leukemia, human colon carcinoma, and human renal cell carcinoma cell lines (*5*–*8*). In addition, panaxynol and panaxydol exhibit anti-inflammatory properties (*9*), inhibition of platelet aggregation (*10*) and lipoxygenase (*11*), cytotoxic activity against leukemia cells (*12*), and inhibition of 15-hydroxyprostaglandin dehydrogenase (*13*). Therefore, in addition to ginsenosides, polyacetylenes are regarded as active compounds in ginseng. Unlike ginsenosides, polyacetylenes are nonpolar components that can be extracted by the solvents *n*-hexane, diethyl ether, and petroleum ether or by steam-distillation.

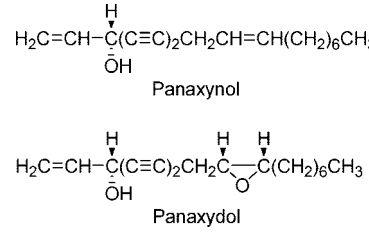

Figure 1. Chemical structures of panxynol and panaxydol in ginseng.

Although many analytical studies on the quantitation of ginsenosides have been conducted, there are only a few reports on the quantitative determination of polyacetylene compounds in ginseng. Nho et al. (*14*) determined the panaxynol and panaxydol contents in ginseng by capillary gas chromatography. Kitagawa et al. (*15*) reported the gas chromatography (GC) determination of polyacetylenes in white ginseng and red ginseng using a derivatization method. Washida et al. (*16*) developed an high-performance liquid chromatography (HPLC)-

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UV method for the quantitation of polyacetylenes in ginseng herbs. To assess the quality of ginseng samples on the basis of their nonpolar constituents and to distinguish ginseng from other closely related herbs, we developed a method that combines gas chromatography with mass spectrometry (GC–MS) for the quantitative analysis of two pharmacologically active polyacetylenes, panaxydol and panaxynol, in three herbs (ginseng, notoginseng, and American ginseng).

MATERIALS AND METHODS

Chemicals, Herbs, and Equipment. Analytical-grade ethyl acetate and *n*-hexane were purchased from Labscan Asia Co. Ltd. (Bangkok, Thailand). A centrifuge (model 5810, Eppendorf) and an ultrasonic instrument (CREST) were employed to extract the herbal samples. Ginseng and notoginseng were collected from the Jilin and Yunnan Provinces of China, respectively. Herbal samples of American ginseng cultivated in Wisconsin were provided by Eu Yan Sang Ltd. (Hong Kong). NMR and electrospray ionization mass spectrometry (ESI-MS) spectra were recorded on a Varian Inova-400 FT-NMR spectrometer and an ESI-Q-TOF mass spectrometer (Bruker Daltonics Inc., Billerica, MA), respectively.

Isolation, Purification, and Structural Identification of Panaxynol and Panaxydol from Ginseng. Dried roots of ginseng (500 g) were powdered mechanically and extracted three times with 1000 mL of *n*-hexane by sonication at room temperature for 1 h. The combined solvent was evaporated under reduced pressure, with the temperature being below 40 °C. The concentrated extract was chromatographically separated by silica gel with a gradient elution of *n*-hexane–EtOAc (19:1–10:1) to yield panaxynol (35 mg) and panaxydol (73 mg). Purities (>98%) were confirmed by thin-layer chromatography (TLC) and GC–MS analyses, and the NMR data were consistent with the published spectroscopic data (*17–20*). The ESI-MS peak for panaxynol is *m*/*z* 267.1734$[M + Na]^+$ (calculated for $C_{17}H_{24}ONa$: 267.1725), and that for panaxydol is *m*/*z* 283.1692$[M + Na]^+$ (calculated for $C_{17}H_{24}O_2Na$: 283.1674).

Sample Preparation for Analysis of Panaxydol and Panaxynol in Ginseng. An accurately weighed amount (0.5 g) of ginseng powder was extracted four times with *n*-hexane (2 × 3 mL, 2 × 2 mL) using sonication for 30 min at room temperature. Each extract was centrifuged separately at 3500 rpm for 10 min The combined supernatant was then transferred to a 10 mL volumetric flask, diluted with *n*-hexane to volume, and mixed well. GC–MS analysis of the fifth extraction (2 mL of *n*-hexane) solution showed that it contained less than 1.5% each of panaxydol and panaxynol based on the total amounts of each compound in the five times of extraction solutions.

GC–MS Conditions. Selected ion monitoring (SIM) GC–MS analysis of the *n*-hexane extracts of the samples was conducted in electron impact (EI) mode using a GC-MS-QP 2010 spectrometer (Shimadzu, Kyoto, Japan). GC separation of the *n*-hexane extracts of the herbs was accomplished by using a 30 mm × 0.25 mm (i.d.), 0.25 μm DM-1 capillary GC column (Dikma Technologies, Beijing, China) coated with cross-linked methyl siloxane. The temperatures for the injector and detector were set at 270 and 280 °C, respectively. The sample solution (1 μL) was introduced into the capillary inlet operated in half-split mode with an equilibrium time of 3.0 min The initial oven temperature was 100 °C, and the temperature was increased to 140 at 8 °C/min, to 180 at 6.0 °C/min, to 220.0 at 2.0 °C/min, and finally to 280 at 4.0 °C/min. The oven temperature was maintained at 280 °C for 20 min with a flow rate of 1.3 mL/min. The split vent was set at 6.9 mL/min, and the septum purge was set at 3.0 mL/min. The mass spectrometer was operated in EI mode. Initially, detection of the two compounds was performed using the full-scan mode in the range *m*/*z* 40–380. Quantification of the compounds was carried out in SIM mode, with scanning at *m*/*z* 159 and *m*/*z* 121 for the detection of panaxynol and panaxydol, respectively.

Method Validation. *Calibration Curves.* The stock solutions of standard panaxynol and panaxydol were prepared freshly in *n*-hexane and serially diluted with the same solvent to prepare the standard solutions. The concentrations were in the range 3.03–60.6 μg/mL for panaxynol and 2.45–78.4 μg/mL for panaxydol. Eight-point calibration curves were acquired by plotting the peak area against the concentration of the calibration standards.

Precision. The intraday precision was estimated by five replicate injections during the same day of standard solutions at three different concentrations (2.10, 8.30, and 16.6 μg/mL for panaxynol; 4.70, 18.8, and 37.6 μg/mL for panaxydol). A relative standard deviation (RSD) within 5% was the criterion for acceptability of data.

Recovery. The recovery studies were conducted by spiking one batch of Radix Ginseng with three different concentrations of recovery standard solution. The recovery standard solutions were prepared by dilution of the stock solution, that is, 1 mL of standard solution containing 4.0, 24.2, or 36.4 μg/mL panaxynol or 28.2, 75.2, or 187.8 μg/mL panaxydol. One milliliter of each recovery standard solution was added to separate aliquots of the ginseng powder. The spiked samples were then extracted, processed, and quantified in accordance with the established method ($n = 3$).

RESULTS AND DISCUSSION

Optimization of Sample Solution Preparation. Although several different methods and solvents have been used to extract polyacetylenes from ginseng herb, in this study, *n*-hexane was selected as the extraction–solvent on the basis of previously reported optimization results (*16*). To determine the optimal particle size of ginseng powders before extraction, six different sizes (30-, 60-, 90-, 120-, 140-, and 160-mesh) of powders were compared with respect to the extraction yield of polyacetylenes. The quantitative GC–MS results showed that 120-mesh powders provided a larger extraction yield of polyacetylenes. When 120-mesh powders were extracted four times with *n*-hexane using sonication (2 × 3 mL, 2 × 2 mL; 30 min each) at room temperature, the extraction yields for both panaxydol and panaxynol were higher than 98.5%.

Optimization of GC–MS Conditions. Typical GC–EI–MS spectra of panaxynol and panaxydol standards in the positive mode are displayed in **Figure 2**. Because each MS spectrum showed the molecular ion in very low abundance, quantification based on detection of the molecular ions, which results in higher limits of detection, would lead to low sensitivities of quantitation of the two polyacetylenes. In general, two factors for the selection of fragment ions suitable for detection and quantification in SIM mode for GC–MS analysis should be considered. First, the fragment ion must be specific to the compound so that the resolution of the GC peak is satisfactory. Second, the fragment ion must be sufficiently abundant for sensitive detection of the compound. In this study, *m*/*z* 159 and *m*/*z* 121 were the ions selected for quantitation of panaxynol and panaxydol, respectively (**Figure 3**). No interference was found near the peaks of these two compounds in the SIM chromatograms of ginseng extract, allowing accurate determination.

Several temperature programs were carefully considered in the optimization of gradient separation. When the rate of temperature increase was set at 2.0 °C/min from 180 to 220 °C and then at 4.0 °C/min from 220 to 280 °C, the respective peaks showed satisfactory resolution from the neighboring peaks.

Parameters for Method Validation. The limit of detection (LOD) was evaluated as the concentration of polyacetylene that generates a signal-to-noise ratio of 3 ($S/N = 3$). The LOD was determined to be 0.014 μg/mL for panaxynol and 0.022 μg/mL for panaxydol. The limit of quantification (LOQ), defined as the lowest concentration of polyacetylene that can be measured with acceptable accuracy and precision, was estimated by replicate injection of the calibration standards. The LOQ was determined to be 0.041 μg/mL for panaxynol and 0.057 μg/mL for panaxydol. The precision (RSD) of six injections was 4.12% for panaxynol and 2.84% for panaxydol.

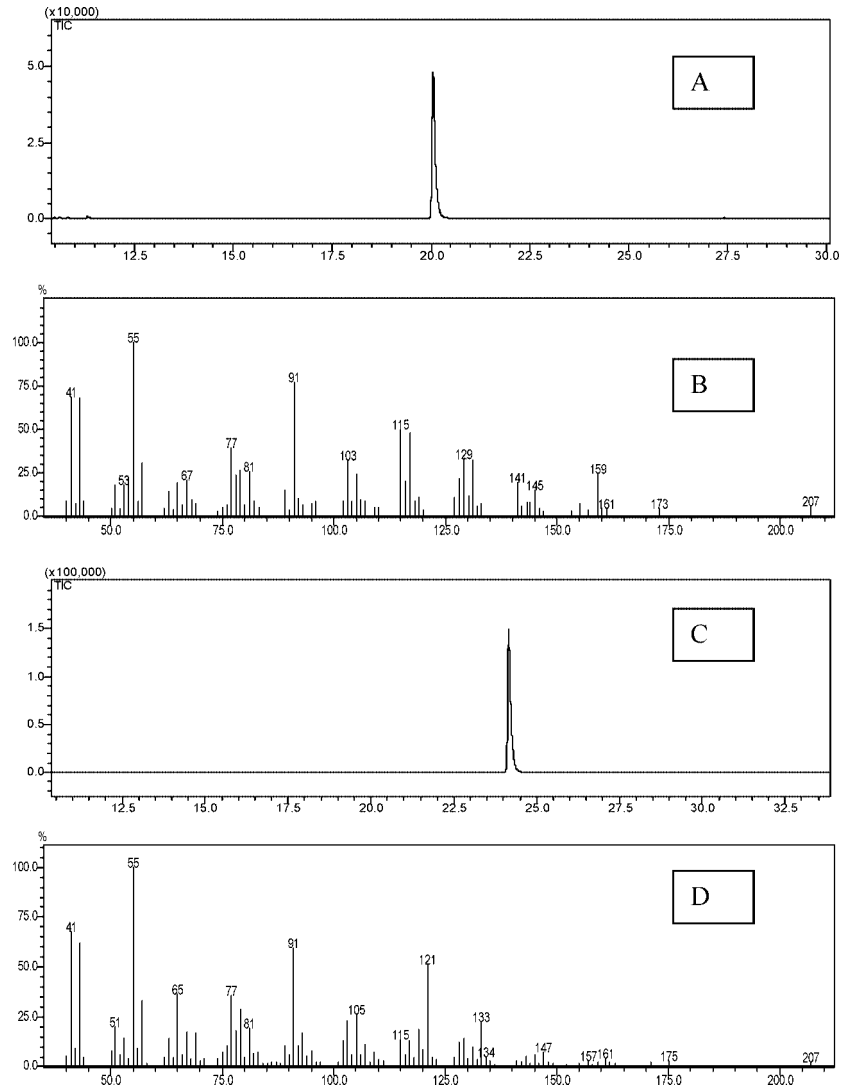

Figure 2. Gas chromatograms and mass spectra of panaxynol and panaxydol: (A) gas chromatogram of a panaxynol standard solution; (B) mass spectrum of panaxynol; (C) gas chromatogram of a panaxydol standard solution; and (D) mass spectrum of panaxydol.

The linear calibration curves were obtained over the entire range of concentrations studied. Regression analysis of the peak area ratios (y) versus concentration (x) for panaxynol and panaxydol was conducted. The equations were as follows: for panaxynol, $y = 11228.4x - 24047.7$ in the range 3.03–60.6 μg/mL with $r^2 = 0.9986$; and for panaxydol, $y = 19991.8x - 56159.4$ in the range 2.45–78.4 μg/mL with $r^2 = 0.9976$.

The precision was measured by performing intraday experiments of five replicate injections of standard solutions at three different concentrations. The RSDs of precision were in the range 1.87–3.31% for panaxynol and 1.36–2.71% for panaxydol (**Table 1**.). The intraday precision was within the acceptable limit of 5% RSD. These results indicated good precision for the developed method at low, medium, and high concentrations of polyacetylene compounds.

The accuracy of the method was determined by the relationship between the amounts of added standards and the amounts detected by the GC–MS assay. As shown in **Table 1**, the recovery values were expressed as the percentage of the calculated concentration that was detected. The recoveries were in the range 94.9–99.0% for panaxynol and 98.2–102.8% for panaxydol, with RSD values of 1.28–2.54% for panaxynol and 2.47–3.20% for panaxydol, which met the criterion for acceptability of accuracy (95–105%) at the analyte concentration of ~0.01%.

Quantitation of Polyacetylenes in Ginseng, Notoginseng, and American Ginseng. Due to different agricultural environments, cultivated ginseng is classified into several types such as Yuan-Ban-Yuan-Lu, Ma-Ya, Shi-Zhu, Chang-Bo, and Bian-Tiao on the basis of morphological characteristics of the rhizome and root, the cultivation conditions and regions, and so forth (*21*). The concentrations of panaxynol and panaxydol in five types of cultivated ginseng and in three forest ginseng samples are summarized in **Table 2**. The panaxynol and panaxydol contents in the main roots of 14 cultivated ginseng samples ranged 84.2–261.8 μg/g and 102.7–247.4 μg/g, respectively. The Yuan-Ban-Yuan-Lu type of ginseng possessed the highest content of total polyacetylenes (413.0 μg/g). The ratio of panaxydol to panaxynol ranged 0.5–1.7 for the cultivated ginseng samples.

Forest ginseng is a type of ginseng that has characteristics between wild ginseng and cultivated ginseng. Like wild ginseng, forest ginseng grows in the natural environment, but its young seedling is transplanted artificially or germinates from artificial sowing of ginseng seeds. The panaxydol and panaxynol contents in two samples of forest ginseng were determined quantitatively by the same GC–MS method used for cultivated ginseng samples. The forest ginseng samples showed much higher panaxynol and panaxydol contents than the cultivated ginseng samples. The ratio of panaxydol to panaxynol for the forest

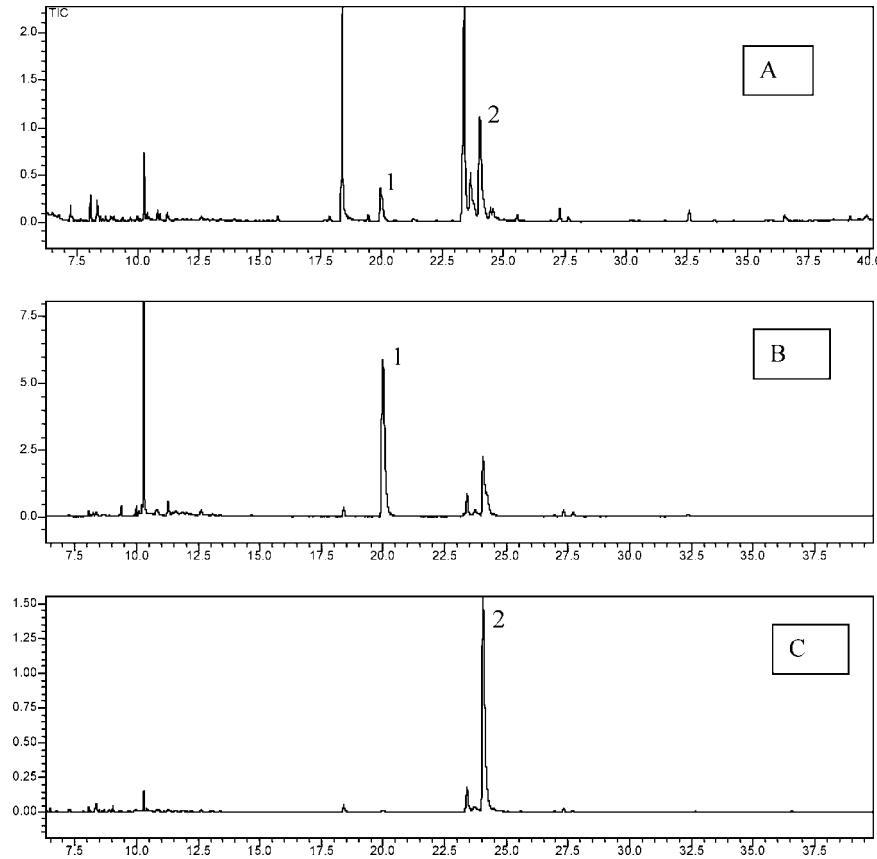

Figure 3. GC–MS chromatograms for the analyses of panaxydol and panaxydol in the *n*-hexane extract of ginseng: (A) TIC of a cultivated Ginseng sample detected with the scan mode; (B) SIM chromatogram targeting ion *m*/*z* 159; and (C) SIM chromatogram targeting ion *m*/*z* 121. Peak 1: panaxynol; peak 2: panaxydol.

Table 1. Intraday Precision for GC–MS Quantitation of Panaxynol and Panaxydol and Recovery Rates of Panaxynol and Panaxydol in GC–MS Analyses

cmpd	conc (μg/mL)	RSD (%)[a]	added (μg)	recovery (%)	RSD (%)[a]
panaxynol	2.10	3.31	4.04	94.9	2.00
	8.30	2.31	24.24	99.0	1.28
	16.60	1.87	36.36	96.0	2.54
panaxydol	4.70	2.71	28.20	100.3	3.20
	18.80	1.56	75.20	102.8	2.47
	37.60	1.36	187.80	98.2	2.79

[a] $n = 5$.

ginseng was 1.0, which is in the middle of the range observed for the cultivated ginseng.

When the concentrations of polyacetylene compounds in notoginseng and American ginseng were analyzed, it was found that both American ginseng and notoginseng have higher polyacetylene contents than ginseng (**Table 3**.). The main roots of American ginseng were found to contain panaxynol and panaxydol at mean concentrations of 486.5 and 1220.1 μg/g, respectively. The main roots of Notoginseng were found to have panaxynol and panaxydol at mean concentrations of 357.1 and 1745.8 μg/g, respectively. Among the three kinds of herbs originating from *Panax* plants, notoginseng had the highest content of total polyacetylenes (2102.9 μg/g). Notoginseng had the highest ratio of panaxydol to panaxynol (5.1), American ginseng ranks in the middle (2.5), and ginseng (cultivated or forest) has the lowest ratio (0.5–1.7). The different ratio of panaxydol to panaxynol may be due to the different kinds and activities of the enzymes in the biosynthetic pathways of these polyacetylenes in different *Panax* species.

Finally, the polyacetylene concentrations in different parts of Radix Ginseng were analyzed. The content of polyacetylenes was highest in the fibrous roots, intermediate in the branch roots, and lowest in the main root (**Table 4**.).

Polyacetylene compounds such as panaxydol and panaxynol are nonpolar constituents of ginseng. Partially responsible for the pharmacological activities of ginseng herb, polyacetylenes are the major components of the essential oils of ginseng. In contrast to the large number of papers describing the quantitative analysis of ginsenosides (the polar components of ginseng), few studies have addressed the quantitation of polyacetylenes in ginseng or related herbs.

This study used panaxydol and panaxynol isolated from ginseng as marker compounds to establish an accurate and reliable GC–MS method for the determination of these polyacetylenes in three herbs of the *Panax* genus. The selected ion monitoring (SIM) technique for GC–MS analysis was adopted for the more sensitive and selective detection of these two polyacetylenes by choosing a specific ion peak in the mass spectrum of each compound compared with that of the GC–flame ionization detection (FID) method (*14*). The method was validated for accuracy, precision, recovery, and selectivity. The method validation showed that GC–MS has 10 times lower quantitation limits than the HPLC-UV method (*16*), demonstrating that this GC–MS method is very sensitive. The GC–MS method in this paper is much simpler than the GC analysis of the derivatives of the polyacetylenes (*15*). Therefore, the method developed here is particularly useful for the quantitative determination of polyacetylene compounds, which have rela-

Table 2. Contents of Polyacetylenes in Different types of Ginseng from the Jilin Province, China

type	source	panaxynol (μg/g)	panaxydol (μg/g)	total (μg/g)	ratio of panaxydol/panaxynol
Yuan-Bang	Fusong	261.8 ± 4.2	132.2 ± 5.1	394.0	0.5
Yuan-Lu	Jingyu	207.3 ± 2.2	171.4 ± 5.0	378.7	0.8
	Dunhua	219.6 ± 5.7	246.8 ± 4.4	466.4	1.1
	mean	**229.6**	**183.5**	**413.0**	**0.8**
Ma-Ya	Fusong	128.6 ± 2.2	155.6 ± 4.5	284.2	1.2
	Fusong	148.9 ± 2.1	218.3 ± 0.9	367.2	1.5
	Jingyu	175.6 ± 3.0	145.1 ± 3.2	320.7	0.8
	Dunhua	183.6 ± 0.9	247.4 ± 2.7	431.0	1.3
	mean	**159.2**	**191.6**	**350.8**	**1.2**
Shi-Zhu	Fusong	244.3 ± 3.6	139.1 ± 4.2	383.4	0.6
	Linjiang	100.5 ± 3.8	102.7 ± 2.9	203.2	1.0
	mean	**172.4**	**120.9**	**293.3**	**0.7**
Chang-Bo	Fusong	84.2 ± 1.6	143.2 ± 2.0	227.4	1.7
	Jingyu	89.2 ± 0.7	140.8 ± 1.1	230.0	1.6
	mean	**86.7**	**142.0**	**228.7**	**1.6**
Bian-Tiao	Ji'an	131.2 ± 3.5	150.4 ± 3.2	281.6	1.1
	Ji'an	146.1 ± 3.9	108.0 ± 3.9	254.1	0.7
	Ji'an	178.3 ± 2.1	135.6 ± 1.2	313.9	0.8
	mean	**151.9**	**131.3**	**283.2**	**0.9**
Forest Ginseng	18-Years	184.8 ± 3.08	204.3 ± 3.59	389.1	1.11
	Market-1	613.6 ± 2.6	587.8 ± 2.7	1201.4	0.96
	Market-2	209.6 ± 3.3	216.8 ± 2.6	426.4.	1.03
	mean	**336.0**	**336.3**	**672.3**	**1.00**

Table 3. Contents of Polyacetylenes in American Ginseng and Notoginseng[a]

source	panaxynol (μg/g)	panaxydol (μg/g)	total (μg/g)	ratio of panaxydol/panaxynol
American Ginseng-1 (Wisconsin)	649.2 ± 3.3	1681.6 ± 4.8	2330.8	2.6
American Ginseng-2 (Wisconsin)	323.7 ± 4.7	758.6 ± 3.5	1082.3	2.3
mean	**486.5**	**1220.1**	**1706.6**	**2.5**
Notoginseng-1 (Wen Shan, Yunnan Province, China)	229.6 ± 3.8	1265.6 ± 11.3	1495.2	5.6
Notoginseng-1 (Ma Guan, Yunnan Province, China)	484.5 ± 5.9	2225.9 ± 9.2	2710.4	4.6
mean	**357.1**	**1745.8**	**2102.9**	**5.1**

[a] $n = 3$.

Table 4. Content of Polyacetylenes in Different Parts of the Ginseng Root[a]

	panaxynol (μg/g)			panaxydol (μg/g)		
ginseng	main root	branch root	fibrous root	main root	branch root	fibrous root
Yuan-Bang Yuan-Lu	207.3 ± 2.2	342.7 ± 3.1	657.9 ± 4.7	171.4 ± 5.0	521.5 ± 4.6	576.4 ± 4.1
Ma-Ya	148.9 ± 2.1	254.2 ± 3.4	579.8 ± 3.3	218.3 ± 0.9	555.8 ± 4.5	575.8 ± 3.4
Bian-Tiao	146.1 ± 3.9	237.0 ± 2.3	593.3 ± 4.5	108.0 ± 3.9	385.9 ± 4.3	580.0 ± 3.2

[a] $n = 3$.

tively low abundances compared with ginsenosides in ginseng samples. Furthermore, the method was confirmed to be applicable for the quantitative analysis of polyacetylenes in the herbs of other *Panax* species, such as American ginseng and notoginseng. This investigation further demonstrated that ginseng, American ginseng, and notoginseng can be distinguished based on the concentration ratio of panaxydol to panaxynol in the herb. Thus, in addition to ginsenosides, the ratio of panaxydol to panxynol can be exploited as a marker for the differentiation of ginseng, American ginseng, and notoginseng. We also conducted quantitative analyses of the two polyacetylene compounds in forest ginseng, a new type of ginseng recorded in the Chinese Pharmacopoeia (2005 edition) (*22*). Compared with cultivated ginseng, forest ginseng is much more expensive and has a higher content of ginsenosides (unpublished data). The present study revealed that the concentrations of polyacetylenes were higher in forest ginseng than in cultivated ginseng, consistent with the higher concentration of ginsenosides in forest ginseng.

Polyacetylenes are the major components of essential oils or *n*-hexane extracts of ginseng, and they represent a group of nonpolar compounds that may be responsible for the anticancer effects of ginseng. Polyacetylenes may be considered as another type of marker compound for the quality assessment of ginseng and related herbs from the *Panax* genus. The quantitative GC–MS method described in this study, the first GC–MS

method for the quantitative analysis of polyacetylenes, can be applied to the quality assessment of ginseng, notoginseng, and American ginseng.

ABBREVIATIONS USED

GC–MS, gas chromatography coupled with mass spectrometry; EI, electron impact; TIC, total-ion-current; SIM, selected ion monitoring; RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification.

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