JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quantification of Protodioscin and Rutin in Asparagus Shoots by LC/MS and HPLC Methods

MINGFU WANG,* YAAKOV TADMOR, QING-LI WU, CHEE-KOK CHIN, STEPHEN A. GARRISON, AND JAMES E. SIMON

New Use Agriculture and Natural Plant Products Program and Department of Plant Biology and Pathology, Cook College, Rutgers University, and New Jersey Agricultural Experiment Station, 59 Dudley Road, New Brunswick, New Jersey 08901

A liquid chromatography/mass spectrometry (LC/MS) method with selected ion monitoring was developed and validated to analyze the contents of protodioscin and rutin in asparagus. The distribution of rutin and protodioscin within the shoots was found to vary by location, with the tissue closest to the rhizome found to be a rich source of protodioscin, at an average level of 0.025% tissue fresh weight in the three tested lines, while the upper youngest shoot tissue contained the highest amount of rutin at levels of 0.03−0.06% tissue fresh weight. The lower portions of the asparagus shoots that are discarded during grading and processing should instead be considered a promising source of a new value-added nutraceutical product.

KEYWORDS: Asparagus; protodioscin; rutin; LC/MS; quantification

INTRODUCTION

Asparagus (*Asparagus officinalis* L., Family Liliaceae) is a popular vegetable that is consumed in many parts of the world for its edible shoots used in salads, vegetable dishes, and soups. Historically, asparagus has been used as a traditional medicine in both European and Asian cultures. In China, asparagus has been used in traditional Chinese medicine as a tonic, antifebrile, antitussive, hair growth stimulator, and diuretic agent (*1*). Asparagus root is also formally listed by the German Commission E (*2*) as an approved medicinal product in the treatment of inflammatory diseases of the urinary tract, for prevention of kidney stones, and with a noted diuretic effect. Modern pharmacological studies have found the extracts of asparagus to possess certain biological activities including antifungal (*3*), diuretic (*4*), cytotoxic (*1*, *5*−*7*), antiviral (*8*), and molluscicide (*9*) properties.

Asparagus is a rich source of phytochemicals. Several types of compounds including flavonoids, sterol saponins, oligosaccharides, carotenoids, sulfur-containing acids, and amino acids have been identified in asparagus (*5*, *10*−*12*). Among this wide group of compounds, protodioscin ($C_{51}H_{84}O_{22}$), the major saponin in asparagus, has attracted more attention. Protodioscin has been associated with bioactivities including cytotoxicity against several human cancer cell lines, especially selectively against one leukemia line (MOLT-4), one NSCLC line (A549/ATCC), and two colon cancer lines (HCT-116 and SW-620) in a brand new pattern when compared in the NCI's anticancer drug screen database (*13*), antiherpes simplex virus type 1 (HSV-1) activity (*14*), cytotoxic activity against human leukemia HL-60 cells (*1*, *5*), cytotoxicity on cultured C6 glioma cells (*15*), xanthine oxidase inhibitors (*16*), antineoplastic activity (*17*, *18*), and improving sexual desire and enhancing erections (*19*). It is of interest to note that protodioscin is the major component in a popular libido-increasing dietary supplement, *Tribulus terrestris* (*20*). Given these activities, this compound became a major focus of our research, but the accurate analysis of protodiosin from botanical sources has limited research and is challenging due to its weak UV absorption. Only a few papers provide reliable procedures for the quantification of protodiosin, and of these, the analytical focus was on the determination of this compound in *Tribulus*. Those protocols focused on the analysis of protodioscin by a colorimetric method (*21*) and quantification of the aglycone diosgenin by high-performance liquid chromatography (HPLC) and then converting it back to protodioscin (*22*) and direct quantification of protodioscin by HPLC using evaporative light scattering detection (*20*). However, these methods may not directly apply to protodioscin analysis in asparagus.

Rutgers University has an active program in asparagus research. Among the goals of this program is to increase the levels of bioactive compounds (protodioscin and rutin) in asparagus by breeding. This requires accurate identification and quantification of these compounds in the parent plants and a large number of the progenies. Here, we report a positive and negative electrospray ionization (ESI)-mass spectrometry (MS) method to characterize the major compounds including saponins and flavonoids in asparagus shoots. We also developed and validated a LC/MS method with selected ion monitoring to quantify the major saponins protodioscin and rutin in asparagus shoots. This method is accurate, reliable, sensitive, and simple and can be used to analyze various asparagus samples with varied amounts of rutin and protodioscin.

* To whom correspondence should be addressed. Tel: 732-932-9711 ext. 367. Fax: 732-932-9441. E-mail: mwang@aesop.rutgers.edu.

10.1021/jf0344587 CCC: $25.00
Published on Web 09/09/2003

MATERIALS AND METHODS

Plant Materials. Asparagus shoots were harvested from three lines of asparagus from Rutgers Agricultural Research and Extension Center at Bridgeton, NJ, in May−June 2000. Shoots were harvested by hand, at the ideal time for the fresh market, cleaned, and prepared as if for sale on the fresh market. Each spear was then immediately frozen after field harvesting and stored under −10 °C.

Chemicals and General Procedures. All solvents used in this research were HPLC grade and purchased from Fisher Scientific (Springfield, NJ). Rutin was purchased from Sigma (St. Louis, MO), protodiosin was purified from tribulus extract, and the structure was validated by NMR spectra and MS data. HPLC analyses were performed on an Agilent 1100 system equipped with an autosampler, a quaternary pump system, a photodiode array and multiple wavelength detector, a thermostated column compartment, a degasser, and a Chemstation software. Negative and positive ESI-MS were measured on an Agilent 1100 LC/MSD system equipped with an electrospray ion source, Bruker Daltonics 4.0 and Data analysis 4.0 software. A prepacked 250 mm × 4.6 mm (5 μm particle size) Luna C_{18} (*2*) column (Phenomenex, Torrance, CA) was selected for HPLC analysis. The absorption spectra were recorded from 200 to 400 nm for all peaks; quantification for rutin was carried out at a single wavelength of 255 nm.

Preparation of Samples for HPLC Analysis and LC/MS Analysis. Six frozen asparagus shoots of each line were separated equally into three parts (top, middle, and bottom, each about 8 cm long). Samples were removed from the freezer, immediately cut into small pieces, and ground under liquid nitrogen to ensure that all samples were uniform. About 2 g of each sample was then separately weighed to 50 mL volumetric flasks, and 35 mL of 70% ethanol aqueous solution was added to the flasks. Samples were sonicated for 45 min and then allowed to cool to room temperature and filled to volume with 70% ethanol. The extraction solution was transferred to the centrifuge tube and centrifuged at 12 000 rpm for 2 min to obtain a clear solution for HPLC analysis.

Preparation of Standards for HPLC Analysis. About 3 mg of each standard compound (rutin and protodioscin) was accurately weighed and placed into a 10 mL volumetric flask. Seven milliliters of 70% ethanol was added, and the solutions were sonicated for 15 min. The flasks were allowed to cool to room temperature and filled to full volume with 70% ethanol solution. Calibration curves were established on 12 data points covering a concentration range of 0.0487−280 μg/mL for rutin and 0.0609−350 μg/mL for protodioscin. Ten microliter aliquots were used for HPLC analysis.

Quantitative Determination of Rutin by HPLC. A Luna C18 (*2*), 5 μm, 4.6 mm × 250 mm column was used in this HPLC analysis. The column temperature was ambient, and the mobile phase included water (containing 0.1% formic acid, solvent A) and acetonitrile (solvent B) in a gradient system. The total running time was 30 min. The gradients were 0 min, 84% solvent A and 16% solvent B, 20 min, 60% A, 40% B, 30 min, 40% A and 60% B, and the postrunning time was 15 min. The flow rate was 0.7 mL/min, the injection volume was 10 μL, and the detection wavelength was set at 255 nm for rutin analysis.

HPLC/MS Conditions for Characterizing Major Compounds in Asparagus Shoot Extract. The mobile phase was the same as the HPLC method described above except mobile phase A was 0.05 M ammonium acetate buffer (pH 4.25) instead of 0.1% formic acid. The electrospray ion mass spectrometer (ESI-MS) was operated under a positive ion mode or negative mode and optimized collision energy level of 60%, scanned from *m*/*z* 200 to 1400. ESI was conducted using a needle voltage of 3.5 kV. High-purity nitrogen (99.999%) was used as drying gas, and the flow rate was at 9 L/min, while the capillary temperature was at 325 °C. Helium was used as the nebulizer at 45 psi. The ESI interface and MS parameters were optimized to obtain maximum sensitivity.

Selection of HPLC/MS Conditions for the Quantification of Protodioscin. As protodioscin lacks a chromophore, it precludes sensitive detection by UV and only can be accurately analyzed by specific detection such as evaporative light scattering detection and MS detection. We tried many different conditions to identify a reliable and accurate method of analysis including LC/MS with selected ion monitoring (SIM) and LC/MS/MS with selected daughter ion monitoring under positive ion mode and negative ion mode. For LC/MS with SIM under the positive ion mode (0.1% formic acid−acetonitrile as mobile phase), we selected the $[M - 18 + 1]^+$ ion peak to quantify protodiosin in asparagus; yet, even under these conditions, we only could achieve good linearity in a very narrow concentration range, not suitable for analyzing asparagus samples with wide protodioscin content variation. Under positive mode, we also tried to quantify protodioscin using an LC/MS/MS method by monitoring the daughter ion 869 from the first stage mass ion peak $[M - 18 + 1]^+$. This method should theoretically be better than LC/MS because it goes through a two stage selection, for well-separated asparagus compounds, the advantage was not significant, and there, too, a linearity was achieved only across a narrow concentration range. We next evaluated a negative mode, although theoretically, formic acid in the mobile phase should suppress the negative ion formation thus lowering the sensitivity of testing. Under this negative mode, at the energy level of 60%, the drying temperature at 325 °C, and the $[M - 1]^-$ was observed as the major ion peak for protodioscin, thereby allowing this ion to be selected for use in the quantification of protodioscin in asparagus. We later found this method to be very sensitive and accurate in achieving a linearity over a wide concentration range. Finally, the LC/MS quantification method was selected to run under negative mode. The instrumental conditions were identical to that described above except the mobile phase A was 0.1% formic acid. SIM was used to record the abundance of the $[M - 1]^-$ molecular ion peaks at *m*/*z* 1047.9 for protodioscin and at *m*/*z* 609 for rutin. Quantification was based on the LC/MS peak areas of protodisocin and rutin, and standard curves were used for calculation.

Validation of LC/MS Method with Selected Ion Monitoring. Accurate assessment of the contents of bioactive compounds in botanical samples requires the validation of certain analytical parameters such as precision, recovery, linearity, and limit of detection. The precision of this LC/MS method with SIM under negative mode was performed by injecting an asparagus shoot bottom extract eight times. The relative standard deviation (RSD) was found to be 2.47% for the content of protodioscin and 3.01% for the content of rutin, suggesting that the method is suitable for protodioscin and rutin analysis. The recovery was validated by spiking one sample with known concentrations of protodioscin and rutin and then calculating recovery rate. Results showed that 95.25 and 92.70% of the theoretical amounts of rutin and protodioscin were recovered. The calibration curves (**Figure 10**) of this method were constructed by injecting the standard solution across 12 different concentrations (0.0000487−0.28 mg/mL for rutin and 0.0000609−0.35 mg/mL for protodioscin). The linearity range of detection was found to be 0.0000487−0.001554 mg/mL for rutin and 0.0000609−0.0777 mg/mL for protodioscin, suggesting that this protocol is a very sensitive method for the quantitative analysis of protodioscin and rutin. The linearity of the HPLC method with UV detection for rutin was found to be 0.000487−0.28 mg/mL (see calibration curve in **Figure 11**). These validation studies show that the recommended method is reliable and sensitive allowing for the phytochemical analysis of rutin and protodioscin from asparagus.

RESULTS AND DISCUSSION

Extraction. Fresh asparagus was ground and extracted for analysis of bioactive phytochemicals. Ethanol (70%) was selected as extraction solvent in this study because in our preliminary work we found that when dissolved in methanol or methanol solution, protodioscin could rapidly convert into methyl protodioscin. In contrast, this compound was stable in ethanol, ethanol solution, and acetonitrile. Methanol was avoided in this study.

Characterization of Major Components in Asparagus by LC/MS Method. Positive and negative ESI-MS were used to identify the major compounds in asparagus. Authentic rutin and protodioscin were run first to confirm that the protonated, sodium-abducted, and deprotonated molecules could be observed under either positive or negative mode. For rutin, at reasonable experimental conditions, *m*/*z* 611 $[M + 1]^+$ or 633 $[M + Na]^+$

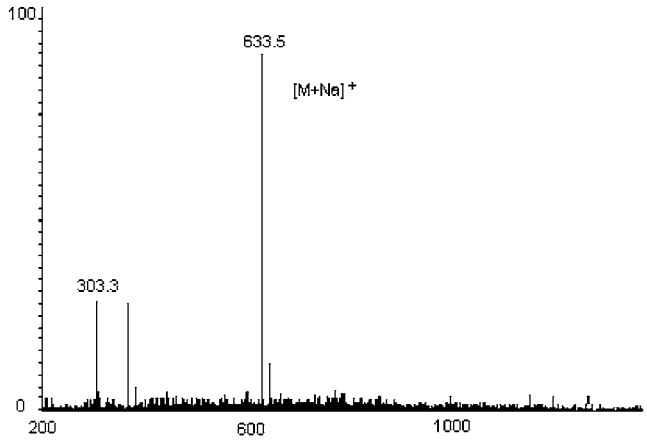

Figure 1. Representative positive ESI mass spectrum of rutin.

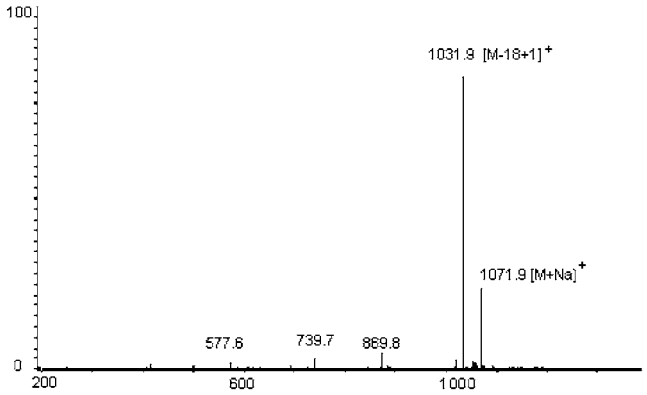

Figure 2. Representative positive ESI mass spectrum of protodioscin.

was always observed in the positive mode and *m*/*z* 609 $[M - 1]^-$ was observed under the negative mode. The representative MS spectrum of rutin under positive mode is illustrated in **Figure 1**. Results were significantly different with protodisocin, where under a negative mode, optimized collision energy level of 60%, scanned from *m*/*z* 200 to 1400, using 0.1% formic acid−acetonitrile as mobile phase; significant $[M - 1]^-$ molecular ion was observed. Under positive mode, the dominant peak was always $[M - 18 + 1]^+$ and neither $[M + 1]^+$ nor $[M + Na]^+$ could be detected under various analytical conditions including low collision energy and low drying temperature. However, when the mobile phase was changed to 0.05 M ammonium acetate buffer (pH 4.25)−acetonitrile gradient, and at the optimum collision energy level of 60%, the $[M + 1]^+$ and $[M + Na]^+$ were finally detected. Other peaks detected include neutral losses of rhamnose (Rha) and glucose (Glu) at *m*/*z*: 869.8 $[M - 18 - Glu + H]^+$, 739.7 $[M - 18 - 2Rha + H]^+$, and 577.6 $[M - 18 - 2Rha - Glu + H]^+$. The mass spectrum of protodioscin is illustrated in **Figure 2**. The major ion peak $[M - 18 + 1]^+$ observed in MS for protodioscin was further subjected to MS/MS analysis, the major second stage mass ion peaks include 869.5 $[M - 18 - Glu + H]^+$, 723.4 $[M - 18 - Glu - Rha + H]^+$, 577.5 $[M - 18 - 2Rha - Glu + H]^+$, and 415.3 $[M - 18 - 2Rha - 2Glu + H]^+$. The MS/MS of ion peak at *m*/*z* 1031.9 was shown in **Figure 3**. All mass data were assignable to the structure of protodisocin.

A representative total ion chromatogram of asparagus shoot bottom parts under positive ion mode (run using 0.05 mM ammonium acetate−acetonitrile gradient) shows five significant peaks (**Figure 4**). Peaks 2 and 3 were identified as rutin and protodioscin, respectively, by comparing the mass spectrum, retention time, and UV absorption with reference standards. Peak

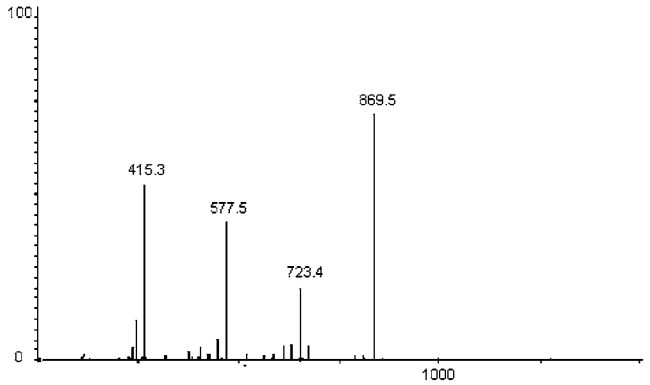

Figure 3. MS/MS spectrum of protodioscin with precursor ion of 1031.9.

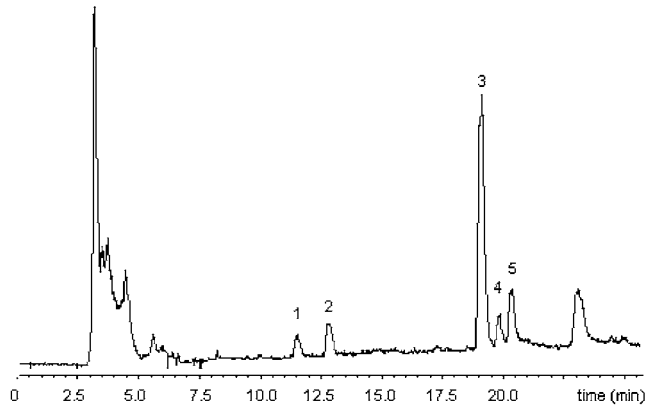

Figure 4. Representative total ion chromatogram of the asparagus bottom part extract under positive ion mode.

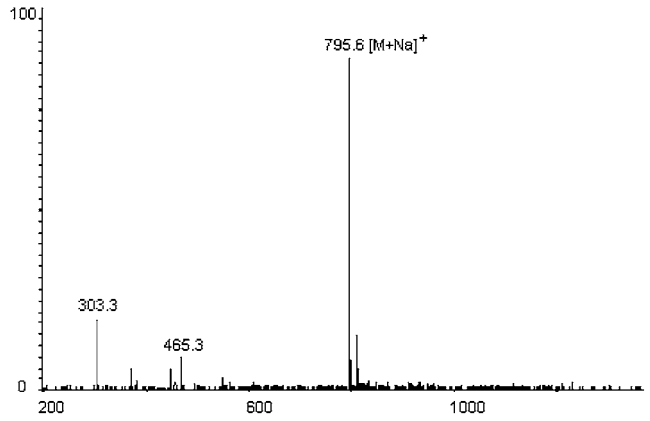

Figure 5. Positive ESI-MS of peak 1.

1 appears to be a rutin type of flavonoid based on its MS spectra (**Figure 5**) as the same aglycone mass peak at *m*/*z* 303.3 was found for this compound as found with rutin and online UV spectrum with maximum absorption at 255 and 355 nm. On the basis of its molecular ion at *m*/*z* $[M + Na]^+$ at 795.6, the compound should have an extra sugar molecule on its structure as compared to rutin alone.

The mass spectrum of the fourth compound showed ion peaks $[M + 23]^+$ at *m*/*z* 925.8, $[M - 18 + 1]^+$ at *m*/*z* 885.9, and $[M - 18 - Glu + 1]^+$ at *m*/*z* 723.8 (**Figure 6**). The major ion peak at *m*/*z* 885.9 was further subjected to MS/MS analysis. In its MS/MS spectra, ion peaks at *m*/*z* 415.4, 579.5, and 723.5 were observed (**Figure 7**). An ion peak at *m*/*z* 415.4 suggests that this compound may have the same aglycone as protodisocin, and *m*/*z* 723.5 $[M - Glu - 18 + 1]^+$ suggests its structure may also have a substituted glucose at the C-26 position. For *m*/*z* 579.5, we suggest that there may be a typical steroidal

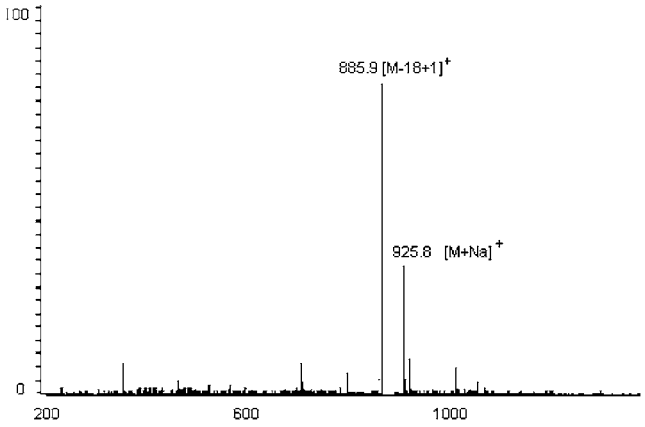

Figure 6. Positive ESI-MS of peak 4.

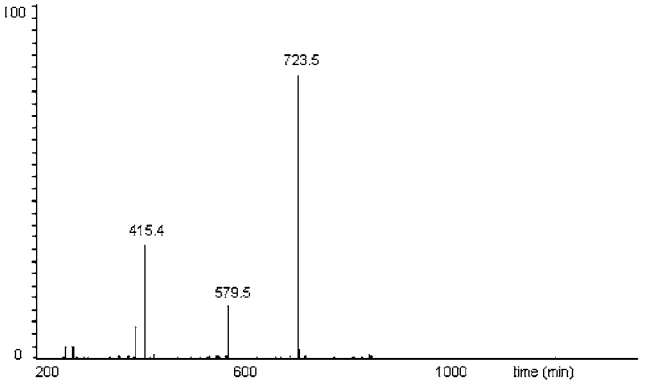

Figure 7. Positive ESI-MS/MS of major ion at *m*/*z* 885.9 for peak 4.

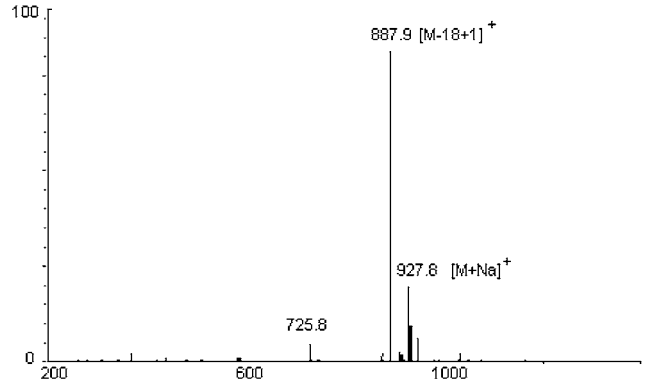

Figure 8. Positive ESI-MS for peak 5.

saponin loss of 144 from *m*/*z* 723.5 (the mechanism was recently proposed for steroidal saponins) (*23*). These mass data suggest that the fourth compound might be asparsaponin II, a saponin previously purified from asparagus (*12*).

The fifth compound showed ion peaks $[M + Na]^+$ at *m*/*z* 927.8, $[M - 18 + 1]^+$ at 887.9, and $[M - 18 - Glu + 1]^+$ at *m*/*z* 725. 8 (**Figure 8**). The MS/MS of the major ion at *m*/*z* 887.9 (**Figure 9**) showed ion peaks at *m*/*z* 417.4, 579.5, and 725.5. An ion peak at *m*/*z* 417.4 suggested that its aglycone might have two extra protons than the aglycone of protodioscin. The ion peak at *m*/*z* 725.5 $[M - 18 - Glu + 1]^+$ suggests that it may also have a substituted glucose at the C-26 position. These data indicate that the fifth compound may be (3β,5β,25*S*)-26-(β-D-glucopyranosyl)-22-hydroxyfurostan-3-yl-6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside, a saponin purified from *Asparagus cochinchinensis* (*24*). On the basis of the retention time and mass spectrum, methylprotodioscin was not detected in the asparagus extract indicating that its presence in

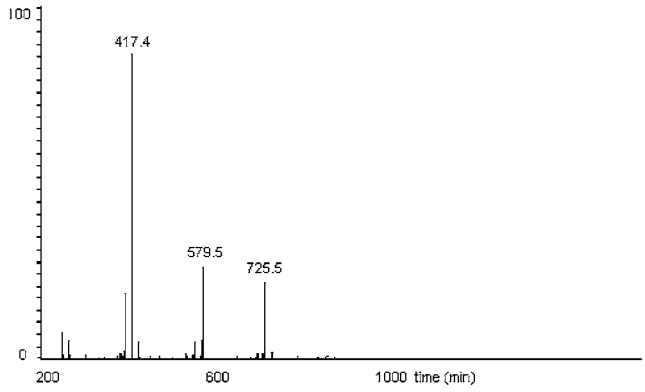

Figure 9. ESI-MS/MS of ion at *m*/*z* 887.9 for peak 5.

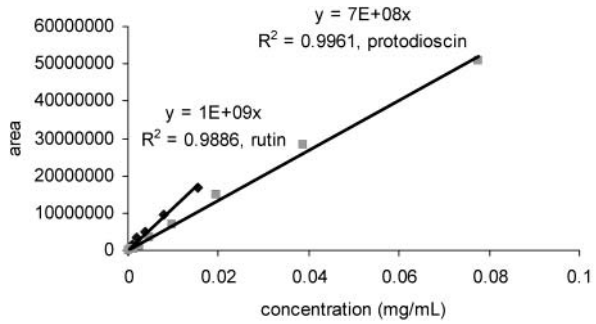

Figure 10. Calibration curve by LC/MS with SIM at *m*/*z* 1047.9 for protodioscin and 609 for rutin.

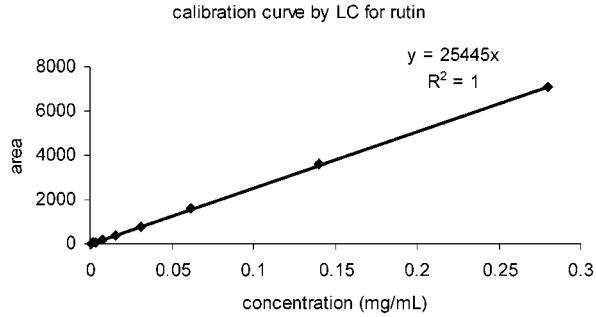

Figure 11. Calibration curve by LC with UV detection at 255 nm for rutin.

asparagus as reported earlier is likely an artifact produced during methanolic extraction and isolation.

Determination of the Contents of Rutin and Protodiosicin in Asparagus Shoots. The sample solutions were subjected to LC/MS analysis under negative ion mode with UV detection at 255 nm for rutin, with monitoring ion peaks at *m*/*z* 609 for rutin and 1047.9 for protodioscin analysis. The RSDs of these analyses were found to be less than 8%. Each section of the shoot tissue differed significantly in the accumulation of rutin and protodioscin (**Figures 12** and **13**). The bottom or older tissue contained >100 times the protodioscin concentration than the top and youngest tissues and >30 times of the middle part. In contrast, the youngest stem tissue of the spear, the top part, contains the most rutin. Asparagus for the fresh market is generally harvested at the length in excess of 23 cm and later trimmed to a length of 23 cm. Results from this research show that those sections normally removed and discarded are an excellent source of protodioscin. The calculated contents of protodioscin at the tops were about 0.24 mg per 100 g in these three lines, while the bottom section, which would be a byproduct of fresh asparagus, contained very high levels of 25 mg of protodioscin per 100 g fresh sample and 250 mg per 100

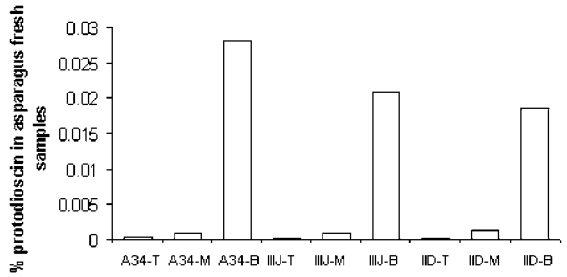

Figure 12. Contents of protodioscin in three asparagus breeding lines (A34, IIIJ, and IID) by LC/MS with SIM (T, top; M, middle; B, bottom).

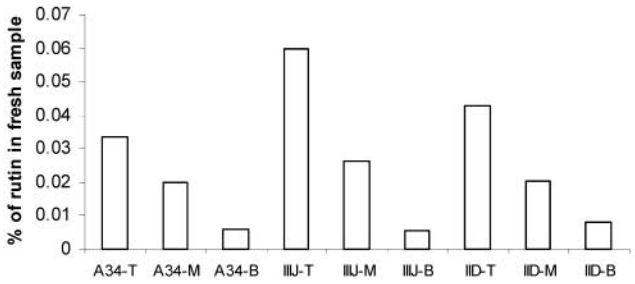

Figure 13. Contents of rutin in three asparagus breeding lines (A34, IIIJ, and IID) by LC/UV (T, top; M, middle; B, bottom).

g dry sample (the moisture content was determined to be 90%). The distribution pattern of rutin is significantly different from protodioscin, and our results here support our prior study (*5*). The youngest tissue was the richest source for rutin, at levels of 0.03−0.06% fresh sample weight. The difference of rutin content by tissue location was not as dramatic as the distribution of protodioscin, suggesting that the oldest stem tissue may serve as a source for both compounds.

SUMMARY

The major chemical components of asparagus shoots were identified by a new LC/MS method. An accurate and sensitive LC/MS with selected ion monitoring was developed to quantify rutin and protodioscin in asparagus shoots. This method showed good linearity across a wide concentration range for protodioscin, and the low detection limit can reach 0.000069 mg per mL. There was a significant differential tissue accumulation pattern by location for both rutin and protodioscin. The oldest tissue in the harvested spears that would normally be discarded during the grading and sorting of fresh or processed asparagus was found to be a rich source of protodiscin and also contained rutin. This waste product could be developed into a new nutraceutical value-added product.

LITERATURE CITED

(1) Shao, Y.; Poobrasert, O.; Kennelly, E. J.; Chin, C. K.; Ho, C.-T.; Huang, M. T.; Garrison, S. A.; Cordell, G. A. Steroidal saponins from *Asparagus officinalis* and their cytotoxic activity. *Planta Med*. **1997**, *63*, 258−262.

(2) Blumenthal, M., Busse, W., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C. W., Rister, R. S., Klein, S., Eds. The Complete German Commission E Monographs. *Therapeutic Guide to Herbal Medicine*; American Botanical Council and Integrative Medicine Communication: Boston, MA, 1998; pp 85.

(3) Shimoyamada, M.; Suzuki, M.; Sonta, H.; Maruyama, M.; Okubo, K. Antifungal activity of the saponin fraction obtained from asparagus and its active principle. *Agric. Biol. Chem.* **1990**, *54*, 2553−2557.

(4) Balansand, J.; Rayband, M. Diuretic action of *Asparagus officinalis*. *ER Soc. Biol*. **1937**, *126*, 954−956.

(5) Chin, C. K.; Garrison, S. A.; Shao, Y.; Wang, M.; Simon, J.; Ho, C. T.; Huang, M. T. Functional elements from asparagus for human health. Proceedings of the Xth ISHS on Asparagus. *Acta Hortic*. **2002**, *589*, 233−241.

(6) Shao, Y.; Chin, C. K.; Ho, C.-T.; Ma, W.; Garrison, S. A.; Huang, M. T. Antitumor activity of the crude saponins obtained from asparagus. *Cancer Lett.* **1996**, *104*, 31−36.

(7) Sati, O. P.; Pant, G.; Nohara, T.; Sato, A. Cytotoxic saponins from asparagus and agave. *Pharmazie* **1985**, *40*, 586.

(8) Aquino, R. Antiviral activity of constituents of *Tamus communis*. *J. Chemother*. **1991**, *3*, 305−307.

(9) Sati, O. P.; Pant, G.; Hostettman, K. Potent mollusicides from asparagus. *Pharmazie* **1984**, *39*, 581.

(10) Goryanu, G. M.; Krokhmalyuk, V. V.; Kintya, P. K.; Glyzin, V. I. Medicinal asparagus as a source of steroidal glycoside. *Farmatsiya* **1976**, *25*, 66−68.

(11) Deli, J.; Matus, Z.; Toth, G. Carotenoid composition in the fruits of *Asparagus officinalis*. *J. Agric. Food Chem*. **2000**, *48*, 2793−2796.

(12) Kawano, K.; Sato, H.; Sakamura, S. A bitter principle of asparagus Part II. Isolation and structure of furostanol saponin in asparagus edible shoots. *Agric. Biol. Chem.* **1977**, *41*, 1−8.

(13) Hu, K.; Yao, X. Protodioscin (NSC-698 796): its spectrum of cytotoxicity against sixty human cancer cell lines in an anticancer drug screen panel. *Planta Med*. **2002**, *68*, 297−301.

(14) Ikeda, T.; Ando, J.; Miyazono, A.; Zhu, X.-H.; Tsumagari, H.; Nohara, T.; Yohomizo, K.; Uyeda, M. Anti-herpes virus activity of *Solanum* steroidal glycosides. *Biol. Pharm. Bull.* **2000**, *23*, 363−364.

(15) Chiang, H. C.; Tseng, T. H.; Wang, C. J.; Chen, C.-F.; Kan, W. S. Experimental antitumor agents from *Solanum indicum* L. *Anticancer Res*. **1991**, *11*, 1911−1917.

(16) Chiang, H. C.; Chen, Y. Y. Xanthine oxidase inhibitors from the roots of eggplant (*Solanum melongena* L.). *J. Enzyme Inhib.* **1993**, *7*, 225−235.

(17) Hu, K.; Dong, A.-J.; Yao, X.; Kobayashi, H.; Iwasaki, S. Antineoplastic steroidal saponins from rhizomes of *Dioscorea collettii* var. *hypoglauca*. *Stud. Plant Sci.* **1999**, *6*, 220−229.

(18) Hu, K.; Dong, A. J.; Yao, X.; Kobayashi, H.; Iwasaki, S. Antineoplastic agents. Part 2. Four furostanol glycosides from Rhizomes of *Dioscorea collettii* var. *hypoglauca*. *Planta Med*. **1997**, *63*, 161−165.

(19) Adimoelja, A. Phytochemicals and the breakthrough of traditional herbs in management of sexual dysfunctions. *Int. J. Androl.* **2000**, *23* (Suppl. 2), 82−84.

(20) Ganzera, M.; Bedir, E.; Khan, I. A. Determination of steroidal saponins in *Tribulus terrestris* by reversed-phase high performance liquid chromatography and evaporative light scattering detection. *J. Pharm. Sci.* **2001**, *11*, 1752−1758.

(21) Gyulemetova, R.; Tomova, M.; Simova, M.; Pangarova, T.; Peeva, S. Determination of furostanol saponins in the preparation Tribestan. *Pharmazie* **1982**, *37*, 296.

(22) Tosun, F.; Tanker, M.; Coskun M.; Tosun, A. Determination of diosgenin in *Tribulus terrestris* L. growing in Turkey by HPLC. *Pharmacia* **1991**, *31*, 90−96.

(23) Liang, F.; Li, L.-J.; Zeper, A.; Yang, Y.-C.; Shi, J.-G. Structural characterization of steroidal saponins by electrospray ionization and fast-atom bombardment tandem mass spectrometry. *Rapid Commun. Mass Spectrom*. **2002**, *16*, 1168−1173.

(24) Konishi, T.; Shoji, J. Studies on the constituents of asparagi radix. I. on the structures of furostanol oligosides of *Asparagus cochinchinensis (Loureio)* Merrill. *Chem. Pharm. Bull.* **1979**, *27*, 3086−3094.

Received for review May 5, 2003. Revised manuscript received July 28, 2003. Accepted August 5, 2003. We acknowledge the New Jersey Agriculture Extension Service, the New Jersey Agriculture Farm Bureau, and the New Crops Farm Bureau for support.

JF0344587