Simultaneous Determination of Furostanol, Pennogenyl, and Diosgenyl Glycosides in Taiwanese Rhizoma Paridis (*Paris formosana* Hayata) by High-Performance Liquid Chromatography with Evaporative Light Scattering Detection

Jau-Tien Lin,[†] Yan-Zin Chang,[‡] Mei-Peng Lu,[†] and Deng-Jye Yang*,[§]

[†]Department of Applied Chemistry, Chung Shan Medical University, 110, Section 1, Jianguo N. Road, Taichung 402, Taiwan

[‡]Institute of Medicine, Chung Shan Medical University, 110, Section 1, Jianguo N. Road, Taichung 402, Taiwan

[§]School of Health Diet and Industry Management and Department of Nutrition, Chung Shan Medical University and Chung Shan Medical University Hospital, 110, Section 1, Jianguo N. Road, Taichung 402, Taiwan

ABSTRACT: A high-performance liquid chromatographic method with an evaporative light scattering detector (HPLC−ELSD) was developed to simultaneously determine 10 steroidal saponins, including 3 furostanol glycosides, 3 pennogenyl glycosides, and 4 diosgenyl glycosides in Taiwanese rhizoma paridis (*Paris formosana* Hayata). The condition was a Cosmosil C18 column kept at 35 °C and a step-gradient solvent system consisting of acetonitrile and water (25:75, v/v) in the first 30 min, 45:55 (v/v) from 31 to 45 min, and 50:50 (v/v) from 45 to 65 min, at a flow rate of 1 mL/min. The separation factors (α) and resolutions (*Rs*) were better than 1, and the limits of detection (LODs) and limits of quantification (LOQs) were 0.01−0.27 and 0.04−0.90 μg, respectively, for these saponins. Moreover, 203 nm UV detection was also used for comparison. The saponins in *P. formosana* Hayata gathered from various areas of Taiwan were determined by applying the established method.

KEYWORDS: Diosgenyl glycoside, ELSD, furostanol glycoside, HPLC, *Paris formosana* Hayata, pennogenyl glycoside, rhizoma paridis, steroidal saponin

1. INTRODUCTION

Rhizoma paridis is a perennial herbaceous plant of the *Paris* genus (Trililiaceae family). This traditional medicinal herb has been used in China for a long time to treat inflammation, intoxication, hemostasis, and wound caused by insect and snake bites.[1,2]

Reports indicated that steroidal saponins, especially spirostanol glycosides, including diosgenyl and pennogenyl glycosides, were the major physiologically active compounds in the herb, e.g., *Paris polyphylla* var. *yunnanensis*,[2] *Paris vietnamensis*,[3] *Paris luquanesis*,[4] and *Paris axialis*.[5,6] Besides, furostanol glycosides could also be found in *Paris polyphylla*.[1]

Steroidal saponins have been reported to be anticancer,[7,8] antithrombotic,[9,10] antiviral,[11] hemolytic,[10,12] hypocholesterolemic,[13,14] and hypoglycemic.[15] However, there are rare reports on the quantitative determination of steroidal saponins in rhizoma paridis plants. Chen et al.[4] used a C18 column, an isocratic solvent system consisting of methanol (MeOH) and water (H₂O), and a refractive index (RI) detector to determine diosgenyl glycosides in rhizoma paridis samples. Wei[16] used a C8 column, an isocratic solvent system consisting of acetonitrile (ACN) and H₂O, and an ultraviolet (UV) detector set at 203 nm to determine diosgenyl and pennogenyl glycosides in rhizoma paridis samples gathered from Yunnan, China. Nevertheless, low-wavelength UV and RI detections cannot offer gradient compatibility, good sensitivity, and a stable baseline; their applications for measurement of nonchromophoric analytes, such as saponins, are limited.[17] The response of evaporative light scattering

detection (ELSD), a mass detection method, does not depend upon the optical characteristics of the samples; it can overcome the limitations for low-wavelength UV and RI detections.[17] Although high-performance liquid chromatography (HPLC)−mass has also been used to determine saponins,[1,18] its steep operating costs and complexity limit its use for routine work.[17,19] HPLC−ELSD is simple and easy for operation and has better sensitivity and moderate operating costs; it is increasingly employed to determine saponins in samples.[17,19−23]

Our previous results demonstrated that steroidal saponins, including furostanol, diosgenyl, and pennogenyl glycosides, could be isolated from *Paris formosana* Hayata, a Taiwanese native rhizoma paridis cultivar.[24] To the best of our knowledge, no report concerning simultaneous determination of furostanol, pennogenyl, and diosgenyl glycosides with HPLC−ELSD has been published.

The investigation is the first time to establish a simple HPLC−ELSD method for simultaneous determination of 10 steroidal saponins, including 3 furostanol glycosides, 3 pennogenyl glycosides, and 4 diosgenyl glycosides. Besides, the composition and content of the steroidal saponins in *P. formosana* Hayata samples gathered from various areas of Taiwan are also the first time to be surveyed by applying the newly established method.
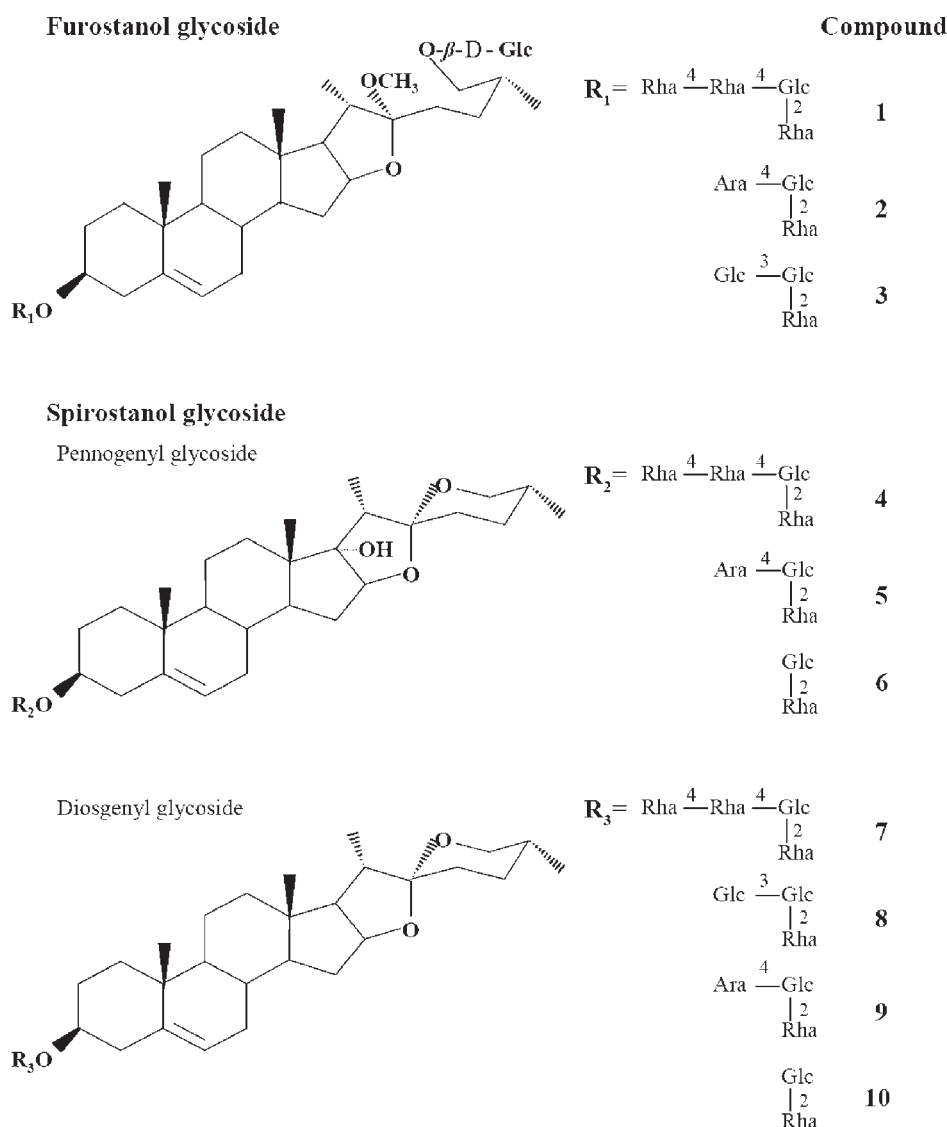
Figure 1. Structures of steroidal saponins.

2. MATERIALS AND METHODS

2.1. *P. formosana* Hayata Samples. Rhizomes of *P. formosana* Hayata were gathered from different areas: Yang-Ming-Shan and Da-Tun-Shan in northern Taiwan, Nan-Tou in central Taiwan, San-Di-Men in southern Taiwan, and Tai-Dong in eastern Taiwan. Samples were cleaned and cut into 4 mm slices with a Cucina slicer (model HR7633, Koninklijke Philips Electronics Co., Suzhou, Jiangsu, China), followed by lyophilization in the freeze-drying system (Vestech Scientific Co., Ltd., Taipei, Taiwan). The dried slices were then ground using a grinder (model RT08, Rong-Tsong Co., Taipei, Taiwan).

2.2. Chemicals. Three furostanol glycosides and six spirostanol glycosides, including two pennogenyl glycosides and four diosgenyl glycosides, were prepared in our laboratory from *P. formosana* Hayata gathered locally in Taiwan according to the method by Yang et al.[24] The furostanol glycosides were 26-*O*-β-D-glucopyranosyl-22α-methoxyl-(25*R*)-furost-5-en-3β,26-diol,3-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (compound **1**), 26-*O*-β-D-glucopyranosyl-22α-methoxyl-(25*R*)-furost-5-en-3β,26-diol,3-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-arabinofuranosyl-(1 → 4)]-β-D-glucopyranoside (compound **2**), and

methyl protogracillin (compound **3**). The pennogenyl glycosides were 25(*R*)-spirost-5-en-3β,17-diol,3-*O*-α-L-rhamnopyranosy-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (compound **4**) and chonglouoside H (compound **5**). The diosgenyl glycosides were formosanin C (compound **7**), gracillin (compound **8**), pollyphyllin D (compound **9**), and prosapogenin A of dioscin (compound **10**). A pennogenyl glycoside, 25(*R*)-spirost-5-en-3β,17-diol,3-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside (compound **6**) prepared from rhizoma paridis was purchased from Nipudak Biotechnology Co., Ltd. (Guangzhou, China). The purity of each saponin was calculated through the peak area on the HPLC–ELSD chromatogram as follows: (saponin peak area/total peak area) × 100%. The purities of these saponins were all above 95%. Figure 1 shows the structures of the saponins. Solvents used for the extraction and analysis of steroidal saponins, including MeOH, *n*-butanol (*n*-BuOH), and ACN were from Tedia Co. (Fairfield, OH). HPLC-grade H_2O [distilled deionized H_2O (ddH_2O)] was prepared with the Ultrapure water purification system (Lotun Co., Ltd., Taipei, Taiwan).

2.3. Analysis of Steroidal Saponins. The equipment used for the determination of steroidal saponins was a PrimeLine gradient model

1588

dx.doi.org/10.1021/jf104839r |*J. Agric. Food Chem.* 2011, 59, 1587–1593

500G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA) with an Alltech ELSD 3300 evaporative light scattering detector (tube temperature, 90 °C; air flow rate, 2.8 L/min) (Alltech Associates, Inc., Deerfield, IL) and a S-3210 UV−vis detector (Schambeck SFD GmbH, Bad Honnef, Germany) (wavelength, 203 nm). The stationary phase was a Cosmogel C18 column (250 × 4.6 mm inner diameter, 5 μm, Nacalai Tesque, Inc., Kyoto, Japan) in a column oven (Hipoint Scientific Co., Kaohsiung, Taiwan) kept at 35 °C. The mobile phase was a step-gradient solvent system consisting of ACN and H_2O (25:75, v/v) in the first 30 min, 45:55 (v/v) from 31 to 45 min, and 50:50 (v/v) from 45 to 65 min, at a flow rate of 1 mL/min. The CHEM-WIN computer software system (Shuen-Hua Co., Taipei, Taiwan) was used to record and process data. HPLC separation efficiency was evaluated by the separation factor (α) and resolution (Rs). The limits of detection (LODs) and limits of quantification (LOQs) of steroidal saponins were evaluated with the signal-to-noise ratio (S/N) of 3 and 10, respectively. The reproducibility of each steroidal saponin was determined as follows: for intraday variability, the assay was performed in triplicate 3 times a day, while for interday variability, the assay was performed in triplicate within 5 consecutive days; variations were expressed by the relative standard deviation (% RSD). Six concentrations (0.05, 0.1, 0.5, 1, 2, and 5 mg/mL) of each saponin were injected at 20 μL into HPLC through an injection valve (Rheodyne 7725i, Rheodyne Co., Rohnert Park, CA); the linear regression equation of the calibration curve for each steroidal saponin was obtained by plotting the injected saponin amount against the peak area. The calibration curves of all steroidal saponins were measured in triplicate, and the mean values were counted.

2.4. Determination of Steroidal Saponins in Rhizoma Paridis Samples. The method used for extraction of steroidal saponins from *P. formosana* Hayata samples was similar to that reported by Lin and Yang.[20] Freeze-dried sample powder (10 g) was extracted with 250 mL of MeOH for 24 h, followed by filtration and concentration in a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan). The residue was suspended in 25 mL of ddH_2O and partitioned against 25 mL of *n*-BuOH for 3 times to yield saponin extract. After the extract was washed with 50 mL of ddH_2O for 3 times, *n*-BuOH was removed by the rotary evaporator. The dried crude extract was then dissolved in 5 mL of MeOH for analysis of steroidal saponins. The steroidal saponins in *P. formosana* Hayata extracts were identified by (1) co-chromatography of steroidal saponin standards with sample extracts and (2) a comparison of the retention time and mass spectrum (compound **1**, MW = 1208; compound **2**, MW = 1048; compound **3**, MW = 1078; compound **4**, MW = 1030; compound **5**, MW = 870; compound **6**, MW = 724; compound **7**, MW = 1014, compound **8**, MW = 884; compound **9**, MW = 854; and compound **10**, MW = 722) obtained on an Agilent HPLC−MS system interfaced to an ion-trap mass spectrometer via an electrospray ion (ESI) source (positive-ion mode) (Agilent Technologies, Palo Alto, CA). Operating conditions: nebulizer pressure, 70 psi; capillary temperature, 350 °C; dry gas flow, 11 L/min; electrospray voltage of the ion source, 3000 V; capillary exit, −159 V; skimmer, 40 V. The recoveries were measured by adding a mixture of the 10 steroidal saponin standards (each weighing 0.1, 0.25, and 0.5 mg) to 10 g of the freeze-dried sample powder, followed by extraction and analysis of steroidal saponins, as described above. The recoveries were obtained from the analytical results, subtracting the original saponin amounts in sample extracts, divided by the amounts of saponins added.

2.5. Statistical Analysis. The determination of recoveries and quantitative analyses for all steroidal saponins were carried out in triplicate, and the mean values were calculated. Statistical analyses of the data were performed through the analysis of variance (ANOVA), and Duncan's test procedures were used to decide significance between means, at a level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. HPLC Separation of Furostanol, Pennogenyl, and Diosgenyl Glycosides. Because of the polar nature of the glycosidated saponins, HPLC is a suitable method for their analysis.[19] As a result of the lack of a strong UV chromophore, RI or lower wavelength UV was used for saponin measurement.[4,16] However, the two methods had large solvent peaks that often interfere with early eluting peaks, lower sensitivity, and restriction of gradient elution, which limited the determination of saponins.[19] Therefore, the development of HPLC−ELSD for saponin determination was encouraged. It has been used to determine steroidal saponins in *Tribulus terrestris*[21] and yam,[19,20] as well as triterpenoidal saponins in ginseng,[22] flos lonicerae,[23] and *Ziziphus jujuba*.[17] The review paper by Olezek and Bialy[25] indicated that C18 columns coupled with ACN−H_2O or MeOH−H_2O solvent systems were usually used for saponin separation. The column temperatures were from ambient temperature to 45 °C.[17,19−23]

In the study, we referred to these reports as described above, tried many analytical conditions, and finally, established a HPLC−ELSD method with the best separation efficiency for simultaneous determination of 10 rhizoma paridis steroidal saponins, including 3 furostanol glycosides, 3 pennogenyl glycosides, and 4 diosgenyl glycosides (Figure 2); its conditions were a Cosmogel C18 column kept at 35 °C, and a step-gradient solvent system consisting of ACN and H_2O (25:75, v/v) in the first 30 min, 45:55 (v/v) from 31 to 45 min, and 50:50 (v/v) from 45 to 65 min, at a flow rate of 1 mL/min. The conditions were developed according to the separation factors (α) and resolutions (Rs). Chen[26] indicated that $\alpha > 1$ is required and the acceptable resolution is $Rs = 1.0$ at the least. Our data showed that the values of α and Rs of the steroidal saponins were all better than 1 (Table 1). Moreover, the condition also presented good reproducibility for the separation of the steroidal saponins, RSD ≤ 1.23% (intraday) and 3.02% (interday) for retention times and RSD < 1.89% (intraday) and 4.07% (interday) for integrated areas (Table 1).

Our investigation also compared ELSD to 203 nm UV for detection of the steroidal saponins. Figure 2 shows that ELSD could tolerate the gradient elution of the mobile phase and the interferences of impurities during determination of the steroidal saponins in *P. formosana* Hayata; nevertheless, an apparent baseline shift following solvent gradient elution and more interferences could be found under 203 nm UV detection. Therefore, ELSD would raise the accuracy for saponin determination. Furthermore, ELSD had higher sensitivity for the measurement of the steroidal saponins than 203 nm UV detection; LODs and LOQs were 0.01−0.27 and 0.04−0.90 μg, respectively, for ELSD and 0.04−0.69 and 0.13−2.12 μg, respectively, for 203 nm UV detection (Table 2). Therefore, the developed HPLC−ELSD method should be suitable for analysis of steroidal saponins in rhizoma paridis samples for routine work.

Chen et al.[4] used a C18 column and an isocratic MeOH−H_2O solvent system at ambient temperature to analyze diosgenyl glycosides. Wei[16] employed a C8 column kept at 40 °C and an isocratic ACN−H_2O solvent system to analyze pennogenyl and diosgenyl glycosides simultaneously. Yang et al.[19] adopted a C18 column kept at 45 °C and a step-gradient MeOH−H_2O solvent system to analyze furostanol and diosgenyl glycosides simultaneously. Our developed method using a Cosmosil C18 column kept at 35 °C and a step-gradient ACN−H_2O solvent system not
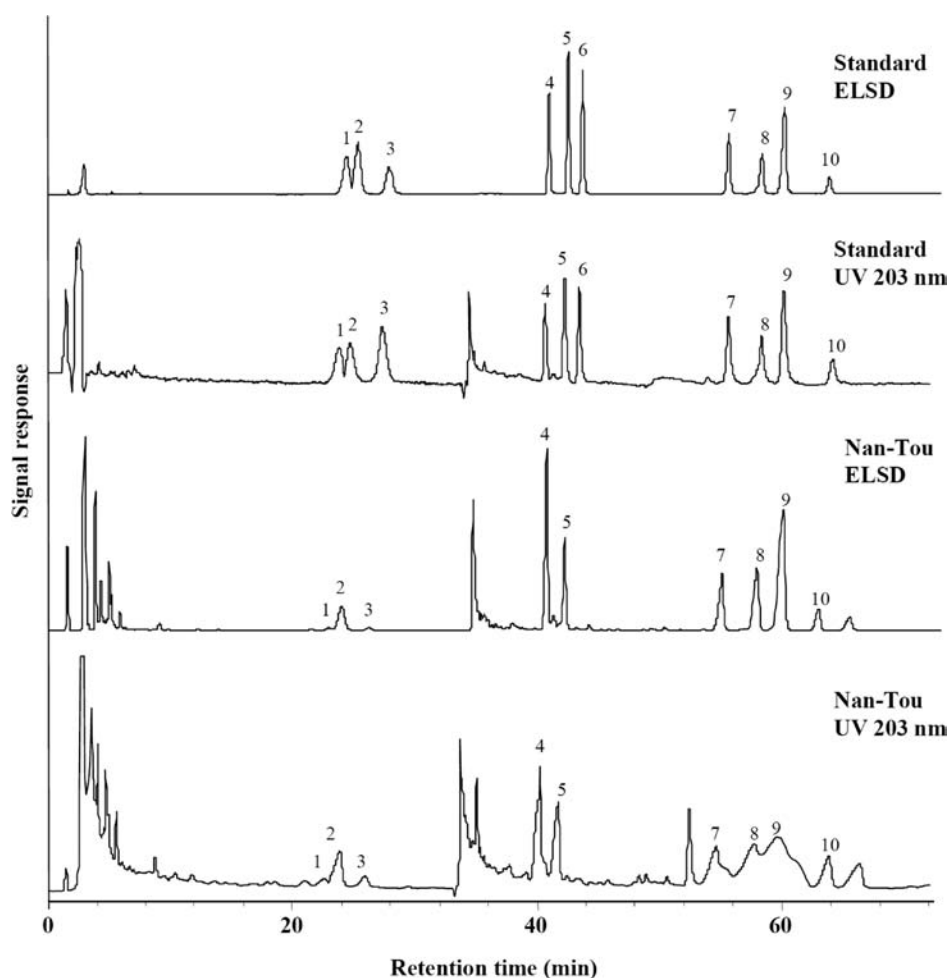
Figure 2. HPLC chromatograms of steroidal saponins in *P. formosana* Hayata detected by ELSD (tube temperature, 90 °C; air flow rate, 2.8 L/min) and 203 nm UV, respectively. Analytical conditions: column, Cosmogel C18 (4.6 mm inner diameter × 250 mm, 5 μm); column temperature, 35 °C; mobile phase, ACN/H$_2$O = 25:75 (v/v) from 0 to 30 min, 45:55 (v/v) from 31 to 45 min, and 50:50 (v/v) from 45 to 65 min; flow rate, 1 mL/min.

Table 1. Separation Factor (α), Resolution (Rs), and Reproducibility of Steroidal Saponins[a]

| | | | | reproducibility (% RSD) | | | |
| | | | | retention time | | integrated area | |
compound	retention time (min)	α[b]	Rs[c]	intraday	interday	intraday	interday
1	22.87			1.23	3.02	1.76	3.64
2	23.99	1.05 (1/2)[d]	1.10 (1/2)	1.14	2.26	1.89	4.07
3	26.22	1.11 (2/3)	2.82 (2/3)	0.85	1.92	1.48	3.47
4	40.42			0.91	1.73	1.35	3.02
5	41.87	1.04 (4/5)	3.75 (4/5)	1.01	1.82	1.26	3.15
6	42.88	1.03 (5/6)	2.80 (5/6)	0.94	2.11	1.47	3.10
7	54.86			0.93	1.86	1.25	3.02
8	57.60	1.06 (7/8)	4.32 (7/8)	0.88	1.79	1.37	3.28
9	59.78	1.04 (8/9)	3.08 (8/9)	0.93	1.68	1.17	3.05
10	62.62	1.05 (9/10)	5.06 (9/10)	0.91	1.80	1.42	3.32

[a] The amount injected to HPLC is 50 μg. The analytical conditions of HPLC−ELSD are described in section 2.3. [b] $\alpha = t_{R2} - t_0/t_{R1} - t_0$, where t_{Rn} is the retention time of an analyte and t_0 is the retention time of an unretained peak. [c] $Rs = 2(t_{R2} - t_{R1})/(w_1 + w_2)$, where w_n is the band width of an analyte at the baseline. [d] Values in parentheses represent two neighboring peaks.

Table 2. LODs, LOQs, and Linear Regression Equations of Steroidal Saponins[a]

	203 nm UV		ELSD				
compound	LOD (μg)	LOQ (μg)	LOD (μg)	LOQ (μg)	linear range (μg)	linear regression equation[b]	correlation coefficient (r^2)
1	0.48	1.90	0.18	0.57	1–100	$y = (2.30x - 2.01)$ E5	0.9981
2	0.69	2.12	0.24	0.81	1–100	$y = (8.30x - 7.28)$ E4	0.9986
3	0.28	0.89	0.27	0.90	1–100	$y = (5.23x - 4.74)$ E4	0.9992
4	0.05	0.17	0.01	0.04	1–100	$y = (11.77x - 8.38)$ E4	0.9992
5	0.04	0.13	0.01	0.05	1–100	$y = (1.69x - 1.33)$ E5	0.9995
6	0.08	0.27	0.03	0.10	1–100	$y = (1.56x - 1.36)$ E5	0.9991
7	0.07	0.24	0.05	0.18	1–100	$y = (8.52x - 3.24)$ E4	0.9991
8	0.12	0.41	0.06	0.19	1–100	$y = (9.32x - 8.72)$ E4	0.9984
9	0.06	0.23	0.03	0.11	1–100	$y = (13.90x - 7.84)$ E4	0.9993
10	0.15	0.53	0.10	0.39	1–100	$y = (3.52x - 2.81)$ E4	0.9991

[a] All data are the means of triplicate analyses. [b] y is the value of the peak area, and x is the value of the sample quantity (μg).

Table 3. Recoveries of Added Steroidal Saponins in *P. formosana* Hayata after Extraction[a]

	% recovery (% CV)[b]		
	added amount (mg)		
compound	0.1	0.25	0.5
1	98.12 (5.52) a[c]	98.72 (5.12) a	99.66 (4.83) a
2	99.46 (4.86) a	99.93 (5.04) a	100.03 (4.72) a
3	99.77 (3.78) a	99.99 (4.28) a	100.04 (3.37) a
4	99.82 (3.68) a	100.04 (3.93) a	99.94 (4.04) a
5	99.88 (3.89) a	100.11 (3.94) a	100.08 (3.75) a
6	99.87 (3.95) a	99.87 (2.98) a	100.13 (3.09) a
7	99.78 (4.73) a	99.75 (5.23) a	99.88 (2.78) a
8	99.82 (4.67) a	100.01 (4.52) a	99.93 (3.37) a
9	99.88 (3.59) a	99.74 (4.75) a	99.79 (2.64) a
10	99.79 (3.48) a	99.54 (4.69) a	99.97 (3.55) a

[a] All values are the means of triplicate analyses. The analytical conditions of HPLC–ELSD are described in section 2.3. [b] Values in parentheses are the coefficient of variation (%). [c] Values bearing different letters in the same column are significantly different ($p < 0.05$).

only can analyze furostanol, pennogenyl, and diosgenyl glycosides simultaneously but also has good separation efficiency and reproducibility.

Solutions containing 1–100 μg of the 10 saponins were used to prepare the calibration curves. They were linear and reproducible. Table 2 shows the linear regression equations; their correlation coefficients (r^2) were all above 0.998.

3.2. Determination of Steroidal Saponins in *P. formosana* Hayata Samples. MeOH was usually used to prepare crude extracts of steroidal saponins from samples.[5,16,19] The dried extracts could further be suspended in H_2O and partitioned against *n*-BuOH to extract saponins.[20] In the investigation, we used the methods as described above to extract steroidal saponins from *P. formosana* Hayata samples for HPLC analysis. Table 3 shows that recoveries of the steroidal saponins (each weighing 0.1–0.5 mg) were all above 98%, regardless of the kind and amount of saponin added.

Figure 3 shows the HPLC chromatograms of the MeOH extracts of *P. formosana* Hayata samples gathered from various

areas of Taiwan. It reveals that the extractive and developed analytical conditions could be successfully applied to determine furostanol, diosgenyl, and pennogenyl glycosides in the samples. Table 4 shows the contents of steroidal saponins in the samples. Only two pennogenyl glycosides (compounds 4 and 5) could be found in Yang-Ming-Shan, San-Di-Men, and Da-Tun-Shan samples. Tai-Dong and Nan-Tou samples contained three furostanol glycosides (compounds 1–3), two pennogenyl glycosides (compounds 4 and 5), and four diosgenyl glycosides (compounds 7–10). The Tai-Dong sample had higher levels of furostanol and diosgenyl glycosides than the Nan-Tou sample, whereas the pennogenyl glycoside level presented the opposite result. The contents [mg/g of dry weight (dw)] of steroidal saponins in *P. formosana* Hayata samples gathered from various areas of Taiwan were in the order of Tai-Dong (31.95 mg/g of dw) > Nan-Tou (22.47 mg/g of dw) > Yang-Ming-Shan (2.42 mg/g of dw) > San-Di-Men (1.88 mg/g of dw) > Da-Tun-Shan (0.40 mg/g of dw).

Dinan et al.[27] illustrated that the saponin content depended upon many factors, such as the cultivar, the age, and the geographic location of the plant. Wei[16] found that contents of steroidal saponins in Yunnanese rhizoma paridis samples were different with different sources. The difference of steroidal saponin contents in *P. formosana* Hayata samples gathered from different areas of Taiwan was shown in our results as well (Table 4).

Chen et al.[6] found that contents of diosgenyl and pennogenyl glycosides in various rhizoma paridis cultivars (*Paris dunniana*, *Paris cronquistii*, *P. vietnamensis*, *P. polyphylla* var. *yunnanensis*, *P. polyphylla* var. *chinensis*, *P. polyphylla* var. *macrosepala*, *Paris mairei*, *P. luquanesis*, *Paris fargesii*, *Paris thibetica*, *P. axialis*, and *P. japonica*) were 0 (not detected), ~2.0 and 0.02–4.0% of dw, respectively. As far as we know, the contents of furostanol glycosides in rhizoma paridis plants have not yet been reported. Our results showed that contents of diosgenyl and pennogeny glycosides in *P. formosana* Hayata samples were 0 (not detected), ~2.0 and 0.04–0.5% of dw, respectively; moreover, contents of furostanol glycosides in the samples were 0 (not detected), ~0.3% of dw.

In conclusion, a simple method using HPLC–ELSD for simultaneous determination of furostanol, pennogenyl, and diosgenyl glycosides was developed in the study. The investigation is the first time to determine composition and content of
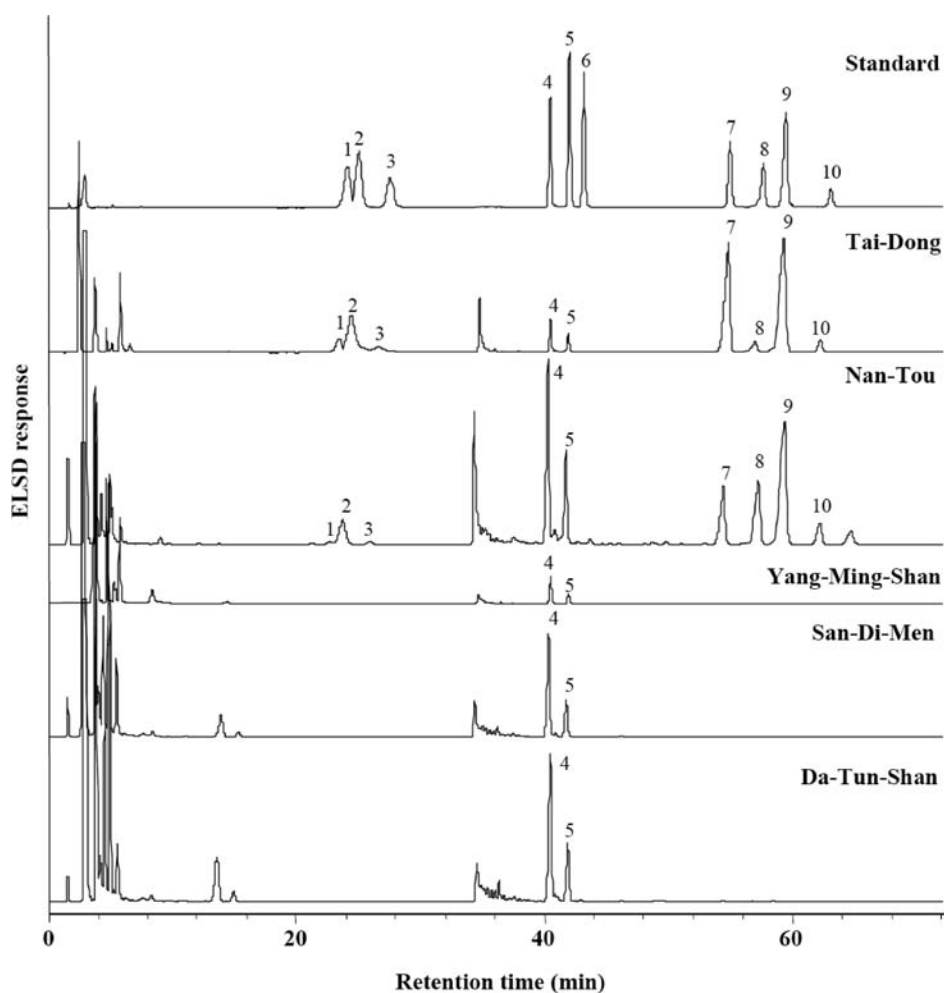
1591

dx.doi.org/10.1021/jf104839r |*J. Agric. Food Chem.* 2011, 59, 1587–1593

Figure 3. HPLC chromatograms of steroidal saponins in *P. formosana* Hayata samples gathered from various areas of Taiwan. Analytical conditions: column, Cosmogel C18 (4.6 mm inner diameter × 250 mm, 5 μm); column temperature, 35 °C; mobile phase, ACN/H$_2$O = 25:75 (v/v) from 0 to 30 min, 45:55 (v/v) from 31 to 45 min, and 50:50 (v/v) from 45 to 65 min; flow rate, 1 mL/min; detection, ELSD (tube temperature, 90 °C; air flow rate, 2.8 L/min).

Table 4. Contents of Steroidal Saponins in *P. formosana* Hayata Gathered from Various Areas in Taiwan[a]

compound	steroidal saponin content (mg/g of dw)				
	Tai-Dong	Nan-Tou	Da-Tun-Shan	San-Di-Men	Yang-Ming-Shan
1	2.50 ± 0.13 a	0.88 ± 0.05 b[b]	ND[c]	ND	ND
2	5.65 ± 0.43 a	1.66 ± 0.10 b	ND	ND	ND
3	1.99 ± 0.11 a	0.41 ± 0.02 b	ND	ND	ND
total fuostanol glycosides	10.14 ± 0.67 a	2.95 ± 0.17 b	ND	ND	ND
4	1.04 ± 0.08 d	3.65 ± 0.24 a	1.90 ± 0.08 b	1.47 ± 0.07 c	0.30 ± 0.01 e
5	0.49 ± 0.03 b	1.55 ± 0.10 a	0.52 ± 0.04 b	0.41 ± 0.02 c	0.10 ± 0.01 d
6	ND	ND	ND	ND	ND
total pennogenyl glycosides	1.53 ± 0.11 d	5.20 ± 0.34 a	2.42 ± 0.12 b	1.88 ± 0.09 c	0.40 ± 0.02 e
7	10.07 ± 0.78 a	2.83 ± 0.18 b	ND	ND	ND
8	0.83 ± 0.04 b	3.45 ± 0.19 a	ND	ND	ND
9	7.14 ± 0.35 a	5.52 ± 0.21 b	ND	ND	ND
10	2.24 ± 0.11 a	2.52 ± 0.13 a	ND	ND	ND
total diosgenyl glycosides	20.28 ± 1.28 a	14.32 ± 0.71 b	ND	ND	ND
total saponins	31.95 ± 2.06 a	22.47 ± 1.22 b	2.42 ± 0.12 c	1.88 ± 0.09 d	0.40 ± 0.02 e

[a] All values are the mean ± standard deviation (SD) obtained by triplicate analyses. The analytical conditions of HPLC−ELSD are described in section 2.3. [b] Values bearing different letters in the same row are significantly different ($p < 0.05$). [c] ND = not detected.

steroidal saponins in *P. formosana* Hayata samples gathered from different areas of Taiwan. These data may serve for a better exploitation of *P. formosana* Hayata.

■ AUTHOR INFORMATION

Corresponding Author
*Telephone: +886-4-24730022, ext. 11867. Fax: +886-4-23248189. E-mail: djyang@csmu.edu.tw.

■ REFERENCES

(1) Man, S.; Gao, W.; Zhang, Y.; Jin, X.; Ma, C.; Huang, X.; Li, Q. Characterization of steroidal saponins in saponin extract from *Paris polyphylla* by liquid chromatography tandem multi-stage mass spectrometry. *Anal. Bioanal. Chem.* **2009**, *395*, 495–505.

(2) Yan, L. L.; Zhang, Y. J.; Gao, W. Y.; Man, S. L.; Wang, Y. *In vitro* and *in vivo* anticancer activity of steroid saponins of *Paris polyphylla* var. *yunnanensis*. *Exp. Oncol.* **2009**, *31*, 27–32.

(3) Hwang, Y.; Cui, L.; Wang, Q.; Ye, W. Separation and identification of active constituents of *Paris vietamensis*. *Acta Pharm. Sin.* **2006**, *41*, 361–364.

(4) Chen, C. X.; Ding, J. K.; Ruan, D. C.; Li, H.; Zhou, J. The determination of the steroidal saponin form *Paris* plants by high performance liquid chromatography. *Acta Bot. Yunnan* **1987**, *9*, 495–502.

(5) Chen, C.; Zhou, J. Studies on the saponin componets of plants in Yunnan X. Two new steroidal glycosides of *Paris axialis* H, Li. (1). *Acta Bot. Yunnan* **1984**, *6*, 111–117.

(6) Chen, C.; Zhou, J. The steroidal saponins of *Paris axialis* (2). *Acta Bot. Yunnan* **1987**, *9*, 230–245.

(7) Ravikumar, P. R.; Hammesfahr, P.; Sih, C. J. Cytotoxic saponins form the Chinese herbal drug Yunnan Bai Yao. *J. Pharm. Sci.* **1979**, *68*, 900–903.

(8) Sung, M. K.; Kendall, C. W. C.; Rao, A. V. Effect of saponins and *Gypsophila* saponin on morphology of colon carcinoma cells in culture. *Food Chem. Toxicol.* **1995**, *33*, 357–366.

(9) Peng, J. P.; Chen, H.; Qiao, Y. Q.; Ma, L. R.; Narui, T.; Suzuki, H.; Okuyama, T.; Kobayashi, H. Two new steroidal saponins form *Allium sativum* and their inhibitory effects on blood coagulability. *Acta Pharm. Sin.* **1996**, *31*, 613–616.

(10) Zhang, J.; Meng, Z.; Zhang, M.; Ma, D.; Xu, S.; Kodama, H. Effect of six steroidal saponins isolated form anemarrhenae rhizoma on platelet aggregation and hemolysis in human blood. *Clin. Chim. Acta* **1999**, *289*, 79–88.

(11) Aquino, R.; Conti, C.; deSimone, F.; Orsi, N.; Pizza, C.; Stein, M. L. Antiviral activity of constituents of *Tamus communis*. *J. Chromatogr.* **1991**, *3*, 305–309.

(12) Santos, W. N.; Bernardo, R. R.; Pecanha, L. M. T.; Palbtnik, M.; Parente, J. P.; deSousa, C. B. P. Haemolytic activities of plant saponins and agjuvants. Effect of *Periandra mediterranea* saponin on the humoral response to the FML antigen of *Leishmania donovani*. *Vaccine* **1997**, *15*, 1024–1029.

(13) Malinow, M. R. Effects of synthetic glycosides on cholesterol absorption. *Ann. N. Y. Acad. Sci.* **1985**, *454*, 23–27.

(14) Sauvaire, Y.; Ribes, G.; Baccou, J. C.; Loubatiéres-Mariani, M. M. Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek. *Lipid* **1991**, *26*, 191–197.

(15) Kato, A.; Miura, T.; Fukunaga, T. Effects of steroidal glycosides on blood glucose in normal and diabetic mice. *Biol. Pharm. Bull.* **1995**, *18*, 167–168.

(16) Wei, J. Determination of steroidal saponins in *Rhizoma paridis* by RP-HPLC. *Acta Pharm. Sin.* **1998**, *33*, 465–468.

(17) Zhao, J.; Li, S. P.; Yang, F. Q.; Li, P.; Wang, Y. T. Simultaneous determination of saponins and fatty acids in *Ziziphus jujuba* (Suanzaoren) by high performance liquid chromatography—evaporative light scattering detection and pressurized liquid extraction. *J. Chromatogr., A* **2006**, *1108*, 188–194.

(18) Kite, G. C.; Porter, E. A.; Simmonds, M. S. J. Chromatographic behaviour of steroidal saponins studied by high-performance liquid chromatography—mass spectrometry. *J. Chromatogr., A* **2007**, *1148*, 177–183.

(19) Yang, D. J.; Lu, T. J.; Hwang, L. S. Simultaneous determination of furostanol and spirostanol glycosides in Taiwanese yam (*Dioscorea* spp.) cultivars by high-performance liquid chromatography. *J. Food Drug Anal.* **2003**, *11*, 10–15.

(20) Lin, J. T.; Yang, D. J. Determination of steroidal saponins in different organs of yam (*Dioscorea pseudojaponica* Yamamoto). *Food Chem.* **2008**, *108*, 1068–1074.

(21) Ganzera, M.; Bedir, E.; Khan, I. A. Determination of steroidal saponins in *Tribulus terrestris* by reverse-phase high-performance liquid chromatography with evaporative light scattering detection. *J. Pharm. Sci.* **2001**, *90*, 1752–1758.

(22) Rark, M. K.; Rark, H. P.; Han, S. B.; Shin, Y. G.; Park, I. H. High-performance liquid chromatographic analysis of ginseng saponins using evaporative light scattering detection. *J. Chromatogr., A* **1996**, *736*, 77–81.

(23) Chai, X. Y.; Li, S. L.; Li, P. Quality evaluation of flos lonicerae through a simultaneous determination of seven saponins by HPLC with ELSD. *J. Chromatogr., A* **2005**, *1070*, 43–48.

(24) Yang, D.J.; Chang, Y. Z.; Liu, S. C.; Lu, M. P.; Lin, J. T. Isolation and identification of steroidal saponins in Taiwanese rhizoma paridis cultivar (*Paris formosana* Hayata). *Taiwan. J. Agric. Food Sci.* **2011**, in press.

(25) Olezek, W.; Bialy, Z. Chromatographic determination of plant saponins—An update (2002−2005). *J. Chromatogr., A* **2006**, *1112*, 78–91.

(26) Chen, G. C. *Chromatographic Series-III Modern High Performance Liquid Chromatography*; Hua-Shiang-Yuan Publishing Co.: Taipei, Taiwan, 1993; pp 7−12.

(27) Dinan, L.; Harmatha, J.; Lafont, R. Chromatographic procedures for the isolation of plant steroids. *J. Chromatogr., A* **2001**, *935*, 105–123.