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Steroidal Sapogenin Contents in Some Domestic Plants

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Abstract □ In order to find out the values of the steroid resources for the future use, the compositions and contents of steroidal sapogenins from 13 domestic plants have been investigated. As a result, *Dioscorea nipponica, D. quinqueloba* and *Smilax china* were found to have large amount of diosgenin. And pennogenin in *Trillium kamtschaticum* and *Paris verticillata*, yuccagenin in *Allium fistulosum*, hecogenin in *Agave americana* and neochlorogenin in *Solanum nigum* were appeared to be major steroidal sapogenins.

Keywords □ Steroid resources, steroidal sapogenin, *Dioscorea, Liriope, Smilax, Trillium, Allium, Agave, Paris, Solanum,* diosgenin, pennogenin, yuccagenin, neochlorogenin, laxogenin, hecogenin, 25(S)-ruscogenin.

Since Marker *et al.*[1] reported the side-chain degradation of steroidal sapogenins to progesterone, sapogenins from various sources have been investigated by many researchers and found to be widely distributed in the plant kingdom, especially in the *Amarylidaceae, Dioscoreaceae, Liliaceae, Scrophulariaceae, Solanaceae* and *Agavaceae* plants.

In addition that steroidal saponins and sapogenins from various plant sources were well known to have various pharmacological activities[2], sapogenins such as diosgenin, hecogenin, solasodin and sterols have now been used as precursor molecules for the partial synthesis of the pharmaceutical steroids, which included anti-inflammatory steroids, mineralocorticoid-like steroids, anabolic steroids, contraceptives and even a steroidal anaethetic[3]. Okanishi *et al.*[4] purified and compared the steroid sapogenins from 16 Liliaceae. And recently, Wang *et al.*[5] studied the contents of diosgenin-type saponin and pennogenin-type saponin from 14 plants of Genus *Paris* and *Trillium*. Besides these publications, there have been numerous reports concerning compositions of the steroid saponin and sapogenin from various sources worldwide[6,7]. However, it may be important to study the steroidal sapogenin contents in the domestic plants for the future use of these resources. Therefore, in this study, the compositions and contents of steroidal sapogenins from domestic plants have been investigated in order to find out the values of the steriod resources in Korea.

EXPERIMENTAL

Materials

The plant materials used in this experiment are listed in Table I-IV. Each plant material was collected in July/August of 1989-1990 from the Kangweon Province of Korea, along with *Solanum nigrum* from Jamaica. Specimens have been identified and deposited in College of Pharmacy, Kangweon National Univ., Korea. Diosgenin and hecogenin were purchased from Steraloids Inc. (Wilton, NH). For the preparation of standard sapogenins, laxogenin[8], neochlorogenin[9], 25(S)-ruscogenin[10] and yuccagenin[11] were isolated and their structures were characterized as previously described. And all redistilled solvents were used.

Isolation of pennogenin

Pennogenin was directly prepared in this experiment using the following methods and used as a

Table I. The list of plant materials and their yields of extracts

Plant material	Yields (%, w/w)		
	A	B	C
Paris verticillata Bieb. (r)[a]	9.11	1.48	0.81
Trillium kamtschaticum Hult. (r)	4.81	1.96	0.98
Dioscorea nipponica Makino (r)	5.26	1.87	0.97
Dioscorea batatas Decaisn. (r)	6.23	1.70	0.61
Dioscorea quinqueloba Thunb. (r)	9.60	2.00	1.06
Liriope spicata Lour. (t)	28.30	0.69	0.31
Smilax china L. (r)	9.70	2.13	1.19
Asparagus schoberioides Kunth. (t)	3.38	1.08	0.40
Solanum nigrum L. (f)	5.00	2.95	1.58

A : MeOH extracts, B : n-BuOH extracts, C : total acid hydrolysates.
[a] r, t and f in parenthesis represented rhizome. tuber and fruit, respectively. The yields for *Solanum nigrum* L. (f) from Jamaica were found to be 9.50, 5.52 and 2.97%, respectively.

Table II. Diosgenin contents

Plant material	Contents (%, w/w)		
	A	B	C
Paris verticillata	3.07×10^{-2}	1.89×10^{-1}	2.80×10^{-3}
Trillium kamtschaticum	6.28×10^{-2}	1.54×10^{-1}	3.02×10^{-3}
Dioscorea nipponica	9.03	25.40	4.75×10^{-1}
Dioscorea batatas	6.29×10^{-2}	2.31×10^{-1}	3.92×10^{-3}
Dioscorea quinqueloba	1.24	5.95	1.19×10^{-1}
Liriope spicata	5.30×10^{-3}	2.17×10^{-1}	1.50×10^{-3}
Smilax china	1.26	5.73	1.22×10^{-1}
Asparagus schoberioides	3.702×10^{-2}	1.16×10^{-1}	1.25×10^{-3}
Solanum nigrum	2.42×10^{-1}	4.10×10^{-1}	1.21×10^{-2}

A : the percentages from the MeOH extracts, B : the percentages from the *n*-BuOH extracts, C : the percentages from the dried materials.
The contents for *Solanum nigrum* L. (f) from Jamaica were found to be 1.55×10^{-1}, 2.66×10^{-1}, and 1.47×10^{-2} % respectively.

reference; The acid hydrolysates of *n*-BuOH soluble fraction from *Trillium kamtschaticum* was subjected to silica gel column chromatography with hexane-EtOAc (4 : 1) as a mobile phase to give pennogenin,

Table III. The list of plant materials and their yields of extracts

Sapogenin, plant material	Yields (%, w/w)		
	A	B	C
Pennogenin			
Paris verticillata Bieb. (r)[a]	9.11	1.48	0.81
Trillium kamtschaticum Hult. (r)	4.81	1.96	0.98
Laxogenin			
Smilax sieboldii Miq. (r)	6.40	2.40	1.47
Yuccagenin			
Allium fistulosum L. (b)	1.17	0.31	0.19
Hecogenin			
Agave americana L. (l)	3.97	1.06	0.22
(25S)-Ruscogenin			
Liriope spicata (t)	28.30	0.69	0.31
Neochlorogenin			
Solanum nigrum (f)	5.00	2.95	1.58

A : MeOH extracts, B : n-BuOH extracts, C : total acid hydrolysates.
[a] r, t and f in parenthesis represented rhizome. tuber and fruit, respectively. The yields for *Solanum nigrum* L. (f) from Jamaica were found to be 9.50, 5.52 and 2.97%, respectively.

mp. 235-237℃, colorless needles from MeOH : ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.82 (3H, s, 18-C$\underline{H}_3$), 1.03 (3H, s, 19-C$\underline{H}_3$), 0.90 (3H, d, J=7.1 Hz, 21-C$\underline{H}_3$), 0.80 (3H, d, J=6.3 Hz 27-C$\underline{H}_3$), 3.34-3.53 (2H, m, 26-C$\underline{H}_2$), 3.97 (1H, t, J=7.7 Hz, 16-$\underline{H}$), 5.34 (1H, br, d, J=4.9 Hz, 6-$\underline{H}$); ^{13}C-NMR ($CDCl_3$, 75.5 Hz), δ 37.2, 31.6[a], 71.7, 42.3, 140.8, 121.3, 32.0, 31.7[a], 49.7, 36.6, 20.9, 31.2, 43.8, 52.9, 30.1[b], 91.0, 90.1, 17.1, 19.4, 44.6, 8.0, 110.1, 30.8[b], 28.1, 29.7, 66.8, 17.1 (signals of C-1 to C-27)[a,b]; interchangeable. This NMR spectrum including mp. was same as the result previously reported[7,12].

Sample preparation

Dried materials were ground into powder. Each powdered sample (10 g) was weighed accurately and extracted twice with MeOH (500 m*l* each) by refluxing for 2 hrs. The combined solutions were evaporated *in vacuo*, weighed and defatted with *n*-hexane (50 m*l* each) for 3 times after suspending with water (100 m*l*). The aqueous solution was then extracted with *n*-BuOH (50 m*l* each) for 3 times and concentrated *in vacuo* to give residues. The *n*-BuOH extracts were weighed and hydrolyzed with 5% metha-

Table IV. Steroidal sapogenin contents

Sapogenin, plant material	Contents (%, w/w)		
	A	B	C
Pennogenin			
Paris verticillata	1.70	10.47	1.55×10^{-1}
Trillium kamtschaticum	6.44	15.82	3.10×10^{-1}
Laxogenin			
Smilax sieboldii	9.22×10^{-1}	2.46	5.90×10^{-2}
Yuccagenin			
Allium fistulosum	2.92	11.03	3.42×10^{-2}
Hecogenin			
Agave americana	7.43	27.83	2.95×10^{-1}
(25S)-Ruscogenin			
Liriope spicata	1.78×10^{-1}	7.32	5.05×10^{-2}
Neochlorogenin			
Solanum nigrum	3.90	6.61	1.95×10^{-1}

A : the percentages from the MeOH extracts, B : the percentages from the *n*-BuOH extracts, C : the percentages from the dried materials.
The contents for *Solanum nigrum* L. (f) from Jamaica were found to be 1.16, 1.99 and 1.10×10^{-1}%, respectively.

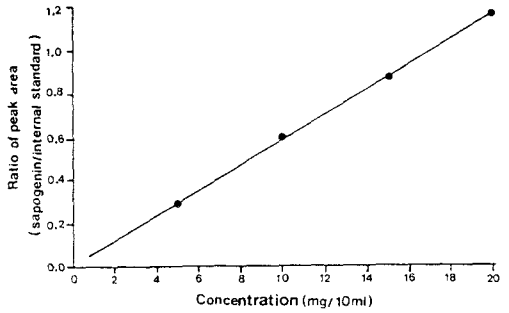

Fig. 1. Calibration curve for diosgenin.

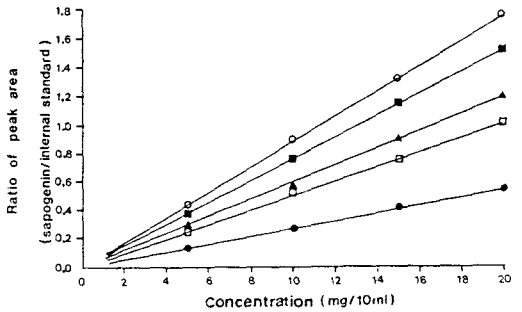

Fig. 2. Calibration curve for steroidal sapogenins. Neochlorogenin (○), hecogenin (■), pennogenin (▲), (25S)-ruscogenin (□), laxogenin (●).

nolic H_2SO_4 (10 m*l* per 100 mg of extract) for 2 hrs. After cooling down, the reaction mixture was evaporated to remove MeOH and diluted with 100 m*l* of water, then extracted with CH_2Cl_2 (50 m*l* each) for 3 times. Each CH_2Cl_2 solution was dried over anhydrous $MgSO_4$ for 10 hrs, filtered and concentrated. The concentrate was transferred into a volumetric flask and adjusted to exactly 10 m*l* with CH_2Cl_2. This solution was passed through a Milipore filter and used for HPLC analysis.

Chromatography

HPLC were performed with a Waters model 440 liquid chromatograph, equipped with a refractive index detector (R401). HPLC were carried out using μ-Bondapak C_{18} reverse column (Waters) and eluted with a solvent mixture containing 83% acetonitrile, 10% methanol and 7% chloroform at a flow rate of 1 ml/min.

Calibration curve

Each authentic standard of steroids was accurately weighed and dissolved in CH_2Cl_2 to give a standard solution (5, 10, 15 and 20 mg/10 m*l*). And an ergosterol solution (40 mg/10 m*l*) was used as an internal standard. Standard calibration curves were prepared by concentration *via* ratio of peak area.

RESULTS AND DISCUSSION

For investigating the steroidal resources in Korea, 13 plants were collected and the steroidal contents were studied. Using authentic steroidal sapogenins, each standard curve was prepared with internal standard, ergosterol (Fig. 1 and 2). The regression equation for diosgenin was $Y=0.0589X+0.0028$, with coefficient $r=0.9998$, which showed good linearity. And the following equations were used for the quantitative analysis of sapogenins present in each plant: pennogenin: $Y=0.0603X-0.0101$ ($r=0.9999$), laxogenin: $Y=0.0509X+0.0075$ ($r=0.9983$), yuccagenin: $Y=0.0279X-0.0020$ ($r=0.9996$), 25(S)-ruscogenin: $Y=0.0561X+0.0018$ ($r=0.9977$), hecogenin: $Y=0.0768X+0.0006$ ($r=0.9999$), neochlorogenin: $Y=0.0884X+0.0038$ ($r=0.9999$). The retention time for the authentic steroid sapogenins were 6.80

Fig. 3. HPLC profile of acid hydrolysate from *D. nipponica*.

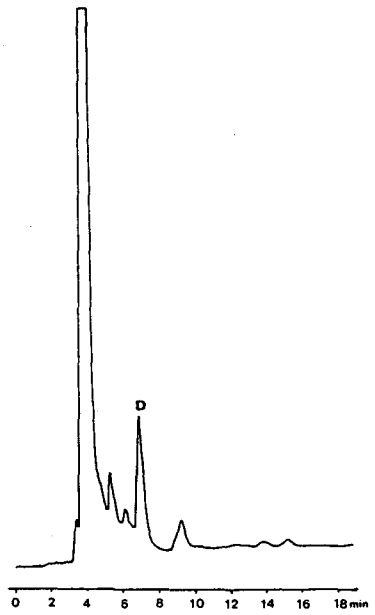

Fig. 4. HPLC profile of acid hydrolysate from *D. quinqueloba*.

min (diosgenin), 5.35 min (pennogenin), 5.15 min (laxogenin), 6.12 min (yuccagenin), 5.31 min ((25S)-ruscogenin), 4.19 min (hecogenin) and 6.40 min

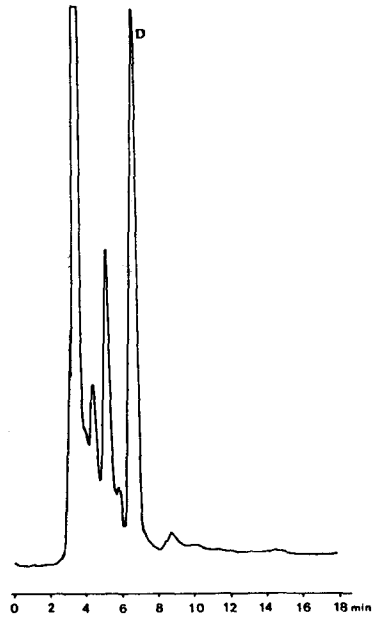

Fig. 5. HPLC profile of acid hydrolysate from *S. china*.

(neochlorogenin). Table I showed the lists of the plants in which their diosgenin contents were determined. Table I also represented the yields of MeOH, *n*-BuOH and acid hydrolysate fractions from these plants. The acid hydrolysate fractions were analyzed for measuring concentrations of diosgenin. Fig. 3, 4 and 5 illustrated three elution profiles of the acid hydrolyzed fractions in which the percentages of diosgenin content is high. And Table II represented the percentages of diosgenin from the MeOH, *n*-BuOH fractions and the dried sample itself, respectively. *Dioscorea nipponica* was found to contain extremely large amounts of diosgenin (≅0.5 % based on the dry wt.). *D. quinqueloba* and *Smilax china* were also rich in this sapogenin accordingly. These results suggest that three species mentioned above could be used as a source of diosgenin. For the steroid sapogenins other than diosgenin, the acid hydrolystates from 8 plant species were analyzed. Table III indicated the lists of the plants and the yields of the fractions from the plants. Their elution profiles of each acid hydrolyzates were shown in Fig. 6. And Table IV showed the percentages of the steroid sapogenins from the MeOH, *n*-BuOH and the dried materials. *Trillium kamtschaticum* and *P. verticillata* were already reported to contain pennogenin glycosides by Nohara *et al.*[13]

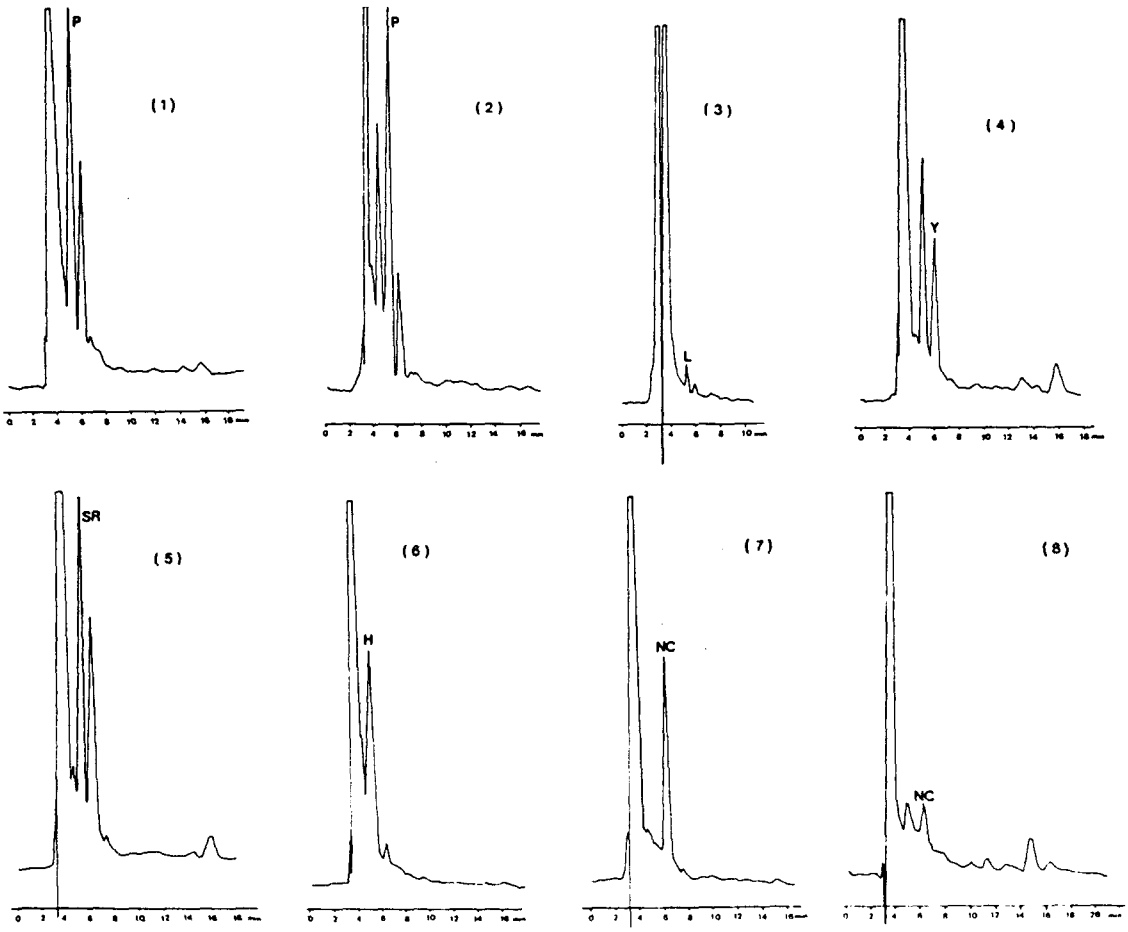

Fig. 6. HPLC profiles of acid hydrolysate fractions
(1) *P. verticillata* (2) *T. kamtschaticum* (3) *S. sieboldii* (4) *A. fistulosum* (5) *L. spicata* (6) *A. americana* (7) *S. nigrum* (Korea) (8) *S. nigrum* (Jamaica).

And laxogenin was first isolated from *S. sieboldii* by Okanishi *et al.* in 1965[4]. Yuccagenin was isolated from *Yucca schotii*[14] and other *Allium* species[15-17], but we recently isolated and characterized yuccagenin glycosides in *A. fistulosum*[11]. (25S)-Ruscogenin was reported to be contained in *Liriope platyphylla*[18] and *L. spicata*[10]. Neochlorogenin, 25(S)-epimer of chlorogenin, is known to distributed in *Solanum hispidum*[19] and *S. torvum*[20], but we isolated this sapogenin from *S. nigrum* and identified[9]. However, only sapogenin contents were measured in this investigation because the objectives of this study was to find out the values of the sapogenin as a resources. And it should be reminded that the measuring procedure of the sapogenin contents in this study after acid hydrolysis would not be adequate for the exact determination of pennogenin because we could not exclude the possibility of its loss due to the transformation of pennogenin to kryptogenin during acid hydrolysis as previously described[13]. Nevertheless, *T. kamtschaticum* and *P. verticillata* showed to be rich in this sapogenin. Of other plants in this table, especially *A. fistulosum, A. americana* and *S. nigrum* could be regarded as excellent sources of steroidal sapogenins.

In conclusion, *D. nipponica, D. quinqueloba* and *S. china* were found to have large amount of diosgenin. And pennogenin in *T. kamtschaticum* and *P. verticillata*, yuccagenin in *A. fistulosum*, hecogenin in *A. americana* and neochlorogenin in *S. nigum* were

appeared to be major steroidal sapogenins. Therefore, these plants might be the resources for the steroidal sapogenins in Korea.

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