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Steroidal Sapogenins of Sixteen Liliaceae Plants[1]

Tameto Okanishi (the late), Akira Akahori, Fumio Yasuda, Yasuyoshi Takeuchi,[2a] and Toru Iwao[2b]

Shionogi Research Laboratory, Shionogi & Co., Ltd.[2a] *and Aburahi Laboratories, Shionogi & Co., Ltd.*[2b]

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Twelve steroidal sapogenins were isolated from sixteen Liliaceae plants: ruscogenin from *Albuca nelsonii*; diosgenin and gentrogenin from *Aletris foliata*; diosgenin from *Aletris formosana*; gitogenin, smilagenin and tigogenin from *Allium grayi*; sarsasapogenin from *Asparagus cochinchinensis*; diosgenin and heloniogenin from *Clintonia udensis*; ruscogenin from *Ophiopogon jaburan*; diosgenin and ruscogenin from *Ophiopogon planiscapus*; diosgenin from *Paris japonica*, *Polygonatum officinale*, *Smilacina hondoensis*, *Tofieldia japonica* and *Trillium smallii*; hecogenin from *Polygonatum falcatum*; rhodeasapogenin and isorhodeasapogenin from *Rohdea japonica*; ruscogenin and neoruscogenin from *Tofieldia japonica*.

Since Marker, *et al.*[3] reported the side-chain degradation of steroidal sapogenins, sapogenins have been investigated by many investigators and found to be widely distributed in the plant kingdom. We have isolated over 40 sapogenins from Japanese Amarylidaceae, Dioscoreaceae, Liliaceae, Scrophulariaceae and Solanaceae plants. However, some sapogenins remained unidentified because of their small quantities or because authentic samples were lacking. The present study was designed to determine the sapogenins of some plants not yet examined in order to obtain data for elucidation of the relationship between the morphological character of the plants and the steroidal sapogenins contained. Further, identification of some sapogenins isolated earlier has been made where it became possible.

Materials and Methods

Materials——Plant materials used in the present study are summarized in Table I. Plants were washed, weighed, air-dried and stored.

Thin-Layer Chromatography (TLC)——Sapogenins were dissolved in methanol, spotted on Silica gel G plates ($20 \times 20 \times 0.03$ cm or $20 \times 5 \times 0.03$ cm) and developed by benzene–acetone–water (70 : 30 : 3) or methylene chloride–acetone (4 : 1). Sapogenins were detected as yellow or reddish orange spots by spraying with cinnamic aldehyde and $SbCl_3$.[4]

Isolation of Steroidal Sapogenins——Materials were first extracted with benzene and then extracted three or four times with methanol under reflux. Methanol solutions were concentrated under reduced pressure and, after addition of HCl to a concentration of 5%, refluxed for 5 hr. The reaction products were extracted with ether and, in some cases, refluxed for 2 hr in 5% methanolic KOH. Unsaponifiable substances were extracted with ether and subjected to column or preparative TLC.

Results and Discussion

Steroidal sapogenins isolated in the present study are summarized in Table II. The sapogenins were identified by comparison of their melting points, mixed melting points, elemental analytical values, optical rotations, infrared (IR) and mass (MS) spectra with those

1) Studies on the steroidal components of domestic plants LXVI. Part LXV: A. Akahori, F. Yasuda, K. Kagawa, and T. Iwao, *Chem. Pharm. Bull.* (Tokyo), 21, 1799 (1973).
2) Location: *a*) *Fukushima-ku, Osaka, 553, Japan*; *b*) *Koka-cho, Shiga, 520-34, Japan*.
3) R.E. Marker and E. Rohrmann, *J. Amer. Chem. Soc.*, **62**, 518 (1940).
4) T. Okanishi, A. Akahori, and F. Yasuda, *Shionogi Kenkyusho Nempo*, **8**, 153 (1958).

TABLE I. Plant Materials Used in the Present Study

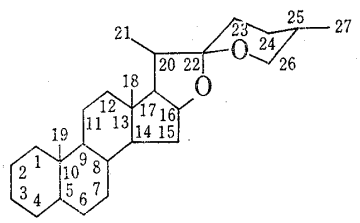
Species	Japanese name	Collected in		Collected at	Part used	Dry weight (g)
Albuca nelsonii N.E.BR.		May	1969	Aburahi, Shiga Pref.[a]	W	7.4
Aletris foliata BUREAU et FRANCH.	nebari-nogiran	July	1961	Shiga-kogen, Nagano Pref.	W	175
A. formosana HAYATA	taiwan-nogiran	Sept.	1968	Daibusan, Taiwan	W	640
Allium grayi REGEL	nobiru	March	1973	Takarazuka, Hyôgo Pref.	W	75
Asparagus cochinchinensis MERR.	kusasugikazura	May	1969	Aburahi, Shiga Pref.[a]	W	2.4
Clintonia udensis TRAUTV. et MEY.	tsubameomoto	Sept.	1972	Mt. Kisokoma, Nagano Pref.	W	353
Ophiopogon jaburan LODL.	noshiran	May	1969	Aburahi, Shiga Pref.[a]	W	14.9
O. planiscapus NAKAI	ôba-janohige	May	1969	Aburahi, Shiga Pref.[a]	W	2.6
Paris japonica FRANCH.	kinugasasô	July	1966	Hachimantai,	W	1700[b]
Polygonatum falcatum A. GRAY	narukoyuri	May	1969	Aburahi, Shiga Pref.[a]	rh	4.7
P. officinale ALL.	amadokoro	March	1973	Takarazuka, Hyôgo Pref.[a]	rh	267
Rohdea japonica ROTH.	omoto	Dec.	1961	Takarazuka, Hyôgo Pref.[a]	W	245[b]
Ruscus aculeatus L.	nagiikada	Sept.	1959	Takarazuka, Hyôgo Pref.[a]	rh	1600[b]
Smilacina hondoensis OHWI	ôba-yukizasa	Aug.	1959	Mt. Kisokoma, Nagano Pref.	W	120
Tofieldia japonica MIQ.	iwashôbu	Sept.	1972	Mt. Togakushi, Nagano Pref.	W	17
Trillium smallii MAXIM.	enreisô	Aug.	1959	Akigani, Gifu Pref.	a	13

a) cultivated *b*) fresh weight
abbreviation: w, whole plant; rh, rhizome; a, aerial part

TABLE II. Steroidal Sapogenins and Sterols isolated from 16 Plants

Species	Sapogenins (mg)	Sterols (mg)
Albuca nelsonii	rusco (1.0)[a]	
Aletris foliata	dios (12.0),[b] gentro (1.0)[a]	β-sito (152)
A. formosana	dios (2.0),[a,b]	β-sito (88),[b,c] unidentified (229)[d]
Allium grayi	smila (1.0), tigo (5.0), gito (0.5)	β-sito (21)[a]
Asparagus cochinchinensis	sarsa (0.5)	
Clintonia udensis	dios (358.0), helonio (83.0)	β-sito (169)
Ophiopogon jaburan	rusco (2.0)	
O. planiscapus	dios (0.5),[a] rusco (0.5)	
Paris japonica	dios (286.0)	
Polygonatum falcatum	heco (0.5)	
P. officinale	dios (22.0)[a]	β-sito[a] (56)
Rohdea japonica	rhodea (128.0), isorhodea (6.0)[a]	
Ruscus aculeatus	rusco (300.0), neorusco (180.0)	
Smilacina hondoensis	dios (100.0)	
Tofieldia japonica	dios (1.0)	
Trillium smallii	dios (25.0)	

a) identified by spectral data only
b) isolated as acetate
c) identified by gass–liquid chromatography
d) Retention time differed from those of β-sitosterol and stigmasterol.
abbreviation: dios, diosgenin; gentro, gentrogenin; gito, gitogenin; heco, hecogenin; helonio, heloniogenin; isorhodea, isorhodeasapogenin; neorusco, neoruscogenin; rhodea, rhodeasapogenin; rusco, ruscogenin; sarsa, sarsasapogenin; smila, smilagenin; tigo, tigogenin, β-sito, β-sitosterol



spirostane

of authentic samples except those noted in the table which were estimated from their spectral data due to insufficient quantities.

The sapogenin isolated from *Ophiopogon jaburan* was detected as one spot by TLC and gave a MS spectrum identical with that of ruscogenin (25D-spirost-5-ene-1β,3β-diol). However, its IR spectrum was slightly different from that of ruscogenin. The intensity of a band at 918 cm^{-1} is almost equivalent to that at 898 cm^{-1}, indicating that the substance is a mixture of 25D and 25L isomers.[5] Neoruscogenin, which was isolated together with ruscogenin and regarded as its 25L isomer,[6] was later clarified to be spirost-5,25(27)-diene-1β,3β-diol[7] and has different IR (Fig. 1) and MS spectra. The sapogenin isolated in the present study is concluded to be a mixture of ruscogenin and its 25L-isomer. 25L-Ruscogenin was not found in sapogenins isolated from *Ruscus aculeatus*.

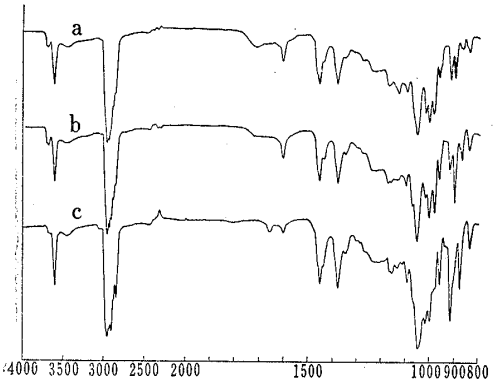


Fig. 1. The Infrared Spectra in $CHCl_3$ of Ruscogenin, Neoruscogenin and Ruscogenin Fraction isolated from *Ophiopogon jaburan*

a) ruscogenin fraction isolated from *O. jaburan*
b) ruscogenin
c) neoruscogenin

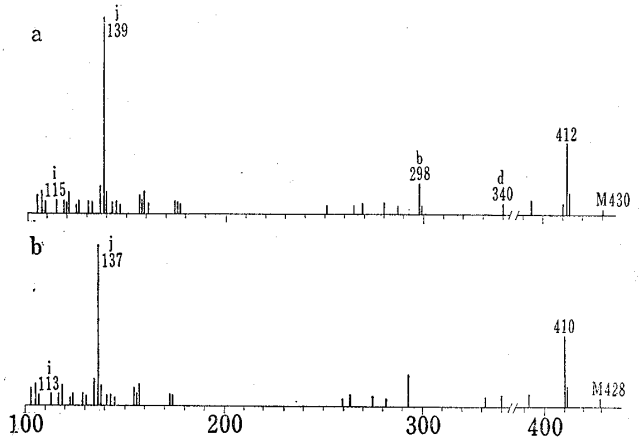


Fig. 2. The Mass Spectra of Neoruscogenin and Ruscogenin Fraction isolated from *Ophiopogeon jaburan*

a) ruscogenin fraction isolated from *O. jaburan*
b) neoruscogenin

The IR spectrum and *Rf* values on TLC of white platelets isolated from *Polygonatum falcatum* are almost identical with those of hecogenin (25D, 5α-spirostan-3β-ol-12-one). In the MS spectrum of the platelets, fragments (i and j[8]) derived from the side chain[9] are identical with those of hecogenin. With regards to fragments derived from the steroid nucleus, besides b, d, f and o which are identical with those of hecogenin, there also exist fragments which have mass number 2 *m/e* less than the above four fragments (Fig. 3). The presence of the fragment (*m/e* 271) further suggests that the second sapogenin is a 12-ketosapogenin.[9b] Thus the platelets are considered to be hecogenin containing small quantities of a monoketomonohydroxyspirostene.

5) M.E. Wall, C.R. Eddy, M.L. McClennan, and M.E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); K. Takeda, and Y. Matsui, *Bunkōkenkyū*, **6**, 1 (1956).
6) D. Burnet, B. Ellis, and V. Petron, *J. Chem. Soc.*, **1958**, 795.
7) J. Robert, R. Vaupré, and G. Poiget, *Compt. Rend.*, **250**, 3187 (1960).
8) The fragments are named according to the report by Budzikiewicz, *et al.*[9b]
9) *a*) F. Yasuda, Y. Nakagawa, A. Akahori, and T. Okanishi, *Tetrahedron*, **24**, 6535 (1968); *b*) H. Budzikiewicz, K. Takeda, and K. Schreiber, *Monatsh. Chem.*, **101**, 1003 (1970).

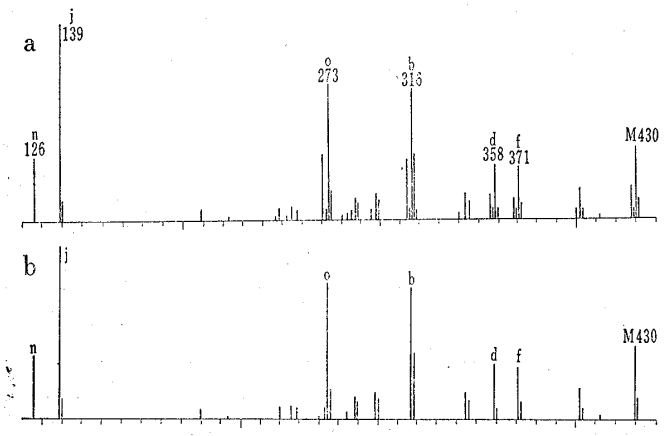


Fig. 3. The Mass Spectra of Hecogenin and Hecogenin Fraction isolated from *Polygonatum falcatum*

a) hecogenin fraction isolated from *P. falcatum*.
b) hecogenin.

Rohdea japonica was reported by Nawa[10] to contain rhodeasapogenin (25L-5β-spirostane-1β,3β-diol). As isorhodeasapogenin isolated in the present study was present in a smaller quantity than rhodeasapogenin, it seems likely that the former is an artifact produced from the latter during the acid hydrolysis.

Hara[11] investigated some Asian species of the genus *Aletris*, and observed a wide variance in the length of the part of the perianth connected with the ovary and proposed reunion of the genus with the genus *Metanarthecium*. Although we could not find 11-hydroxysapogenins in *Aletris spicata* (Thunb.) Franch., we isolated isonarthogenin (25D-spirost-5-ene-3β,27-diol), which had been found only in two plants, *Metanarthecium luteo-viride* Maxim.[12] and *Dioscorea quinqueloba* Thunb.,[13] from the plant in support of Hara's proposal.[14] However, the 27-hydroxysapogenin was not found in *Aletris foliata* and *A. formosana*. The principal sapogenin of these two plants, diosgenin (25D-spirost-5-en-3β-ol), was also detected in *A. spicata*[14] and *A. farinosa*[15] but was not found in *M. luteo-viride*.[16] From a phytochemical standpoint, the separation of *Metanarthecium* from *Aletris* probably occurred at a fairly early stage, even though they are closely related.

From *Allium* plants, seven sapogenins have been reported: diosgenin,[17,18] hecogenin,[19] ruscogenin,[20] β-chlorogenin (25D, 5α-spirostane-3β,6β-diol),[18] tigogenin (25D, 5α-spirostan-3β-ol),[21] alliogenin (25D, 5α-spirostane-2α,3β,5α,6β-tetraol)[22] and neoagrigenin.[23] Allium plants are divided into two groups; one containing diosgenin and the other not. *A. grayi* is a first *Allium* plant shown to contain smilagenin (25D, 5β-spirostan-3β-ol) and gitogenin (25D, 5α-spirostane-2α,3β-diol) and belongs to the second group.

Plants of the genus *Asparagus* are also divided into two groups: one containing

10) H. Nawa, *Yakugaku Zasshi*, **73**, 1192, 1195, 1197 (1953); *idem*, *Chem. Pharm. Bull.* (Tokyo), **6**, 255 (1958).
11) H. Hara, *J. Jap. Bot.*, **42**, 312 (1967).
12) H. Minato and A. Shimaoka, *Chem. Pharm. Bull.* (Tokyo), **11**, 876 (1963).
13) A. Akahori, F. Yasuda, K. Kagawa, and T. Iwao, *Chem. Pharm. Bull.* (Tokyo), **19**, 846 (1971).
14) A. Akahori, F. Yasuda, and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2409 (1971).
15) R.E. Marker, D.L. Turner, A.C. Shabica, E.M. Jones, and J. Krueger, *J. Amer. Chem. Soc.*, **62**, 2620 (1940).
16) K. Hamamoto, *Shionogi Kenkyusho Nempo*, **15**, 1 (1965).
17) E.V. Kereselidze, T.A. Pkheidze, and E.P. Kemertelidze, *Khim. Prir. Soedin.*, **6**, 378 (1970); L.I. Eristani, *Soobshch. Akad. Nauk. Gruz. SSR*, **66**, 93 (1972).
18) M.B. Gorovits, F.S. Khristulas, and N.K. Abubakirov, *Khim. Prir. Soedin.*, **7**, 434 (1971); L.I. Eristani, M.B. Gorovits and N.K. Abubakirov, *ibid.*, **3**, 124 (1967).
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20) T.A. Pkheidze, E.V. Kereselidze, and E.P. Kemertelidze, *Khim. Prir. Soedin.*, **7**, 841 (1971).
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22) F.S. Khistulas, M.B. Gorovits, V.N. Luchanskaya, and N.K. Abubakirov, *Khim. Prir. Soedin.*, **6**, 489 (1970).
23) A.N. Abubakirov, M.B. Gorovits, and S.A. Khamidkhodzarov, *Khim. Prir. Soedin.*, **9**, 438 (1973).

diosgenin[24,25] and the other sarsasapogenin (25L, 5β-spirostan-3β-ol).[25,26] Etiolated bud[25] of *A. officinalis* is the only *Asparagus* known to contain both these sapogenins. In the present study, *A. cochinchinensis* was found to belong to the group containing only sarsasapogenin.

The second sapogenin of *Clintonia udensis* remained unidentified in our previous work[27] and is now identified with heloniogenin (25D-spirost-5-ene-3β,12α-diol) by comparison of its physical properties with those of an authentic sample. This sapogenin has been found only in *Heloniopsis orientalis* C. TANAKA,[28] which contains also diosgenin.

Ruscogenin isolated from two *Ophiopogon* species in the present study was also found in *O. japonicus* KER-GAWL.,[29] but diosgenin was detected only in *O. planiscapus* NAKAI. Morphologically, *Ophiopogon* plants resemble each other very closely and identification of the species is often difficult. Diosgenin may be used as one of useful taxonomical criteria.

Hecogenin isolated from *Polygonatum falcatum* has never been found in other *Polygonatum* species,[30] while diosgenin isolated from *Paris japonica*, *Smilacina hondoensis*, *Tofieldia japonica* and *Trillium smallii* has been reported also from plants of these genera.[26a,31]

A spot corresponding to pennogenin (25D-spirost-5-ene-3β,17-diol) isolated by Huang[31b] from *Paris dunniona* LIUL var. *oligophylla* WANG et TANG was also detected in *P. japonica* by TLC.

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25) G.Y. Held, D. Vagujfalvi, and F. Uresch, *Phytochem.*, **8**, 493 (1969).
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