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Studies on the Constituents of Asparagi Radix. I.[1] On the Structures of Furostanol Oligosides of *Asparagus cochinchinensis* (Loureio) Merrill

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Asp-IV, V, VI and VII, the major furostanol oligosides, have been isolated from the methanol extract of *Asparagus cochinchinensis* (Loureio) Merrill (Liliaceae). The structures of Asp-IV, V, VI and VII have been established as 26-*O*-*β*-D-glucopyranosyl-22-methoxyfurostane-3*β*,26-diol 3-*O*-*β*-D-xylopyranosyl(1→4)-*β*-D-glucopyranoside (**1**), 26-*O*-*β*-D-glucopyranosyl-22-methoxyfurostane-3*β*,26-diol 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (**2**), 26-*O*-*β*-D-glucopyranosyl-22-methoxyfurostane-3*β*,26-diol 3-*O*-*β*-D-xylopyranosyl(1→4)-[*α*-L-rhamnopyranosyl(1→6)]-*β*-D-glucopyranoside (**3**) and 26-*O*-*β*-D-glucopyranosyl-22-methoxyfurostane-3*β*,26-diol 3-*O*-[*β*-D-glucopyranosyl(1→2)][*β*-D-xylopyranosyl(1→4)][*α*-L-rhamnopyranosyl(1→6)]-*β*-D-glucopyranoside (**4**), respectively. A methoxyl group at C-22 of each furostanol oligoside is converted to a hydroxyl group by boiling in aqueous acetone. The interconversions between methoxyl and hydroxyl groups at C-22 of the furostanol oligosides were investigated and the genuine furostanol oligosides of Asparagi radix appear to be of the hydroxyl type, based on comparative studies of the methanol and pyridine extracts.

Keywords——Asparagi radix; *Asparagus cochinchinensis*; Liliaceae; furostanol oligoside; steroidal saponin; sarsasapogenin; C-13 NMR

Recently, Sakamura *et al.*[3,4] reported four steroidal saponins, officinalisnin I and II, and asparasaponin I and II, of the storage root and base of *Asparagus officinalis* L. Goryanu *et al.*[5] have also studied the same plant and they reported the isolation of nine steroidal saponins designated asparagosides A-I, and described the structures of asparagosides A, B, C, E, F and H.

Asparagi radix (天門冬; Japanese name, tenmondo), the dried root of *Asparagus cochinchinensis* (Loureio) Merrill, is a well-known Chinese medicine used as a tonic, and exhibiting antifebrile, antitussive and diuretic activities. The constituents of this drug had been investigated by Tomoda *et al.*[6] and they reported the isolation of some amino acids and seven fructo-oligosaccharides. This paper describes the isolation and structure elucidation of Ehrlich-positive[7] furostanol oligosides named Asp-IV (**1**), $C_{45}H_{76}O_{18}$, Asp-V (**2**), $C_{46}H_{78}O_{18}$, Asp-VI (**3**), $C_{51}H_{86}O_{22}$, and Asp-VII (**4**), $C_{57}H_{96}O_{27}$ from Asparagi radix.

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2) Location: *Hatanodai, Shinagawa-ku, Tokyo, 142, Japan.*
3) H. Saito and S. Sakamura, *Agr. Biol. Chem.*, **37**, 225 (1973).
4) K. Kawano, K. Sakai, H. Sato, and S. Sakamura, *Agr. Biol. Chem.*, **39**, 1999 (1975); *idem, ibid.*, **41**, 1 (1977).
5) G.M. Goryanu, G.V. Lazur'evskii, and P.K. Kintya, *Khim. Prir. Soedin*, **3**, 400 (1976) [*C.A.*, **85**, 90178*w* (1976)]; *idem, ibid.*, **6**, 823 (1976) [*C.A.*, **86**, 103064*d* (1977)]; *idem, ibid.*, **6**, 810 (1977) [*C.A.*, **88**, 13690*u* (1978)]; G.V. Lazur'evskii, G.M. Goryanu, and P.K. Kintya, *Dokl. Akad. Nauk. SSSR*, **231**, 1479 (1976) [*C.A.*, **86**, 68399*t* (1977)].
6) M. Tomoda and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **22**, 2306 (1974); M. Tomoda, N. Satoh, and M. Tanaka, *Kyoritsu Daigaku Kenkyu Nempo*, **20**, 9 (1975).
7) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968); T. Kawasaki, T. Komori, T. Nohara, I. Hosokawa, and K. Mihashi, *ibid.*, **22**, 2164 (1974).

The four new furostanol oligosides were obtained from the methanolic extract of the commercial cut root of *A. cochinchinensis* MERRILL as shown in Chart 1.

Thin-layer chromatography (TLC) of the butanol extract revealed the presence of more than seven saponins; the major spots were named Asp-I—VII in order of nicreasing polarity. The general properties of **1**, **2**, **3**, **4**, and the related compounds are given in Table I.

Proton magnetic resonance (PMR) spectroscopy (δ 3.28 ppm, 3H, s, –O–C$\underline{H}_3$) and ^{13}C nuclear magnetic resonance (CMR) spectroscopy (δ 56.5 ppm, –O–$\underline{C}H_3$) suggested the presence of a 22-methoxyl furostanol structure in each oligoside. On boiling in aqueous acetone,[8] **2** and **3** were converted to Asp-V′ (**2′**), $C_{45}H_{76}O_{18}$, and Asp-VI′ (**3′**), $C_{50}H_{84}O_{22}$, respectively, which are more polar than **2** and **3**; the PMR spectra of **2′** and **3′** do not show any methoxyl

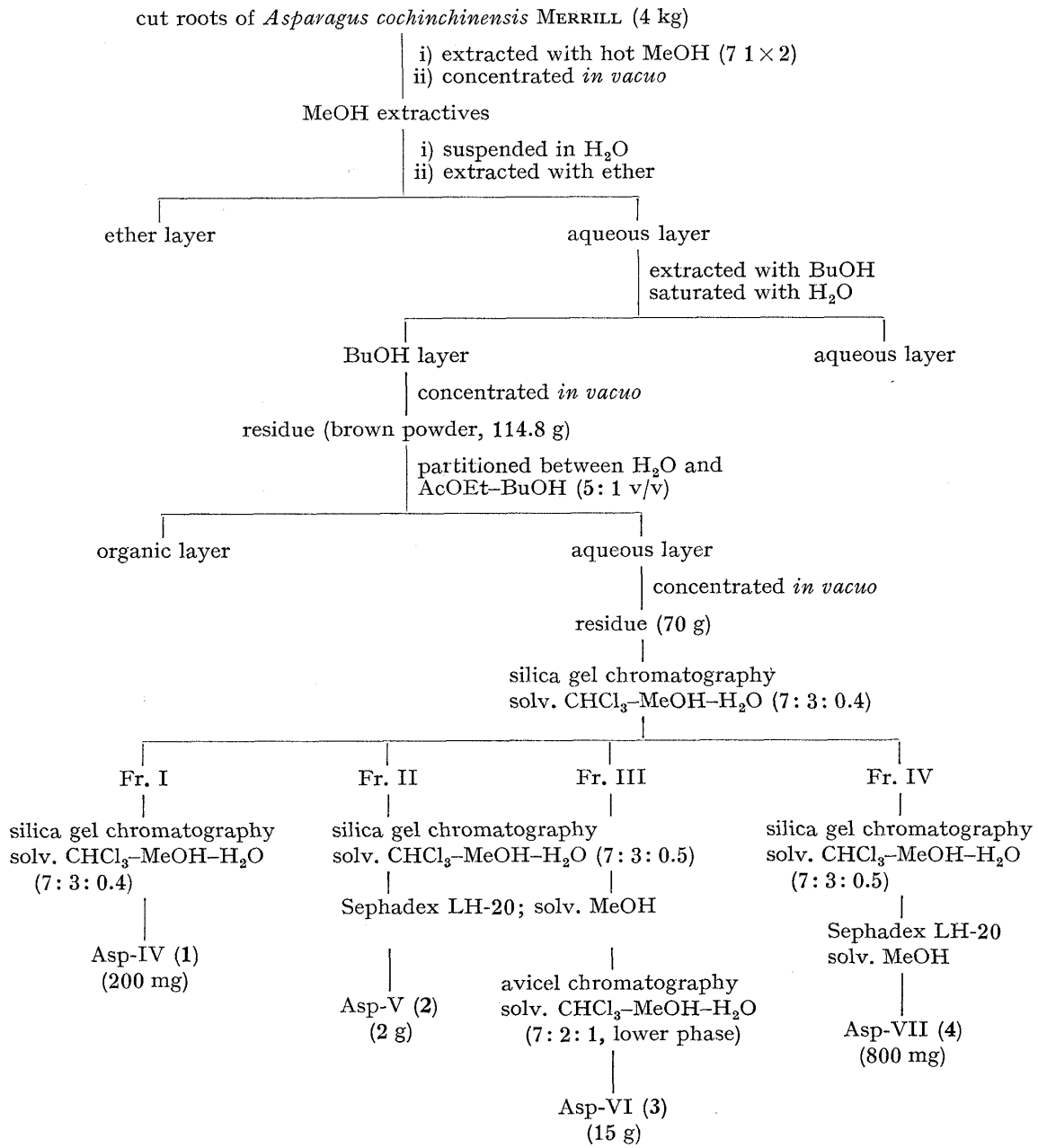

Chart 1

8) H. Hirschmann and F.B. Hirschmann, *Tetrahedron*, **3**, 243 (1958).

signal. Similar conversions were also observed in the cases of **1** and **4**. Interconversion between a hydroxyl group and a methoxyl group at C-22 of furostanol oligosides has been reported.[7,8] Therefore, **2'** and **3'** are considered to be 22-hydroxy derivatives of **2** and **3**, respectively.

On acidic hydrolysis with 2 N sulfuric acid in 50% ethanol, **1**, **2**, **3**, and **4** gave sarsasapogenin (**5**) as a common aglycone together with the monosaccharides listed in Table II.

On the other hand, enzymatic hydrolyses of **3** and **3'** with almond emulsin (Sigma Chem. Co.) afforded D-glucose and a prosapogenin (**6**), $C_{44}H_{72}O_{16}$, which showed 25S spiroketal

Table I

Oligoside	Properties	mp (°C)	$[\alpha]_D$ (c, solv., °C)	Formula	PMR (δ ppm in C_5D_5N) (OCH_3 and anomer H)
Asp-IV (**1**)	A white powder (MeOH)	165—167 (dec.)	−22.9° (2.1, MeOH, 26.5)	$C_{45}H_{76}O_{18}$	3.28 (3H, s, $OC\underline{H}_3$) 4.82 (1H, d, J=7 Hz, anomer H) 4.88 (1H, d, J=6 Hz, anomer H) 5.10 (1H, d, J=6.5 Hz, anomer H)
Asp-IV' (**1'**)	A white powder (H_2O)	146—149 (dec.)	−30.9° (0.87, Py, 22)	$C_{44}H_{47}O_{18}$	4.81 (1H, d, J=7 Hz, anomer H) 4.90 (1H, d, J=6 Hz, anomer H) 5.13 (1H, d, J=7 Hz, anomer H)
Asp-V (**2**)	A white powder (acetone–MeOH)	150—156 (dec.)	−56.5° (1.0, MeOH, 24.5)	$C_{46}H_{78}O_{18}$	3.28 (3H, s, $OC\underline{H}_3$) 4.83 (1H, d, J=7.5 Hz, anomer H) 4.96 (1H, d, J=7.5 Hz, anomer H) 5.52 (1H, s, anomer H)
Asp-V' (**2'**)	Colorless needles (aqueous acetone)	270—275 (dec.)	−45.5° (0.33, Py, 21)	$C_{45}H_{76}O_{18}$	4.81 (1H, d, J=7 Hz, anomer H) 4.86 (1H, d, J=7 Hz, anomer H) 5.49 (1H, s, anomer H)
Asp-VI (**3**)	A white powder (acetone–MeOH)	165—168.5 (dec.)	−50° (1.0, MeOH, 24.5)	$C_{51}H_{86}O_{22}$	3.29 (3H, s, $OC\underline{H}_3$) 4.84 (3H, d, J=7 Hz, 3×anomer H) 5.50 (1H, s, anomer H)
Asp-VI' (**3'**)	A white powder (aqueous acetone)	169—172 (dec.)	−49.5° (1.0, Py, 21)	$C_{50}H_{84}O_{22}$	4.83 (3H, d, J=7 Hz, 3×anomer H) 5.51 (1H, s, anomer H)
Asp-VII (**4**)	A white powder (AcOEt–MeOH)	179—181 (dec.)	−26.8° (1.2, MeOH, 26.5)	$C_{57}H_{96}O_{27}$	3.28 (3H, s, $OC\underline{H}_3$) 4.83 (3H, d, J=7 Hz, 3×anomer H) 5.39 (1H, d, J=7 Hz, anomer H) 5.53 (1H, s, anomer H)
Asp-VII' (**4'**)	A white powder (aqueous acetone)	187—190 (dec.)	−31.6° (1.2, Py, 22)	$C_{56}H_{94}O_{27}$	4.79 (2H, d, J=7 Hz, 2×anomer H) 4.83 (1H, d, J=7 Hz, anomer H) 5.38 (1H, d, J=7 Hz, anomer H) 5.51 (1H, s, anomer H)

Table II

Saponins	Aglycone	Sugar components
Asp-IV (**1**)	Sarsasapogenin	D-Glucose D-Xylose
Asp-V (**2**)	Sarsasapogenin	D-Glucose L-Rhamnose
Asp-VI (**3**)	Sarsasapogenin	D-Glucose L-Rhamnose D-Xylose
Asp-VII (**4**)	Sarsasapogenin	D-Glucose L-Rhamnose D-Xylose

absorption bands at 985, 920, 897 and 850 cm^{-1} (intensity, 920>897, 25-spiroketal) in the infrared (IR) spectrum. Consequently, **3** and **3'** were inferred to be 22-methoxy- and 22-hydroxyfurostanol 3,26-*O*-bisglycosides related to **6**, and this view was confirmed by Baeyer-Villiger oxidation[9] of **3** to provide methyl γ-methyl δ-hydroxypentanoate β-D-glucopyranoside tetraacetate (**7**) and 5β-pregnane-3β,16β,20α-triol (**8**). The latter compound, **8**, was identical with an authentic sample synthesized from sarsasapogenin and the structure of **7** was inferred from the PMR and mass spectral data, and the results of enzymatic hydrolysis.[10]

Methylation of **3** by Hakomori's method[11] afforded the permethylate (**9**), $C_{63}H_{110}O_{22}$, which was methanolyzed to yield methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside and methyl 2,3-di-*O*-methyl-D-glucopyranoside. On hydrolysis with 0.1 N sulfuric acid, **6** gave three products, $C_{33}H_{54}O_8$ (**10**), $C_{38}H_{62}O_{12}$ (**11**) and $C_{39}H_{64}O_{12}$ (**12**), which were permethylated by Hakomori's method to afford $C_{37}H_{62}O_8$ (**13**), $C_{44}H_{74}O_{12}$ (**14**) and $C_{45}H_{77}O_{12}$ (**15**), respectively. The permethylates (**13**, **14**, **15**) were subjected to methanolysis to afford sarsasapogenin and the following methylated monosaccharides: methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside from **13**, methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside and methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside from **14**, and methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside from **15**. As a result of sugar analysis, it was found that **3** has one glucose residue and a trisaccharide, D-xylopyranosyl(1→4)-[L-rhamnopyranosyl(1→6)]-D-glucopyranose, in the molecule. The anomeric configuration of each monosaccharide of **3** has been assigned by the application of Klyne's rule[12] to **10**, **11** and **12** (Table III). In addition, a β-configuration of the glucose residue at the C-26 hydroxyl group of **3** is suggested by the results of enzymatic hydrolysis and PMR analysis of **7** (δ 4.50, 1H, d, J=7 Hz). Accordingly, the structure of Asp-VI (**3**) was elucidated as 26-*O*-β-D-glucopyranosyl-22-methoxyfurostane-3β,26-diol 3-*O*-β-D-xylopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside.

Table III. Conparison of the Molecular Optical Rotations of Prosapogenins of Asp-VI (3)

Prosapogenins	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
11	−54°	−382°	−74°
10	−53°	−308°	−125°
12	−60°	−433°	
Methyl α-D-xylopyranoside		: $[M]_D$ +249°	
Methyl β-D-xylopyranoside		: $[M]_D$ −107°	
Methyl α-L-rhamnopyranoside		: $[M]_D$ −111°	
Methyl β-L-rhamnopyranoside		: $[M]_D$ +170°	

Asp-V (**2**) and Asp-V' (**2'**) were hydrolyzed with almond emulsin to afford a prosapogenin (**12**) and D-glucose. On methylation by Hakomori's method, **2** gave a permethylate (**16**), $C_{59}H_{98}O_{18}$, which was methanolyzed to generate sarsasapogenin and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside. The above observations and inspections of the anomeric configurations of each monosaccharide established the structure of Asp-V (**2**) as 26-*O*-β-D-glucopyranosyl-22-methoxyfurostane-3β,26-diol 3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranos-

9) Von Baeyer and Villiger, *Ber.*, **32**, 3625 (1899); R.E. Marker and D.L. Turner, *J. Am. Chem. Soc.*, **62**, 2540 (1940); K. Morita, S. Noguchi, H. Kono, and T. Miki, *Chem. Pharm. Bull.* (Tokyo), **11**, 90, 144 (1963).
10) R. Tschesche, G. Ludke, and G. Wulff, *Tetrahedron Lett.*, **29**, 2785 (1967); *idem*, *Chem. Ber.*, **102** 1253 (1969).
11) S. Hakomori, *J. Biochem.*, **55**, 209 (1964).
12) W. Klyne, *Biochem. J.*, **47**, xii (1950).

	R_1	R_2	R_3	R_4
1:	H	β-D-xylose	H	CH_3
1′:	H	β-D-xylose	H	H
2:	H	H	α-L-rhamnose	CH_3
2′:	H	H	α-L-rhamnose	H
3:	H	β-D-xylose	α-L-rhamnose	CH_3
3′:	H	β-D-xylose	α-L-rhamnose	H
4:	β-D-glucose	β-D-xylose	α-L-rhamnose	CH_3
4′:	β-D-glucose	β-D-xylose	α-L-rhamnose	H

	R_1	R_2	R_3
6:	H	β-D-xylose	α-L-rhamnose
10:	H	H	H
11:	H	β-D-xylose	H
12:	H	H	α-L-rhamnose
17:	β-D-glucose	β-D-xylose	α-L-rhamnose

Fig. 1

ide.

On enzymatic hydrolysis with almond emulsin, Asp-IV (**1**) gave D-glucose and a prosapogenin (**11**), which was identical with a partial hydrolysis product of the prosapogenin of **6**. Furthermore, comparative CMR spectra studies with **2**, **3** and **10** led to elucidation of the structure of Asp-IV (**1**) as 26-*O*-*β*-D-glucopyranosyl 22-methoxyfurostane-3*β*,26-diol 3-*O*-*β*-D-xylopyranosyl(1→4)-*β*-D-glucopyranoside (Table IV).

Table IV. Carbon-13 Chemical Shifts (δ)[a] of Anomeric Carbons and *O*-methyl Carbons of Saponins in C_5D_5N

Saponins	Carbons					
	O–CH_3	Glucose			Rhamnose	Xylose
		C-3	C-26	C-6′		
Asp-IV (**1**)	56.5	102.8	104.7	—	—	105.3
Asp-V (**2**)	56.5	103.3	104.8	—	102.2	—
Asp-VI (**3**)	56.5	104.6	104.6	—	101.7	104.9
Asp-VII (**4**)	56.5	101.7	104.7	105.2	101.7	104.9
Prosapogenin (**10**)	—	103.1	—	—	—	—

a) Chemical shifts are given in δ(ppm) with tetramethylsilane as an internal standard.

Finally, Asp-VII (**4**) was treated with almond emulsin to afford a prosapogenin (**17**), $C_{50}H_{82}O_{21}$, and D-glucose. On the other hand, enzymatic hydrolysis of **4** with crude hesperidinase (Tanabe Seiyaku Co., Ltd.) afforded two prosapogenins, **6** and **10**, which had already been obtained from Asp-VI (**3**). Further, a permethylate (**18**), $C_{72}H_{126}O_{27}$, prepared by Hakomori's methylation of **4** was subjected to methanolysis and the resulting methylated monosaccharides were identified as methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and methyl 3-*O*-methyl-D-glucopyranoside. These data, and comparison of the CMR spectra of **4** with those of **1**, **2**, **3** and **10** established the structure of Asp-VII (**4**) as 26-*O*-*β*-D-glucopyranosyl-22-methoxyfurostane-3*β*,26-diol 3-*O*-[*β*-D-glucopyranosyl(1→2)] [*β*-D-xylopyranosyl(1→4)][*α*-L-rhamnopyranosyl(1→6)]-*β*-D-glucopyranoside.

As described above, the structures of four 22-methoxyl furostanol oligosides from the methanol extract of Asparagi radix have been established. Interconversion between 22-hydroxyl and 22-methoxyl furostanol oligosides is known to occur, so we tried to obtain the genuine oligosides of Asparagi radix by extracting the crude drug without methanol. A thin-layer chromatogram of the extract of Asparagi radix with pyridine showed only the spots of the 22-hydroxyl furostanol oligosides, **1′**, **2′**, **3′** and **4′**, with no spots corresponding to the 22-methoxyl furostanol oligosides, **1**, **2**, **3** and **4**. Furthermore, the oligoside fraction of the pyridine extract was collected by preparative TLC and the oligoside mixture was examined by PMR. No 22-methoxyl signals corresponding to those of **1**, **2**, **3** and **4** were found in the PMR spectrum of the oligoside mixture and it was concluded that the genuine oligosides are of the 22-hydroxyl furostanol type in the crude drug.

Studies of the pharmacological action of these oligosides are in progress.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Yanagimoto OR-5 automatic polarimeter. IR spectra were obtained with a Hitachi EPI-2 spectrometer and PMR spectra were taken at 90 MHz with a Hitachi R-22 NMR spectrometer. CMR spectra were measured with a JEOL PFT-100 spectrometer (22.15 MHz) and chemical shifts are given in δ (ppm) scale with tetramethylsilane as an internal standard. Gas liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector. Mass spectra were recorded on a Hitachi RMS-4 mass spectrometer. Paper partition chromatography (PPC) of sugars was conducted on Tōyō Roshi No. 51 paper using a mixture of pyridine (1) and the upper phase of BuOH–pyridine–

water (6: 2: 3). Detection was carried out with aniline hydrogen phthalate as a spray reagent. TLC was performed on pre-coated Kieselgel F_{254} plates (Merck) and the detection was carried out by spraying the Ehrlich reagent or 10% H_2SO_4, followed by heating. Column chromatography was carried out on Kieselgel (0.02—0.2 mm, Merck), Wakō C-100 and Wakō C-200. Unless otherwise noted, solvents used for TLC and column chromatography were as follows; solv. a: $CHCl_3$–MeOH–H_2O (7: 3: 0.4), solv. b: $CHCl_3$–MeOH–H_2O (7: 3: 0.5), solv. c: $CHCl_3$–MeOH (10: 1), solv. d: hexane–AcOEt (1: 2), solv. e: benzene–acetone (3: 1).

Extraction and Isolation of Furostanol Glycosides——The commercial cut roots of *Asparagus cochinchinensis* MERRILL (4 kg) were extracted with MeOH (7 1×2) and the extract was concentrated *in vacuo*. The residue was dissolved in water and extracted with ether. The aqueous layer was extracted with BuOH saturated with water and the BuOH soluble fraction was concentrated *in vacuo* to afford a brown powder (114.8 g), which was partitioned between water and ethyl acetate–BuOH (5: 1 v/v). The aqueous layer was evaporated to dryness *in vacuo* and the residue (70 g) was subjected to column chromatography over silica gel (800 g) with solv. a, yielding four fractions (Fr. I—IV). Fr. I was purified by rechromatography on silica gel with solv. a to give pure Asp-IV (200 mg). Fr. II and III were subjected to column chromatography on silica gel with solv. b, followed by column chromatography on Sephadex LH-20 with MeOH. Asp-V (2 g) was isolated from Fr. II and Asp-VI (15 g) was isolated by rechromatography on cellulose powder (Avicel) with $CHCl_3$–MeOH–H_2O (7: 2: 1, lower phase) from Fr. III. Fr. IV was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7: 3: 0.6) followed by Sephadex LH-20 column chromatography using MeOH to afford pure Asp-VII (800 mg).

Properties of Asp-IV (1), V (2), VI (3) and VII (4)——The general properties of **1**, **2**, **3** and **4** are listed in Table I. Asp-IV (**1**): *Anal.* Calcd. for $C_{45}H_{76}O_{18}$: C, 59.71; H, 8.46. Found: C, 59.44; H, 8.78. Asp-V (**2**): *Anal.* Calcd. for $C_{46}H_{78}O_{18}\cdot 5/2H_2O$: C, 57.30; H, 8.68. Found: C, 57.48; H, 8.82. Asp-VI (**3**): *Anal.* Calcd. for $C_{51}H_{86}O_{22}\cdot 2H_2O$: C, 56.34; H, 8.34. Found: C, 56.27; H, 8.30. Asp-VII (**4**): *Anal.* Calcd. for $C_{57}H_{96}O_{27}\cdot 3H_2O$: C, 54.01; H, 8.11. Found: C, 54.01; H, 7.99.

Conversion of 1, 2, 3, 4 to 1′, 2′, 3′, 4′ with Aqueous Acetone——Compounds **1**, **2**, **3**, and **4** (100 mg each) were each boiled with acetone–H_2O (7: 3 v/v, 30 ml) for 10 hr, then the reaction mixture was evaporated down *in vacuo*. The general properties of **1′**, **2′**, **3′** and **4′** are listed in Table I. Asp-IV′ (**1′**): *Anal.* Calcd. for $C_{44}H_{74}O_{18}\cdot H_2O$: C, 58.13; H, 8.21. Found: C, 58.03; H, 8.00. Asp-V′ (**2′**): *Anal.* Calcd. for $C_{45}H_{76}O_{18}\cdot 3/2H_2O$: C, 57.98; H, 8.38. Found: C, 58.16; H, 8.18. Asp-VI′ (**3′**): *Anal.* Calcd. for $C_{50}H_{84}O_{22}\cdot H_2O$: C, 55.97; H, 8.22. Found: C, 56.16; H, 8.05. Asp-VII′ (**4′**): *Anal.* Calcd. for $C_{56}H_{91}O_{27}\cdot 2H_2O$: C, 53.63; H, 8.00. Found: C, 53.38; H, 7.81.

Acid Hydrolysis of 1, 2, 3 and 4——A solution of each oligoside, **1** (20 mg), **2** (20 mg), **3** (200 mg) or **4** (20 mg), in 2 N H_2SO_4–50% EtOH was refluxed for 5 hr. Each reaction mixture was diluted with water. The precipitates were collected by filtration and purified by column chromatography on silica gel with solv. c or by recrystallization from MeOH to afford colorless needles, mp 197—198°, $[\alpha]_D^{25}$ −78.1° (c=1.0, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 3640 (OH), 986, 920, 896, 850 (intensity 920>896, 25S spiroketal); this compound was found to be identical with sarsasapogenin and its acetate by TLC, mixed fusion and by comparing the IR and PMR spectra.

Each filtrate was neutralized with Amberlite MB-3 and evaporated to dryness *in vacuo*, then the residue was examined by PPC, TLC and GLC. PPC: *Rf* 0.53 (glucose), 0.60 (xylose), 0.78 (rhamnose), GLC (column: 5% SE-52 on Chromosorb W 3 mm×2 m, column temp.: 175°, injection temp.: 210°, carrier gas: N_2 1.0 kg/cm^2, sample: trimethylsilane (TMS) derivatives): t_R(min) 10.2, 15.2 (glucose), 4.3, 5.8 (xylose), 3.4, 4.3 (rhamnose). Furthermore, the residue was chromatographed on silica gel with solv. b to isolate each monosaccharide, and the optical rotation was measured. D-Glucose: $[\alpha]_D^{20}$ +51.3° (c=2.6, H_2O) (lit.[13] $[\alpha]_D$ +52.7°); D-xylose: $[\alpha]_D^{20}$ +19.2° (c=1.56, H_2O) (lit. $[\alpha]_D$ +18.8°); L-rhamnose: $[\alpha]_D^{20}$ +8.1° (c=4.3, H_2O) (lit. $[\alpha]_D$ +8.9°).

Enzymatic Hydrolysis of 3 and 3′——Asp-IV (**3**) (400 mg) in H_2O was incubated with almond emulsin (80 mg) at 37° for 24 hr. The precipitate was collected by filtration, dried and chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (7: 2: 0.2) to afford a prosapogenin (**6**). The prosapogenin (**6**) was recrystallized from EtOH to give colorless needles, mp 269—270° (dec.), $[\alpha]_D$ −51.0° (c=1.0, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 985, 920, 897, 850 (intensity 920>897 25S spiroketal), *Anal.* Calcd. for $C_{44}H_{72}O_{16}\cdot H_2O$: C, 60.39; H, 8.52. Found: C, 60.40; H, 8.89.

The aqueous filtrate was evaporated to dryness *in vacuo*. The residue was examined by TLC and only glucose was detected as a sugar component.

Enzymatic hydrolysis of **3′** was carried out by the same procedure. Glucose and the prosapogenin (**6**) were detected by TLC.

Baeyer-Villiger Oxidation of 3——A suspension of **3** (1.1 g) in dichlorethane (15 ml) was treated with 85% formic acid (30 mg) and 30% H_2O_2 (3.2 ml). The reaction mixture was heated at 55° for 1 hr and evaporated down *in vacuo*. Next, 3% KOH in MeOH (40 ml) was added to the residue and the solution was left to stand at 50° for 30 min. The reaction mixture was neutralized with dil. HCl and evaporated down *in vacuo*. The residue was acetylated with acetic anhydride and pyridine (1: 1) by heating on a water bath for 2 hr. The

13) J. Stanek, M. Cerny, J. Kocourek, and J. Pacak, "The Monosaccharides," Academic Press, New York and London, 1963, p. 83.

reaction mixture was diluted with water and then extracted with AcOEt. The organic layer was evaporated to dryness and the residue was purified by chromatography on silica gel with solv. d. Methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside tetraacetate (**7**) was obtained from the first fraction as a colorless oil (122 mg), $[\alpha]_D^{21}$ −18.1° (c=1.88, MeOH), PMR ($CDCl_3$) δ: 0.90 (3H, d, >CH–C$\underline{H}_3$), 3.66 (3H, s, –O–C$\underline{H}_3$), 4.50 (1H, d, J=7 Hz, C_1-anomer $\underline{H}$ of glucose). Mass spectrum *m/e*: 417 (M^+−$COOCH_3$), 331, 243, 242, 200, 169, 157, 145, 140, 129 (base peak), 115, 109, 98, 97. **7** was treated with NH_3 in MeOH on a water bath for 5 min. After standing for 1 hr at room temperature the reaction mixture was concentrated *in vacuo*. The residue was washed with AcOEt and then hydrolyzed with almond emulsin to give glucose (PPC).

5β-Pregnane-3β,16β,20α-triol triacetate was obtained from the second fraction as a white powder (200 mg). A half of the triacetate was boiled with 1.8 N H_2SO_4 in 50% EtOH for 4 hr and the reaction mixture was neutralized with aqueous 4% KOH. After removing EtOH by evaporation *in vacuo* the neutral aqueous solution was diluted with water to form a precipitate, which was purified by chromatography on silica gel with $CHCl_3$–MeOH (2: 1). **8** was crystallized from aqueous MeOH to give colorless needles, 30 mg, mp 224—228°, $[\alpha]_D^{22}$ +45.4° (c=1.1, pyridine), *Anal.* Calcd. for $C_{21}H_{36}O_3$: C, 74.95; H, 10.78. Found: C, 74.33; H, 11.02. **8** was identified by direct comparison with an authentic sample synthesized from sarsasapogenin by the same procedure.

Methylation of 3 by Hakomori's Method——According to Hakomori's method, NaH (250 mg) was warmed with dimethylsulfoxide (DMSO, 15 ml) at 70° for 45 min with stirring under an N_2 flow, then a solution of **3** (200 mg) in DMSO (5 ml) was added and the mixture was stirred for 1 hr under an N_2 flow. CH_3I (3 ml) was added to the solution and the reaction mixture was allowed to stand at room temperature for 3 hr with stirring. After dilution with water, the reaction mixture was extracted with ether and the organic layer was washed with water, dried and evaporated to dryness. The residue was chromatographed on Sephadex LH-20, eluting with MeOH, to afford a homogeneous per-*O*-methylate (**9**, 90 mg), obtained as a white powder from MeOH, mp 70—71°, $[\alpha]_D^{22}$ −37° (c=1.0, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH and spiroketal (nil), PMR ($CDCl_3$) δ: 0.81 (3H, s, C$\underline{H}_3$), 0.98 (3H, s, C$\underline{H}_3$), 1.27 (3H, d, J=7 Hz, C$\underline{H}_3$), 3.39 (3H, s, OC$\underline{H}_3$), 3.48—3.59 (3H×12, OC$\underline{H}_3$), 4.30 (H×3, d, J=7 Hz, anomer $\underline{H}$), 4.88 (1H, s, anomer $\underline{H}$), *Anal.* Calcd. for $C_{63}H_{110}O_{22}$: C, 62.04; H, 9.09. Found: C, 62.55; H, 9.01.

Methanolysis of 9——A solution of **9** (200 mg) in methanolic 2 N HCl (5 ml) was refluxed for 2 hr. After cooling, the precipitate was filtered and crystallized from MeOH to afford colorless needles identical with an authentic sample of sarsasapogenin. The filtrate was neutralized with Ag_2CO_3 and the neutral solution was concentrated to dryness. The residue was examined by TLC (solv. e) and GLC (column: 5% NPGS glass column on Chromosorb W, 3 mm×2 m, column temp.: 155°, carrier gas: N_2 1.0 kg/cm^2). *Rf* 0.6, 0.69; t_R(min): 1.9, 2.8 (methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside), *Rf* 0.55, 0.63; t_R(min): 1.9, 2.3 (methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside), *Rf* 0.56, 0.67; t_R(min): 4.1, 5.8 (methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside), *Rf* 0.09, 0.05; t_R(min): 3.5, 4.8 (methyl 2,3-di-*O*-methyl-D-glucopyranoside).

Partial Hydrolysis of 6 with 0.1 N H_2SO_4——The prosapogenin **6** (500 mg) was refluxed with 0.1 N H_2SO_4 in 50% dioxane for 10 hr. After cooling, the reaction mixture was diluted with water and the precipitate was collected by filtration, dried and chromatographed on silica gel using solv. c to afford three kinds of prosapogenins (**10** (100 mg), **11** (30 mg), **12** (200 mg)).

10: Colorless needles from MeOH, mp 239—240° (dec.), $[\alpha]_D^{21}$ −53.3° (c=1.0, MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3300—3500 (OH), 985, 910, 895, 849 (intensity, 910>895 25S spiroketal), *Anal.* Calcd. for $C_{33}H_{54}O_8 \cdot H_2O$: C, 66.41; H, 9.46. Found: C, 66.78; H, 9.26.

11: Colorless needles from aqueous MeOH, mp 246—247.5° (dec.), $[\alpha]_D^{17}$ −53.8° (c=1.0, $CHCl_3$–MeOH (1: 1)), IR ν_{max}^{Nujol} cm^{-1}: 3300—3500 (OH), 986, 919, 895, 850 (intensity, 919>895 25S spiroketal), *Anal.* Calcd. for $C_{38}H_{62}O_{12} \cdot H_2O$: C, 62.61; H, 8.85. Found: C, 62.31; H, 8.50.

12: Colorless needles from aqueous MeOH, mp 255—256° (dec.), $[\alpha]_D^{16}$ −59.8° (c=1.0, MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3300—3500 (OH), 985, 920, 895, 850 (intensity, 920>895 25S spiroketal), *Anal.* Calcd. for $C_{39}H_{64}O_{12} \cdot H_2O$: C, 63.05; H, 8.95. Found: C, 62.98; H, 8.68.

Methylation of 10, 11 and 12——Each prosapogenin, **10**, **11** and **12**, was methylated by Hakomori's method and the products (**13**, **14** and **15**) were purified by crystallization.

13: Colorless needles from MeOH, mp 140.5—141.5°, $[\alpha]_D^{19}$ −70.0° (c=1.0, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 985, 920, 895, 850 (intensity 920>895, 25S spiroketal), PMR ($CDCl_3$) δ: 0.75 (3H, s, C$\underline{H}_3$), 0.95 (3H, s, C$\underline{H}_3$), 3.38, 3.51, 3.59, 3.62 (each s, 4×OC$\underline{H}_3$), 4.26 (1H, d, J=7 Hz, anomer $\underline{H}$), *Anal.* Calcd. for $C_{37}H_{62}O_8$: C, 69.99; H, 9.84. Found: C, 69.49; H, 10.23.

14: Colorless needles from MeOH, mp 178—180°, $[\alpha]_D^{20}$ −54.7° (c=0.8, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 985, 919, 890, 850 (intensity 919>890, 25S spiroketal), PMR ($CDCl_3$) δ: 0.76 (3H, s, CH_3), 0.96 (3H, s, C$\underline{H}_3$), 3.38—3.60 (each s, 6×OC$\underline{H}_3$), 4.30 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.31 (1H, d, J=6 Hz, anomer $\underline{H}$), *Anal.* Calcd. for $C_{44}H_{74}O_{12}$: C, 66.47; H, 9.38. Found: C, 66.42; H, 9.75.

15: A white powder from aqueous MeOH, mp 83—84°, $[\alpha]_D^{20}$ −62.5° (c=1.0, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 985, 920, 897, 850 (intensity, 920>897, 25S spiroketal), PMR ($CDCl_3$) δ: 0.77 (3H, s, C$\underline{H}_3$), 0.97 (3H, s, C$\underline{H}_3$), 1.28 (3H, d, J=6 Hz, C$\underline{H}_3$), 3.48, 3.50, 3.52, 3.54, 3.60, 3.63 (each s, 6×OC$\underline{H}_3$), 4.27 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.84 (1H, s, anomer $\underline{H}$), *Anal.* Calcd. for $C_{45}H_{77}O_{12}$: C, 66.72; H, 9.58. Found: C, 66.68; H, 9.54.

Methanolyses of 13, 14 and 15——The per-*O*-methyl derivatives **13**, **14** and **15** were methanolyzed by the method described above. The residue was examined by GLC (column: 5% NPGS glass column on Chromosorb W, 3 mm×2 m, column temp.: 155° (for **13**, **14**), 165° (for **15**), carrier gas: N_2 1.2 kg/cm² for **13**, **14** and 1.0 kg/cm² for **15**: t_R(min) **13**: 4.1, 5.8 (methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside; **14**: 1.9, 2.3 (methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside), 12.6, 17.3 (methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside); **15**: 1.5, 2.1 (methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside), 6.9, 9.6 (methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside).

Enzymatic Hydrolysis of 1, 2 and 2′——Solutions of **1** (50 mg), **2** (100 mg) or **2′** (100 mg) in H_2O (15 ml) were each incubated with almond emulsion (30 mg) at 37° for 24 hr. The solution was extracted with BuOH and the organic solution was concentrated *in vacuo*. The prosapogenin of **1** was recrystallized from MeOH to afford colorless needles (**11**, 20 mg) and those of **2** and **2′** were recrystallized from aqueous MeOH to give colorless needles (**12**, 40 mg). These prosapogenins were found to be identical with authentic samples by mixed fusion and TLC and by comparing the IR and PMR spectra.

Methylation of 2 and 4 by Hakomori's Method——Asp-V (**2**, 180 mg) and VII (**4**, 200 mg) were methylated by Hakomori's method as described above to afford the per-*O*-methylates, **16** (100 mg) and **18** (150 mg), respectively.

16: A white powder from aqueous MeOH, mp 62.5—64°, $[\alpha]_D^{25}$ −54.6° (c=1.1, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH and spiroketal (nil), PMR ($CDCl_3$) δ: 0.89 (3H, s, C$\underline{H}_3$), 0.96 (3H, s, C$\underline{H}_3$), 1.27 (3H, d, J=6 Hz, C$\underline{H}_3$), 3.15 (3H, s, OC$\underline{H}_3$), 3.40—3.60 (each s, 10×OC$\underline{H}_3$), 4.20 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.27 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.86 (1H, s, anomer $\underline{H}$), *Anal.* Calcd. for $C_{59}H_{98}O_{18}$: C, 63.49; H, 9.33. Found: C, 63.71; H, 8.95.

18: A white powder from MeOH, mp 73—74°, $[\alpha]_D^{27}$ −43.2° (c=1.0, MeOH), IR ν_{max}^{Nujol} cm^{-1}: OH and spiroketal (nil), PMR ($CDCl_3$) δ: 0.79 (3H, s, C$\underline{H}_3$), 0.99 (3H, s, C$\underline{H}_3$), 1.27 (3H, d, J=6 Hz, C$\underline{H}_3$), 3.14 (3H, s, OC$\underline{H}_3$), 3.38—3.65 (each s, 15×OC$\underline{H}_3$), 4.19 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.25 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.33 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.70 (1H, d, J=7 Hz, anomer $\underline{H}$), 4.87 (1H, s, anomer $\underline{H}$), *Anal.* Calcd. for $C_{72}H_{126}O_{27}\cdot H_2O$: C, 59.98; H, 8.95. Found: C, 59.82; H, 8.99.

Methanolysis of the Per-*O*-methylates 16 and 18——The per-*O*-methylates, **16** and **18**, were methanolyzed with methanolic 2 N HCl under reflux for 2 hr and the reaction mixtures were worked up as described for **9**. The *O*-methylated sugars were examined by TLC (solv. e) and GLC (column: 5% NPGS glass column on Chromosorb W, 3 mm×2 m, column temp.: 155°, carrier gas: N_2 1.0 kg/cm² for **16**, and 1.2 kg/cm² for **18**). **16**: *Rf* 0.60, 0.69; t_R(min) 1.9, 2.8 (methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside); *Rf* 0.56, 0.67; t_R(min) 4.1, 5.8 (methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside); *Rf* 0.27, 0.30; t_R(min) 9.8, 10.4 (methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside). **18**: *Rf* 0.55, 0.63; t_R(min) 3.2, 3.9 (methyl 2,3,4-*tri*-*O*-methyl-D-xylopyranoside), *Rf* 0.60, 0.69; t_R(min) 3.2, 4.5 (methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside), *Rf* 0.56, 0.67; t_R(min) 6.8, 9.4 (methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside), *Rf* 0.02; t_R(min) 8.1 (TMS derivative of methyl 3-*O*-methyl-D-glucopyranoside).

Enzymatic Hydrolysis of 4 with Almond Emulsin and Crude Hesperidinase——a) A solution of **4** (700 mg) in H_2O (25 ml) was incubated with almond emulsin at 37° for 24 hr, then the reaction mixture was extracted with BuOH. The BuOH solution was washed with H_2O and concentrated *in vacuo*. The extractive was diluted with MeOH to afford a precipitate, which was collected by filtration. After drying, the precipitate was recrystallized from MeOH to afford colorless needles (**17**, 130 mg), mp 261—265° (dec.), $[\alpha]_D^{27}$ −45.1° (c=0.95, pyridine), IR ν_{max}^{Nujol} cm^{-1}: 3300—3500 (OH), 985, 920, 895, 850 (intensity 920>895, 25S spiroketal), PMR (pyridine-d_5) δ: 0.83, 0.98 (3H, each s, 2×C$\underline{H}_3$), 1.62 (3H, d, J=5 Hz, C$\underline{H}_3$), 4.79 (2H, d, J=6 Hz, 2×anomer $\underline{H}$), 5.32 (1H, d, J=7 Hz, anomer $\underline{H}$), 5.47 (1H, s, anomer $\underline{H}$), *Anal.* Calcd. for $C_{59}H_{82}O_{21}\cdot 2H_2O$: C, 56.90; H, 8.22. Found: C, 56.72; H, 8.09.

b) A solution of **4** (100 mg) in $NaHPO_4$–citric acid buffer (pH 4.0, 20 ml) was incubated with crude hesperidinase (20 mg) at 32° for 24 hr. The reaction mixture was extracted with BuOH and the extract was concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (10: 1), followed by elution with $CHCl_3$–MeOH–H_2O (7: 3: 0.2) to give **6** (10 mg), **10** (10 mg) and **4** (30 mg). These prosapogenins were found to be identical with authentic samples by mixed fusion and TLC, and by comparison of the IR spectra.

Extraction of Asparagi Radix with Pyridine and Isolation of the Furostanol Oligosides——Asparagi radix (300 g) was extracted with pyridine (150 ml) at room temperature for one week with occasional testing by TLC. The pyridine and methanol extracts were spotted on the same TLC plate, which was developed with $CHCl_3$–MeOH–H_2O (7: 3: 0.5) or BuOH–AcOEt–H_2O (4: 1: 5, upper phase). After spraying with Ehrlich reagent, the plate was heated to visualize the spots of **1**, **2**, **3**, **4**, **1′**, **2′**, **3′** and **4′** in the methanol extract and those of **1′**, **2′**, **3′** and **4′** in the pyridine extract. The oligoside fraction in the pyridine extract was isolated by preparative TLC with solvent b and the oligoside mixture was examined by PMR spectroscopy in pyridine-d_5; no methoxyl signal was seen for **1**, **2**, **3** or **4**.

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