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Entada Saponins (ES) II and IV from the Bark of *Entada phaseoloides*

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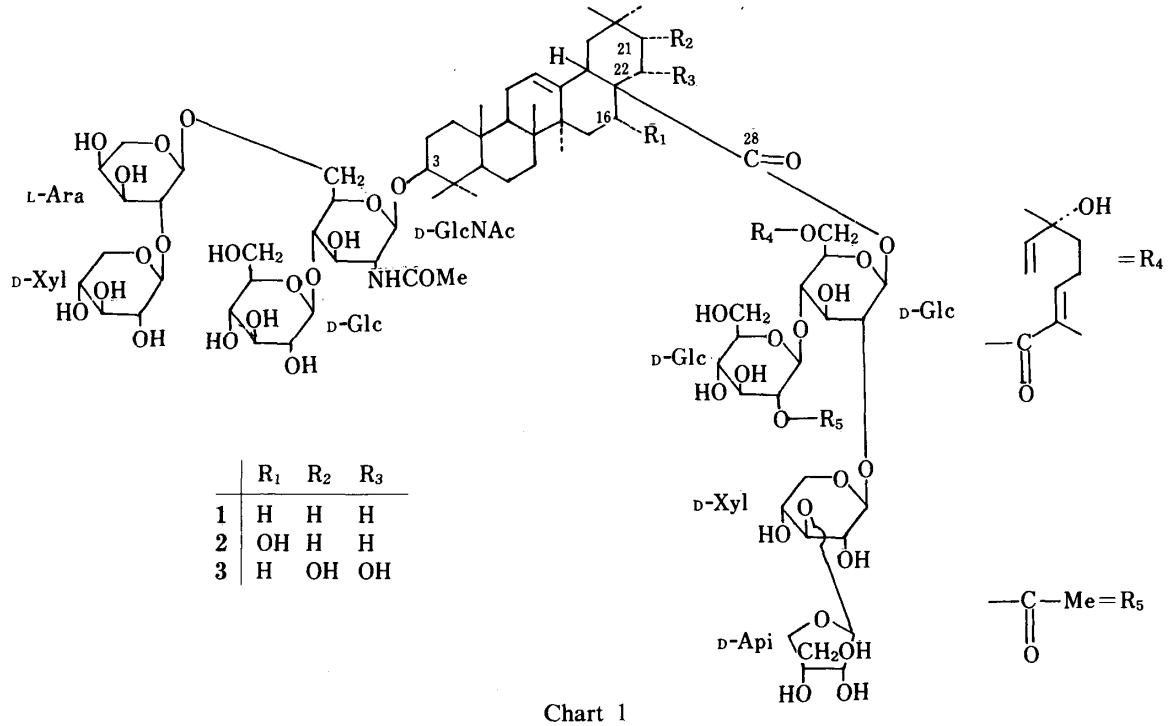
The structures of entada saponins II and IV, isolated from the bark of *Entada phaseoloides* (L.) MERRILL (Leguminosae), were elucidated as 3-*O*-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl-(1→6)][β-D-glucopyranosyl(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-28-*O*-[β-D-apiofuranosyl(1→3)-β-D-xylopyranosyl(1→2)][(2-*O*-acetyl)-β-D-glucopyranosyl(1→4)]-6-*O*-((6*R*)-6-hydroxy-2,6-dimethyl-(2*E*)-2,7-octadienoyl)-β-D-glucopyranosyl oleanolic acid (**1**) and entagenic acid (**3**).

Keywords——*Entada phaseoloides*; Leguminosae; triterpene saponin; ^{13}C-NMR chemical shift; entada saponin II; entada saponin IV

In the previous paper,[2)] we reported the isolation of three triterpenoid saponins, entada saponin II (ES-II) (**1**), ES-III (**2**) and ES-IV (**3**) from the bark of *Entada phaseoloides* (L.) MERRILL. (Leguminosae). The structure of the main saponin, **2**, was established to be 3-*O*-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)][β-D-glucopyranosyl(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-28-*O*-[β-D-apiofuranosyl(1→3)-β-D-xylopyranosyl(1→2)][(2-*O*-acetyl)-β-D-glucopyranosyl(1→4)]-6-*O*-((6*R*)-6-hydroxy-2,6-dimethyl-(2*E*)-2,7-octodienoyl)-β-D-glucopyranosyl echinocystic acid by ^{1}H- and ^{13}C-nuclear mag-



	R_1	R_2	R_3
1	H	H	H
2	OH	H	H
3	H	OH	OH

Chart 1

netic resonance (^{1}H- and ^{13}C-NMR) spectrometry and methylation analysis. In this paper, we elucidate the structures of **1** and **3**.

From the methanolic extracts of the bark of *E. phaseoloides*, the pure saponins, **1** and **3**, were isolated by high performance liquid chromatography (HPLC) using an Aquasil[3] and an ODS column. On refluxing with 2 N sulfuric acid in ethanol, **1** and **3** afforded the sapogenins **4** and **5**, respectively. The sapogenins **4** and **5** were identified, as oleanolic acid[4] and entagenic acid[4] respectively, from their physical data.

ES-II (**1**), a white powder, mp 214—216 °C (dec.), $[\alpha]_D^{25}$ $-15.2°$ (methanol), and ES-IV (**3**), a white powder, mp 222—225 °C (dec.), $[\alpha]_D^{25}$ $-22.7°$ (methanol), were presumed to be bisdesmosidic saponins, based on their infrared (IR) and ^{13}C-NMR spectra, which were very similar to those of **2**. Alkaline hydrolysis of **1** and **3** with 20% potassium hydroxide in ethanol afforded the prosapogenins, **6** and **7**, respectively.

Compound **6**, a white powder, $C_{54}H_{87}NO_{21}$, mp 232—235 °C (dec.), $[\alpha]_D^{25}$ $+3.8°$, gave oleanolic acid (**4**) on hydrolysis with 2 N hydrochloric acid.

Compound **7**, a white powder, $C_{54}H_{87}NO_{23}$, mp 235.5—238 °C (dec.), $[\alpha]_D^{25}$ $-8.2°$, gave entagenic acid (**5**) on HCl hydrolysis.

The elementary analysis of **6** also showed the presence of one N-atom, and the ^{1}H-NMR spectrum of **6** revealed a signal at δ 9.03 ppm, ascribable to the proton of an NH group, while the IR spectrum showed absorptions at 1645 and 1550 cm^{-1} ascribable to a CONH group. The ^{13}C-NMR spectrum of **6** showed the characteristic signal at δ 58.3 ppm due to the C-2 carbon of *N*-acetyl-β-D-glucosamine, at δ 23.7 ppm due to a methyl group and at δ 170.2 ppm

TABLE I. Prosapogenins Produced from Entada Saponins with Alkali and the Products by Their Stepwise Acid Hydrolysis

Saponin		Prosapogenin		Products	No.
ES-II (**1**)	$\xrightarrow{OH^-}$	Oleanolic acid —3— Glc$\frac{1\ 4}{}$GlcN (6—1) Ara (2—1) Xyl (**6**)	$\xrightarrow{H^+}$	Oleanolic acid	(**4**)
				4$\frac{3}{}$GlcN	(**8**)
				4$\frac{3}{}$GlcN$\frac{4\ 1}{}$Glc	(**9**)
				4$\frac{3}{}$GlcN$\frac{4\ 1}{}$Glc (6—1) Ara	(**10**)
ES-III (**2**)	$\xrightarrow{OH^-}$	Echinocystic acid —3— Glc$\frac{1\ 4}{}$GlcN (6—1) Ara (2—1) Xyl (**15**)	$\xrightarrow{H^+}$	Echinocystic acid	(**14**)
				14$\frac{3}{}$GlcN	(**19**)
				14$\frac{3}{}$GlcN$\frac{4\ 1}{}$Glc	(**20**)
				14$\frac{3}{}$GlcN$\frac{4\ 1}{}$Glc (6—1) Ara	(**21**)
ES-IV (**3**)	$\xrightarrow{OH^-}$	Entagenic acid —3— Glc$\frac{1\ 4}{}$GlcN (6—1) Ara (2—1) Xyl (**7**)	$\xrightarrow{H^+}$	Entagenic acid	(**5**)
				5$\frac{3}{}$GlcN	(**11**)
				5$\frac{3}{}$GlcN$\frac{4\ 1}{}$Glc	(**12**)
				5$\frac{3}{}$GlcN$\frac{4\ 1}{}$Glc (6—1) Ara	(**13**)

due to a carbonyl carbon. Accordingly, the amino sugar in **6** is also *N*-acetyl *β*-glucosamine as contained in **2**. The field desorption-mass spectrum (FD-MS) and fast atom bombardment (FAB)-MS gave the $[M+H]^+$ ion at *m/z* 1086, and $[M+Na]^+$ ion at *m/z* 1108. From these results, the molecular weight (M_r 1085) and the molecular formula, $C_{54}H_{87}NO_{21}$, of **6** were confirmed.

Compound **7** also gave similar results to those of **6** in its elementary analysis, and IR, ^{1}H-NMR and ^{13}C-NMR spectral analyses. The FAB-MS of **7** gave the $[M+H]^+$ ion at *m/z* 1118, and $[M+Na]^+$ ion at *m/z* 1140 with NaCl, indicating the molecular formula $C_{54}H_{87}NO_{23}$ (M_r 1117) for **7**.

The partial hydrolysis of **6** with 0.5 N sulfuric acid afforded **8**, **9** and **10** together with oleanolic acid (**4**) and unchanged prosapogenin (**6**), while under the same conditions, **7** gave **11**, **12** and **13**, together with entagenic acid (**5**) and unchanged prosapogenin (**7**) in a small amount. The products of acid hydrolysis of prosapogenins are summarized in Table I. These results suggested that the sugar components of the prosapogenin and their sequence might be identical with those of **15** obtained from ES-III (**2**) under the same reaction conditions. This was confirmed by the following reactions. Compounds **6**, **7** and **15** were methylated by Hakomori's method[5] to afford the permethylates **16**, **17** and **18**, respectively, which were subjected to methanolysis with 5% hydrochloric acid in dried methanol to yield methyl 2,3,4-tri-*O*-methyl-*β*-D-xylopyranoside, methyl 2,3,4,6-tetra-*O*-methyl-*β*-D-glucopyranoside and methyl 3,4-di-*O*-methyl-*α*-L-arabinopyranoside, which were identified by means of gas-liquid chromatography (GLC).

All the ^{13}C-NMR chemical shifts due to sugar moieties of each prosapogenin **6**, **7** and **15** gave almost the same values, as shown in Table II, indicating that the sequences of sugar

TABLE II. ^{13}C-NMR Chemical Shift Values (ppm) of Sugar Moieties of **6**, **15** and **7**

		6	**15**	**7**
GlcNAc	1	104.2	104.3	104.2
	2	58.3	58.1	58.4
	3	73.2	73.3	73.4
	4	82.3	82.7	81.7
	5	75.1	75.2[a)]	75.2[a)]
	6	68.3	68.3	68.4
$\underline{C}H_3CONH$		23.7	23.8	23.6
$CH_3\underline{C}ONH$		170.2	170.2	170.2
Glc	1	104.6	104.6	104.6
	2	75.1	75.0[a)]	75.1[a)]
	3	78.1	78.2[b)]	78.0[b)]
	4	72.0	71.9	72.2
	5	78.6	78.6	78.6
	6	62.8	62.7	63.0
Ara	1	103.3	103.4	103.2
	2	81.5	81.4	81.6
	3	73.6	73.7	73.4
	4	68.7	68.8	68.7
	5	65.9	66.1	65.5
Xyl	1	107.1	107.4	106.8
	2	76.0	76.2	75.8
	3	78.1	78.1[b)]	78.0[b)]
	4	71.0	71.0	71.1
	5	67.3	67.4	67.3

a, b) The assignments might have to be reversed in each column.

Table III. ^{13}C-NMR Chemical Shift Values (ppm) of Anomeric Carbon Atoms of the Sugar Moieties of **1**, **2** and **3**

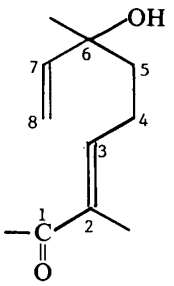
1	2	3
93.3	93.2	93.3
102.5	102.4	102.4
103.3	103.1	103.1
104.2	104.0	104.1
104.7	104.5	104.5
105.3	105.2	105.2
106.9	106.8	106.8
111.6	111.4	111.4

Table IV. ^{13}C-NMR Chemical Shift Values (ppm) of Acetyl and Monoterpene Moieties of **1**, **2** and **3**

1	2	3	
12.7	12.6	12.6	C-2-CH_3
20.8	20.7	20.7	$\underline{C}H_3COO$
24.3	24.2	24.2	C-4
28.5	28.4	28.4	C-6-CH_3
41.7	41.6	41.6	C-5
72.4	72.4	72.4	C-6
111.8	111.7	111.7	C-8
128.0	127.9	127.9	C-2
144.1	144.0	144.0	C-3
146.8	146.6	146.6	C-7
167.5	167.4	167.6	C-1
170.3	170.3	170.2	$CH_3\underline{C}OO$

Table V. ^{13}C-NMR Chemical Shifts (δ Values) of C-28-O–Sugar Moieties of **1**, **2** and **3**

1	2	3
62.7	62.6	62.6
63.5	63.4	63.4
66.1	65.9	65.9
66.9	66.7	66.9
69.9	69.7	69.6
72.4	72.2	72.3
73.8	73.7	73.6
75.1	74.9	74.8
75.2	75.0	75.0
75.6	75.4	75.4
76.5	76.3	76.4
76.7	76.5	76.5
78.1	77.9	77.9
78.6	78.5	78.5
78.9	78.9	79.0
80.3	80.2	80.2
81.2	81.0	80.9
85.4	85.2	85.3
93.3	93.2	93.3
102.5	102.4	102.4
105.3	105.2	105.2
111.6	111.4	111.4



linkages of **6** and **7** are identical with that of **15**.

The presence of a monoterpenyl moiety (**22**) and an acetyl group in ES-III was established as reported in the previous paper.[2] The ^{1}H-NMR and ^{13}C-NMR spectra of **1** and **3** also suggested the presence of these two acyl groups in their molecules.

Compounds **1** and **3** were hydrolyzed with 1% potassium hydroxide in dioxane to give two kinds of oily substances, which were identified by thin layer chromatography (TLC) as the monoterpene, 2,6-dimethyl-6-hydroxy-2-*trans*-2,7-octadienoic acid (**22**), and its transformed compound, 2-(1-carboxyethyl)-5-methyl-5-vinyltetrahydrofuran (**23**). On the other hand, the sugar components attached to the C-28 carboxyl group of the sapogenins, **4** and **5**, were examined by TLC and GLC to confirm the presence of glucose (2 mol), xylose and apiose, which are identical with those of ES-III (**2**) attached at the same position. Furthermore, the ^{13}C-NMR signals showed almost the same values (Table V). This spectral evidence showed

that sugar moieties attached to the C-28 carbonyl group of the sapogenins, **4**, **14** and **5**, have the same sequences. Therefore, **1**, **2** and **3** possess the same sugar moieties involving an acetyl group and the same monoterpenyl group.

Consequently the structures of **1** and **3** were established to be 3-*O*-[*β*-D-xylopyranosyl-(1→2)-*α*-L-arabinopyranosyl(1→6)][*β*-D-glucopyranosyl(1→4)]-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-28-*O*-[*β*-D-apiofuranosyl(1→3)-*β*-D-xylopyranosyl(1→2)][(2-*O*-acetyl)-*β*-D-glucopyranosyl(1→4)]-6-*O*-((6*R*)-6-hydroxy-2,6-dimethyl-(2*E*)-2,7-octadienoyl)-*β*-D-glucopyranosyl oleanolic acid, and 3-*O*-[*β*-D-xylopyranosyl(1→2)-*α*-L-arabinopyranosyl(1→6)][*β*-D-glucopyranosyl(1→4)]-2-acetamido-2-deoxyl-*β*-D-glucopyranosyl-28-*O*-[*β*-D-apiofuranosyl(1→3)-*β*-D-xylopyranosyl(1→2)][(2-*O*-acetyl)-*β*-D-glucopyranosyl(1→4)]-6-*O*-((6*R*)-6-hydroxy-2,6-dimethyl-(2*E*)-2,7-octadienoyl)-*β*-D-glucopyranosyl entagenic acid, respectively.

Experimental

Melting points were determined in a Yanagimoto melting point apparatus or by using a silicone bath, and are uncorrected. IR spectra were measured with a JASCO DS-701G. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were measured with a JEOL GX-400 in C_5D_5N at 80 °C, and recorded in δ values with TMS as an internal reference. Optical rotations were measured with a JASCO J-20. Electron impact (EI)-MS were measured at 70 eV on a JEOL D-300 combined gas chromatograph-mass spectrometer (GC-MS). FAB and FD-MS were measured with a JEOL DX-300/FAB-FD apparatus with a xenon atom beam. In HPLC, detection was done with a refractive index (RI) detector.

ES-II (1)——White powder, mp 214—216 °C (dec.). $[\alpha]_D^{25}$ −15.2° (c=0.66, MeOH). *Anal.* Calcd for $C_{88}H_{139}NO_{42}\cdot 5H_2O$: C, 53.08; H, 7.64; N, 0.70. Found: C, 52.77; H, 7.54; N, 0.70. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (COOR), 1640, 1550 (CONH). ^{1}H-NMR (pyridine-d_5) δ: 0.77, 0.87, 0.91, 0.93, 0.95, 1.26 (21H, s, 7×CH_3), 1.47 (3H, s, $C_{(6)}CH_3$ of **22**), 1.95 (3H, s, $C_{(2)}CH_3$ of **22**), 2.03 (3H, s, CH_3COO), 2.16 (3H, s, CH_3CONH), 9.20 (1H, NH). FAB-MS m/z: 1920 (M+K) with KI, 1904 (M+Na) with NaCl.

ES-IV (3)——White powder, mp 222—225 °C (dec.). $[\alpha]_D^{25}$ −22.7° (c=7.0, MeOH). *Anal.* Calcd for $C_{88}H_{139}NO_{44}\cdot 6H_2O$: C, 52.24; H, 7.52; N, 0.69. Found: C, 52.17; H, 7.47; N, 0.66. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (COOR), 1640, 1550 (CONH). ^{1}H-NMR (pyridine-d_5) δ: 0.92, 0.97, 1.07, 1.12, 1.14, 1.17 (21H, s, 7×CH_3), 1.42 (3H, s, $C_{(6)}CH_3$ of **22**), 1.95 (3H, s, $C_{(2)}CH_3$ of **22**), 2.01 (3H, s, CH_3COO), 2.06 (3H, s, CH_3CONH), 8.25 (1H, d, NH). FAB-MS m/z: 1952 (M+K) with KI, 1936 (M+Na) with NaCl.

Isolation of Oleanolic Acid (4) and Entagenic Acid (5)——A solution of **1** (300 mg) in EtOH (20 ml) was treated with 2 N H_2SO_4 (20 ml), and the mixture was refluxed for 4 h. The solution was concentrated to 20 ml. The precipitate was collected, washed and dried. The solid was chromatographed over silica gel with $CHCl_3$–MeOH (100:1) to afford a powder, which was recrystallized from EtOH to give oleanolic acid (**4**) (41 mg). mp 308—310 °C. $[\alpha]_D^{25}$ +80° (c=0.4, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{48}O_3\cdot 0.5H_2O$: C, 77.37; H, 10.61. Found: C, 77.83; H, 10.83. IR ν_{max}^{KBr} cm^{-1}: 3423 (OH), 1694 (COOH). ^{1}H-NMR (pyridine-d_5+$CDCl_3$) δ: 0.88—1.00 (totally 15H, 5×CH_3), 1.15 (3H, s, CH_3), 1.22 (3H, s, CH_3), 5.37 (1H, t-like). MS m/z: 456 (M^+), 438 (M^+-H_2O), 423 ($M^+-H_2O-CH_3$), 248 ($C_{16}H_{24}O_2^+$), 203 (248−COOH). High MS: M_r. Calcd for $C_{30}H_{48}O_3$: 456.3603. obs. 456.3602.

On similar treatment, **3** gave a solid. This solid was chromatographed over silica gel using $CHCl_3$–MeOH (100:3) as the solvent to afford a colorless solid, which was recrystallized from EtOH to give entagenic acid (**5**) (60 mg). mp 313—315 °C. $[\alpha]_D^{25}$ +32.5° (c=0.8, EtOH). *Anal.* Calcd for $C_{30}H_{48}O_5\cdot 0.5H_2O$: C, 72.39; H, 9.92. Found: C, 71.93; H, 10.15. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1694 (COOH). ^{1}H-NMR (pyridine-d_5+$CDCl_3$) δ: 0.84—1.08 (totally 18H, 6×CH_3), 1.45 (3H, s, CH_3), 4.18 (1H, d, J=4 Hz), 4.62 (1H, d, J=4 Hz), 5.50 (1H, t-like). MS m/z: 488 (M^+), 470 (M^+-H_2O), 280 ($C_{16}H_{24}O_4^+$), 235 (280−COOH). High MS: M_r. Calcd for $C_{30}H_{48}O_5$: 488.3502. obs. 488.3502.

Hydrolysis of ES-II (1) and IV (3) with 20% KOH——A solution of **1** (600 mg) in 20% KOH (30 ml) and EtOH (30 ml) was refluxed for 2 h. The reaction mixture was cooled to room temperature, neutralized with 10% HCl and concentrated to 30 ml, then frozen and dried. The MeOH-soluble fraction of the product was chromatographed on a column of Sephadex LH-20 with MeOH to afford a crude prosapogenin, which was further purified by HPLC on an Aquasil column (30 cm×20 mm i.d.) using $CHCl_3$–MeOH–H_2O (30:20:4) as the solvent to give a colorless powder **6** (142 mg). mp 232—235 °C (dec.). $[\alpha]_D^{25}$ +3.8° (c=1.6, MeOH). *Anal.* Calcd for $C_{54}H_{87}NO_{21}\cdot 3H_2O$: C, 56.87; H, 8.22; N, 1.23. Found: C, 56.82; H, 8.25; N, 1.21. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1693 (COOH), 1640, 1555 (CONH). ^{1}H-NMR (pyridine-d_5) δ: 0.76, 0.95, 0.98, 1.02, 1.21, 1.32 (21H, s, 7×CH_3), 2.18 (3H, s, CH_3CONH), 4.84 (1H, d, J=7.3 Hz), 5.12 (1H, d, J=8.2 Hz), 5.26 (1H, d, J=7.0 Hz), 5.52 (1H, d, J=7.9 Hz), 9.03 (1H, d, J=8.5 Hz, NH). FAB-MS m/z: 1086 (M+H), 1108 (M+Na).

Similarly, **3** (600 mg) gave a colorless powder (**7**) (162 mg). mp 235.5—238 °C (dec.). $[\alpha]_D^{25}$ −8.2° (c=0.7, MeOH). *Anal.* Calcd for $C_{54}H_{87}NO_{23}\cdot 5.5H_2O$: C, 53.28; H, 8.11; N, 1.15. Found: C, 53.23; H, 7.91; N, 1.21. IR

ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (COOH), 1645, 1555 (CONH). ^{1}H-NMR (pyridine-d_5) δ: 0.87, 0.92, 1.03, 1.09, 1.15, 1.75 (21H, s, 7 × CH_3), 2.08 (3H, s, CH_3CONH), 8.29 (1H, NH). FAB-MS *m/z*: 1118 (M+H), 1140 (M+Na) with NaCl.

Hydrolysis of 6 and 7 with 2 N HCl——A solution of **6** (20 mg) in 2 N HCl (5 ml) was allowed to stand on a water bath at 100 °C for 4 h, and the precipitates formed were collected by filtration, washed with water and dried to give a solid. The solid was recrystallized from EtOH to give oleanolic acid (**4**). After neutralization, the filtrate was examined by HP-TLC and/or GLC. Glucosamine hydrochloride, glucose, arabinose and xylose were detected.

Similarly, **7** (20 mg) gave entagenic acid (**5**) as the sapogenin and glucosamine hydrochloride, glucose, arabinose and xylose.

Hydrolysis of 6 and 7 with 0.5 N H_2SO_4——A solution of **6** (100 mg) in 0.5 N H_2SO_4 (10 ml)–EtOH (10 ml) was refluxed for 2 h. The reaction mixture was cooled at room temperature, neutralized with 5% NaOH and concentrated to 10 ml under reduced pressure, then frozen and dried. The MeOH-soluble fraction of the product was chromatographed on a column of Sephadex LH-20 with MeOH to afford the prosapogenin fraction, which was further separated by HPLC on an Aquasil column (30 cm × 20 mm i.d.) using $CHCl_3$–MeOH–H_2O (10 : 6 : 1) as the solvent to give three colorless, amorphous substances, **8**, **9** and **10**, together with the sapogenin **4** and the unchanged prosapogenin **6**. These compounds gave sugars as shown in Table I on hydrolysis with 2 N HCl.

Similarly, **7** gave compounds **11**, **12** and **13**, together with the sapogenin **5** and the unchanged prosapogenin **7**. The hydrolysates are summarized in Table I.

Methylation of 6, 7 and 15——According to Hakomori's method, NaH (1 g) was stirred with dimethyl sulfoxide (DMSO) (50 ml) at 60 °C for 1 h under N_2 gas stream. A solution of **6**, **7**, or **15** (100 mg each) in DMSO (5 ml) was added to the above reagent (5 ml), and the mixture was stirred for 1 h at room temperature under N_2 gas stream. CH_3I (5 ml) was added, and the reaction mixture was allowed to stand at room temperature for 3 h under stirring. After dilution with water, the mixture was extracted with $CHCl_3$, and the organic layer was washed with water, dried and concentrated to afford a syrup. The residue was chromatographed over silica gel with *n*-hexane–acetone (3 : 1) to afford **16** (82 mg), **17** (69 mg) or **18** (72 mg), respectively.

Compound **16**: $[\alpha]_D^{25}$ −8.6° (*c* = 0.81, $CHCl_3$). Compound **17**: $[\alpha]_D^{25}$ −24.0° (*c* = 0.5, $CHCl_3$). Compound **18**: $[\alpha]_D^{25}$ −30.2° (*c* = 3.2, $CHCl_3$).

Methanolysis of 16, 17 and 18——A solution of **16**, **17** or **18** (5 mg each) in 5% methanolic HCl (1 ml) was refluxed for 2 h in a sealed tube. The reaction mixture was neutralized with Ag_2CO_3 and the filtrate was evaporated *in vacuo*. Each *O*-methylated sugar fragment was identified by GLC comparison with an authentic sample.

GLC: Column, 15% BDS Celite 545 (AW-DMCS) 80—100 mesh, 1 m × 2 mm; N_2 gas, 0.9 kg/cm^2; column temp., 178 °C. t_R: 2′39″, 3′18″ (methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside); 5′34″, 7′48″ (methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside); 11′06″ (methyl 3,4-di-*O*-methyl-L-arabinopyranoside).

Hydrolysis of 1 and 3 with 1% KOH——A solution of **1** (400 mg) in dioxane (50 ml) was treated with 1% KOH (50 ml), and the mixture was stirred at 0 °C for 3 h under N_2 gas. The reaction mixture was acidified with 10% HCl and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water and evaporated to dryness. The formation of **22** and **23** was confirmed by TLC. Similarly, **3** (400 mg) gave **22** and **23**.

TLC (Silica): Solvent, *n*-hexane–acetone (3 : 2); *Rf* 0.38 (compound **22**), 0.52 (compound **23**).

Hydrolysis of 1 and 3 with 2 N HCl——A solution of **1** or **3** (10 mg each) in 2 N HCl (2 ml) was allowed to stand on a water bath at 100 °C for 4 h. The filtrate was repeatedly evaporated at 40 °C until the solution became neutral. The residue was identified by HP-TLC (A) and GLC as a neutral sugar mixture of glucose, arabinose, xylose and apiose, and HP-TLC (B) revealed D-glucosamine hydrochloride.

HP-TLC (Silica) (A): Solvent, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, the lower layer), *n*-BuOH–iso-PrOH–H_2O (5 : 3 : 1), iso-PrOH–*n*-BuOH–H_2O (7 : 1 : 2); color reagent, 10% H_2SO_4, aniline hydrogen phthalate.

GLC: Column, Silicone OV-1 3% on Chromosorb W (DMCS) 60—80 mesh, 3.0 m × 2.0 mm column temp., 168 °C N_2 gas, 1.75 kg/cm^2; samples, trimethylsilyl (TMS) derivatives t_R 5′24″ (apiose), 5′30″, 6′06″ (arabinose), 7′54″, 8′00″ (xylose), 17′54″, 27′48″ (glucose).

HP-TLC (Cellulose) (B): Solvent, EtOAc–C_5H_5N–AcOH–H_2O (5 : 5 : 1 : 3); color reagent, ninhydrin.

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