Chem. Pharm. Bull. 30(3) 810—821 (1981)

Studies on the Constituents of the Root of *Polygala tenuifolia* Willdenow. II.[1] On the Structures of Onjisaponins A, B and E

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(Received July 29, 1981)

The structures of three triterpenoidal saponins, onjisaponins A (**1**), B (**2**) and E (**3**), were determined on the basis of spectral and chemical evidence as presenegenin-(3)-β-D-glucopyranosido-(28)-2-*O*-{[β-D-apio-D-furanosyl(1→3)][β-D-galactopyranosyl(1→4)-β-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl}-3-*O*-(α-L-rhamnopyranosyl)-4-*O*-(4′-methoxycinnamoyl)-β-D-fucopyranoside, presenegenin-(3)-β-D-glucopyranosido-(28)-2-*O*-[β-D-galactopyranosyl(1→4)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl]-3-*O*-(α-L-rhamnopyranosyl)-4-*O*-(4′-methoxycinnamoyl)-β-D-fucopyranoside(=senegin III) and presenegenin-(3)-*O*-β-D-glucopyranosido-(28)-2-*O*-[β-D-galactopyranosyl(1→4)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl]-4-*O*-(3′,4′,5′-trimethoxycinnamoyl)-β-D-fucopyranoside, respectively. The ^{13}C nuclear magnetic resonance spectra of onjisaponins A, B, E and their derivatives were investigated and each carbon signal was assigned as shown in Table I.

Keywords——triterpenoidal saponin; onjisaponin A; onjisaponin B; onjisaponin E; *Polygala tenuifolia*; Polygalae Radix; Polygalaceae; ^{13}C NMR

As we reported in the previous paper[1] seven triterpenoidal saponins, onjisaponins A, B, C, D, E, F and G, were isolated from the root of *Polygala tenuifolia* Willdenow, and the structures of onjisaponins G and F have been established. The present paper describes the structure elucidation of onjisaponins A, B and E, which led to the assignment of the structures **1**, **2** and **3**, respectively.

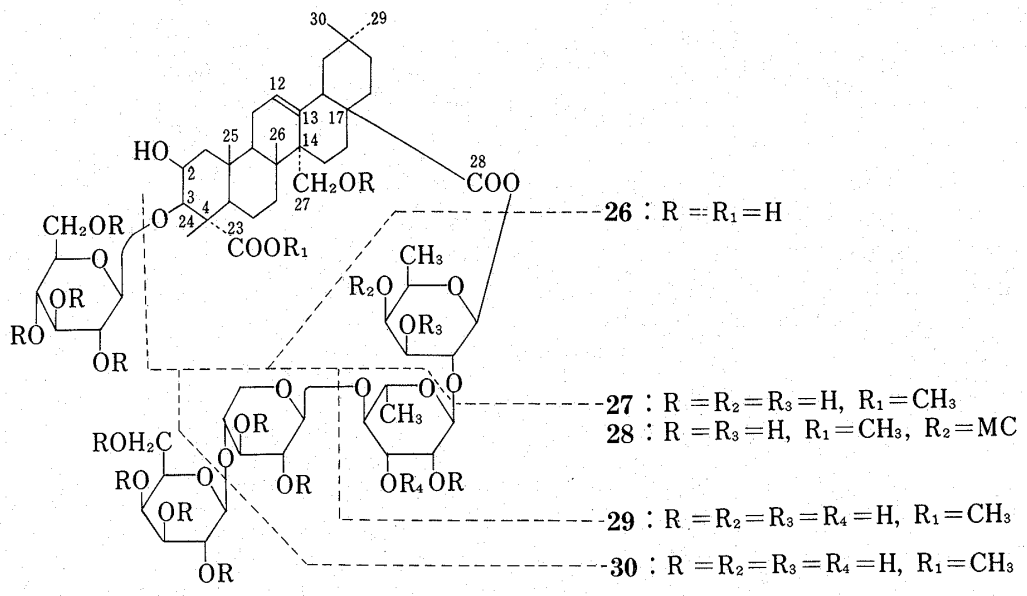
Onjisaponin A (**1**), $C_{80}H_{120}O_{39}$, contains hydroxyl groups, two ester groups, a carboxylic group, a double bond and a benzenoid system as judged from the infrared (IR) spectrum. The ultraviolet (UV) spectrum of **1** showed the absorption maximum at 316 nm (log ε 4.19), and the ^{13}C nuclear magnetic resonance (CMR) spectrum showed seven anomeric carbon signals at δ 95.0, 101.9, 104.2, 104.5, 104.8, 105.0 and 111.6.

On hydrolysis with 1 N potassium hydroxide, **1** gave tenuifolin (**16**: presenegenin 3-β-D-glucopyranoside),[2] 4-methoxycinnamic acid and an unidentified oligosaccharide. On hydrolysis with 4 N hydrogen chloride–dioxane–benzene (3:1:2 v/v), **1** afforded rhamnose, fucose, xylose, galactose and glucose. Furthermore, **1** gave apiose on hydrolysis with 0.2 N hydrogen chloride–dioxane (1:1 v/v).

On methylation with diazomethane, **1** gave a monomethyl ester (**4**), $C_{81}H_{122}O_{39}$, which was further methylated by Kuhn's method[3] to afford an octadeca-*O*-methyl ether monomethyl ester (**7**), $C_{99}H_{158}O_{39}$.

As the IR spectrum of **1** suggests the presence of two kinds of ester groups (1750 and 1730 cm^{-1}), the alkali treatment of **4** was examined. When **4** was treated with 0.5% potassium hydroxide, des-4-methoxycinnamoyl onjisaponin A monomethyl ester (**10**), $C_{71}H_{114}O_{37}$, was obtained and characterized by methylation according to Hakomori's method[4] to form des-4-methoxycinnamoyl onjisaponin A nonadeca-*O*-methyl ether monomethyl ester (**13**), $C_{90}H_{152}O_{37}$. To prove the location of an esteric linkage between the aglycone and oligosaccharide, **4** was hydrolyzed with 1 N potassium hydroxide to afford **17**, $C_{37}H_{58}O_{12}$, which was identified as tenuifolin 23-monomethyl ester by comparing with an authentic sample obtained from senegin II[5] (mixed fusion, IR and CMR spectra). The formation of **17** from **4** suggests that the esteric linkage between presenegenin and oligosaccharide is located at the C-17 car-

boxylic acid. The location of 4-methoxycinnamic acid in onjisaponin A was confirmed by comparative analyses of the methanolysates of **7** and **13** with methanolic 2 N hydrogen chloride. Methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside, methyl 2,3,4-tri-*O*-methylapiofuranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3-di-*O*-methylxylopyranoside, methyl 2-*O*-methylrhamnopyranoside were commonly detected in the methanolysates of compounds **7** and **13**. Methyl fucopyranoside was also obtained from **7**, and methyl 4-*O*-methylfucopyranoside from **13**. Consequently, the 4-methoxycinnamoyl group in **1** was deduced to be located at the C-4 hydroxyl group of fucose.



1: R=R$_1$=H, R$_2$=MC, R$_3$=Rha, R$_4$=Api
2: (=senegin III (**32**))
R=R$_1$=R$_4$=H, R$_2$=MC, R$_3$=Rha
3: R=R$_1$=R$_3$=R$_4$=H, R$_2$=TC
4: R=H, R$_1$=CH$_3$, R$_2$=MC, R$_3$=Rha, R$_4$=Api
5: R=R$_4$=H, R$_1$=CH$_3$, R$_2$=MC, R$_3$=Rha
6: R=R$_3$=R$_4$=H, R$_1$=CH$_3$, R$_2$=TC
7: R=R$_1$=CH$_3$, R$_2$=MC, R$_3$=Rha, R$_4$=Api
8: R=R$_1$=R$_4$=CH$_3$, R$_2$=MC, R$_3$=Rha
9: R=R$_1$=R$_3$=R$_4$=CH$_3$, R$_2$=TC
10: R=R$_2$=H, R$_1$=CH$_3$, R$_3$=Rha, R$_4$=Api
11: (=des-4-methoxycinnamoylsenegin III methyl ester (**33**))
R=R$_2$=R$_4$=H, R$_1$=CH$_3$, R$_3$=Rha
12: (=des-3,4-dimethoxycinnamoylsenegin II methyl ester (**31**))
R=R$_2$=R$_3$=R$_4$=H, R$_1$=CH$_3$
13: R=R$_1$=R$_2$=CH$_3$, R$_3$=Rha, R$_4$=Api
14: R=R$_1$=R$_2$=R$_4$=CH$_3$, R$_3$=Rha
15: R=R$_1$=R$_2$=R$_3$=R$_4$=CH$_3$

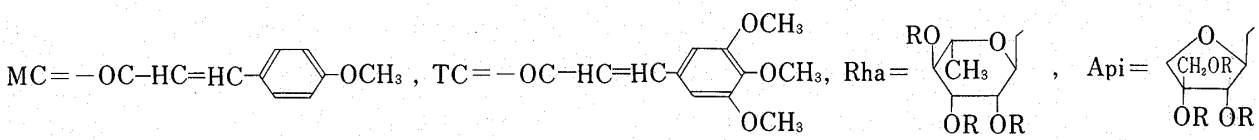


Chart 1

The structure of the oligosaccharide moiety of **1** was established as follows. On reduction with lithium aluminum hydride, compound **13** afforded compound **19**, $C_{48}H_{88}O_{26}$, and compound **18**, $C_{41}H_{68}O_{10}$, and the latter was identified as olean-12-ene-27-*O*-methyl-2β,23,28-trihydroxy-3β-(tetra-*O*-methyl)-β-D-glucopyranoside, obtained from senegin II. The proton magnetic resonance (PMR) spectrum of the other product, **19**, indicated the presence of three secondary methyl groups, fourteen *O*-methyl groups and five anomeric protons. On methanolysis with 2 N hydrogen chloride, **19** afforded methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside, methyl 2,3,4-tri-*O*-methylapiofuranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3-di-*O*-methylxylopyranoside, methyl 2-*O*-methylrhamnopyranoside and 4-*O*-methylfucitol. The formation of 4-*O*-methylfucitol from **19** indicated that the oligosaccharide moiety of

onjisaponin A links to the C-17 carboxyl group of presenegenin through an acetal hydroxyl group of fucose.

On treatment with methanolic 0.1 N hydrogen chloride in dried methanol, **19** afforded methyl 2,3,4-tri-*O*-methyl-D-apio-D-furanoside and an *O*-methylated oligosaccharide (**20**), $C_{40}H_{74}O_{22}$. The PMR spectrum of **20** showed the presence of four anomeric protons, three secondary methyl groups and eleven *O*-methyl groups. On methanolysis with methanolic 2 N hydrogen chloride, **20** afforded methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside, methyl

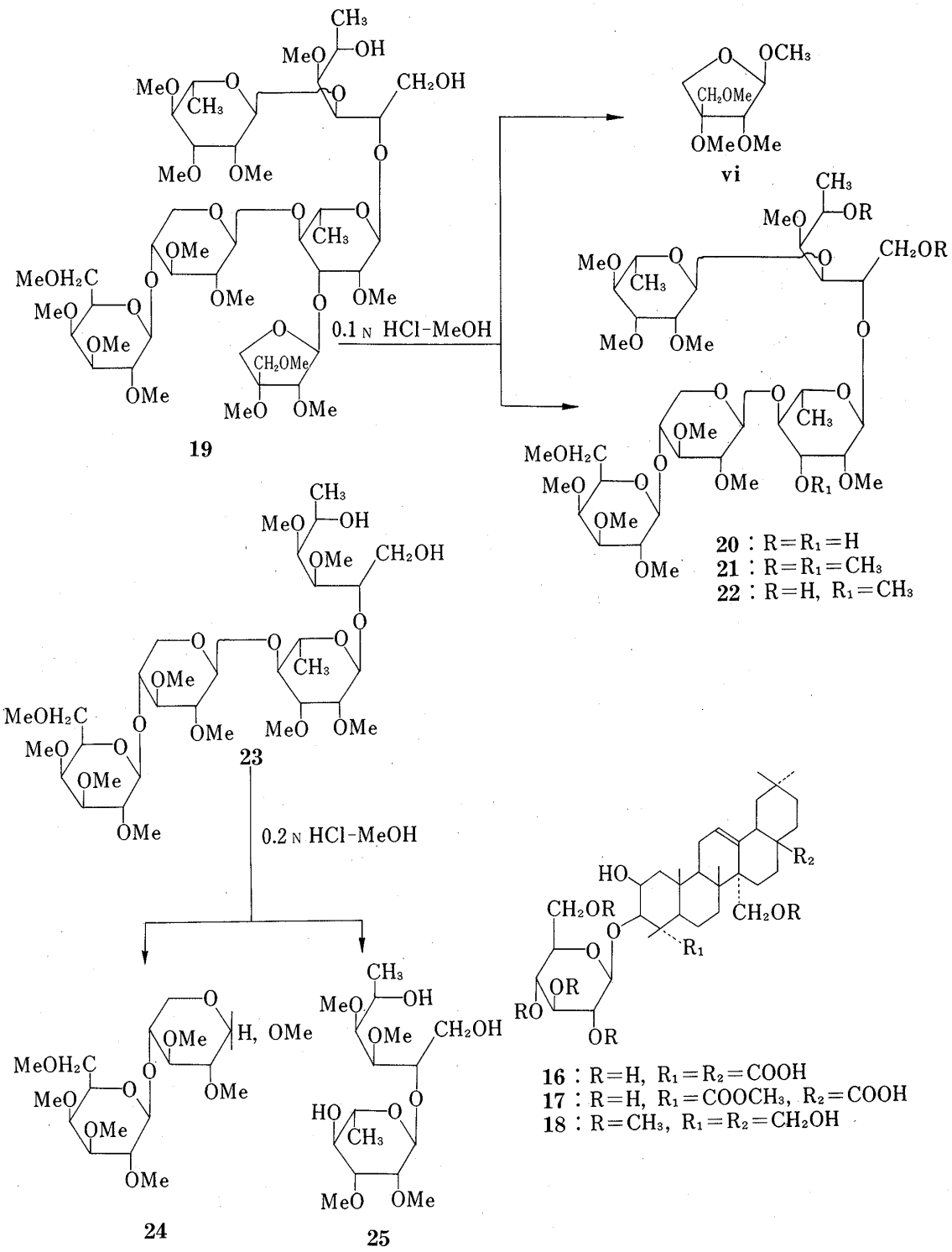


Chart 2

2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3-di-*O*-methylxylopyranoside, methyl 2-*O*-methylrhamnopyranoside and 4-*O*-methylfucitol. After methylation of **20** by Hakomori's method, the resulting per-*O*-methylated oligosaccharide (**21**) was methanolyzed with methanolic 2 N hydrogen chloride to afford methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3-di-*O*-methylxylopyranoside, methyl 2,3-di-*O*-methylrhamnopyranoside and 1,4,5-tri-*O*-methylfucitol (unidentified). By comparing the methanolysis products of **20** and **21**, it was concluded that the apiosyl moiety is attached to the C-3 hydroxyl group of the rhamnose, which is branched at the C-4 hydroxyl group.

The physical properties and results of methanolysis experiments of per-*O*-methylated oligosaccharide (**21**) suggest the similarity of **21** to a per-*O*-methylated oligosaccharide of senegin III,[6] and the identity of both compounds was proved by thin-layer chromatography (TLC), as well as IR and PMR spectral comparisons.

The configuration of the terminal D-apiose in **1** was assigned as β-form from the molecular optical rotation difference[7] between **19** and **20** ($\Delta[M]_D = -181.7°$, methyl 2,3,4-tri-*O*-methyl-α-D-apio-D-furanoside $[M]_D = +239°$, methyl 2,3,4-tri-*O*-methyl-β-D-apio-D-furanoside $[M]_D = -163°$).

From these experimental data, the structure of onjisaponin A has been established to be presenegenin-(3)-*O*-β-D-glucopyranosido-(28)-2-*O*-{[β-D-apio-D-furanosyl(1→3)][β-D-galactopyranosyl(1→4)-β-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl}-3-*O*-(α-L-rhamnopyranosyl)-4-*O*-(4′-methoxycinnamoyl)-β-D-fucopyranoside, formula **1**.

Onjisaponin B (**2**), $C_{75}H_{112}O_{35}$, contains hydroxyl groups, two ester groups, a carboxylic group, a double bond and a benzenoid system as judged from the IR spectrum. The UV spectrum of **2** showed the absorption maximum at 316 nm (log ε 4.17), and the CMR spectrum showed six anomeric carbon signal at δ 94.9, 101.8, 104.2, 104.5, 105.0 and 106.6.

On basic hydrolysis with 1 N potassium hydroxide, **2** gave tenuifolin (**16**), 4-methoxycinnamic acid and an unidentified oligosaccharide. Furthermore, rhamnose, fucose, xylose, galactose and glucose were found in acid hydrolysis products of **2**, but, in contrast to the case of onjisaponin A, D-apiose was not found among the mild acid hydrolysis products.

The physical properties and chemical composition of **2** described above suggest that onjisaponin B (**2**) is similar in structure to known saponins of Polygalae Radix and Senegae Radix. Among them, senegin III, which is composed of tenuifolin, 4-methoxycinnamic acid, two mol of L-rhamnose and one mol each of D-fucose, D-xylose and D-galactose, is inferred to be identical with **2**. To prove the structure of **2**, investigations similar to those for onjisaponin A described above were carried out in comparison with senegin III. The results are described in detail in the experimental section.

Finally, the structure of onjisaponin B has been established to be presenegenin-(3)-β-D-glucopyranosido-(28)-2-*O*-[β-D-galactopyranosyl(1→4)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl]-3-*O*-(α-L-rhamnopyranosyl)-4-*O*-(4′-methoxycinnamoyl)-β-D-fucopyranoside as formula **2** (=senegin III).

Onjisaponin E (**3**), $C_{71}H_{106}O_{33}$, contains hydroxyl groups, two ester groups, a carboxylic group, a double bond and a benzenoid system as judged from the IR spectrum. The UV spectrum of **3** showed absorption maxima at 232 nm (log ε 4.21) and 312 nm (log ε 4.21), and the CMR spectrum showed five anomeric carbon signals at δ 94.8, 101.6, 104.1, 105.0 and 106.7. The composition of **3** was deduced to comprise one mol each of rhamnose, fucose, xylose, galactose, 3,4,5-trimethoxycinnamic acid and **16** from the results of hydrolyses of **3** with 1 N potassium hydroxide and 2 N hydrogen chloride.

On methylation with diazomethane, **3** gave a monomethyl ester (**6**), $C_{72}H_{108}O_{33}$, which was treated with 0.5% potassium hydroxide to afford des-3,4,5-trimethoxycinnamoyl onjisaponin E monomethyl ester (**12**), $C_{60}H_{96}O_{29}$. Compounds **6** and **12** were methylated by Kuhn's method or Hakomori's method to afford onjisaponin E tetradeca-*O*-methyl ether monomethyl ester (**9**), $C_{86}H_{136}O_{33}$, and descinnamoyl onjisaponin E pentadeca-*O*-methyl ether monomethyl

TABLE I. ^{13}C Chemical Shifts of Onjisaponins A, B, E and Related Compounds (in C_5D_5N)

Carbon No.		26	16	17	27	28	29	30	3	12	31	2	32	11	33	1	10
	1	45.0	44.1	44.0	45.1	45.0	44.9	44.8	44.3	44.1	44.5	44.3	44.3	44.0	44.3	44.3	44.3
	2	71.6	70.2	70.1	71.5	71.5	71.3	71.4	70.3	70.1	70.1	70.3	70.2	70.3	70.3	70.1	70.2
	3	75.8	86.0	86.0	75.8	75.7	75.5	75.6	86.0	86.3	86.4	86.0	86.3	85.8	86.0	86.1	86.1
	4	54.0	52.8	53.2	54.5	54.4	54.2	54.2	52.9	53.2	53.2	52.9	53.1	53.3	53.3	52.9	53.1
	5	52.3	52.3	52.5	52.5	52.1	52.4	52.3	52.3	52.7	52.5	52.4	52.3	52.4	52.3	52.4	52.2
	6	21.6	21.2	21.2	21.8	21.5	21.8	21.6	21.5	21.2	21.3	21.5	21.6	21.5	21.4	21.3	21.4
	7	34.1	34.2	34.2	34.0	33.8	33.8	33.7	34.1	33.9	34.3	34.0	34.1	33.9	34.0	34.1	34.1
	8	40.9	40.9	40.9	41.2	41.1	40.9	41.0	41.3	41.1	41.1	41.3	41.3	41.0	41.0	41.3	41.4
	9	49.5	49.4	49.3	49.5	49.4	49.2	49.2	49.5	49.3	49.3	49.5	49.4	49.1	49.1	49.5	49.5
	10	37.1	37.0	37.0	37.2	37.0	37.0	36.9	37.1	37.2	37.0	37.1	37.1	36.9	36.9	37.1	37.2
	11	24.0[a]	24.0[a]	24.0[a]	23.9[a]	23.7[a]	23.8[a]	23.5[a]	24.0[a]	23.9[a]	24.0[a]	24.1[a]	24.1[a]	23.9[a]	23.9[a]	24.2[a]	24.0[a]
	12	127.6	127.7	127.5	127.8	127.8	127.5	127.4	127.9	128.0	127.8	127.8	127.8	127.7	127.7	127.8	127.8
	13	139.7	139.8	139.8	139.3	139.0	138.8	139.0	139.2	139.1	139.0	139.1	139.2	138.8	138.9	139.3	139.3
	14	46.5	46.5	46.5	46.9	46.8	46.7	46.9	47.1	46.8	46.9	47.1	47.2	46.8	46.8	47.1	47.0
	15	24.7[a]	24.7[a]	24.5[a]	24.6[a]	24.6[a]	24.3[a]	24.3[a]	24.6[a]	24.4[a]	24.5[a]	25.4[a]	25.6[a]	25.0[a]	25.0[a]	25.6[a]	25.5[a]
	16	24.1[a]	24.0[a]	24.0[a]	23.9[a]	23.7[a]	23.9[a]	23.7[a]	24.1[a]	24.1[a]	24.1[a]	24.1[a]	24.1[a]	24.3[a]	24.3[a]	24.2[a]	24.2[a]
	17	48.1	48.2	48.1	48.1	48.0	47.9	47.9	48.3	48.1	48.1	48.2	48.3	47.9	47.9	48.2	48.2
	18	41.8	41.8	41.8	41.6	41.5	41.8	41.9	42.0	41.9	41.9	42.0	41.9	41.9	41.9	42.1	42.2
	19	45.5	45.7	45.6	45.4	45.2	45.3	45.3	45.8	45.7	45.3	45.8	45.8	45.8	45.7	45.8	45.8
	20	31.0	30.9	30.9	30.8	30.7	30.6	30.6	30.8	30.7	30.7	30.8	30.8	30.7	30.7	30.8	30.9
	21	33.5	33.5	33.4	33.5	33.4	33.3	32.9	33.7	33.7	33.6	33.7	33.7	33.6	33.7	33.1	33.1
	22	33.2	33.2	33.1	33.1	32.9	33.0	32.3	32.5	32.4	32.4	32.5	32.9	32.3	32.3	32.4	32.4
	23	180.8	180.6	178.3	178.9	178.8	178.7	179.4	180.6	179.2	179.3	180.7	180.5	179.1	179.2	180.8	178.3
	24	13.7	14.0	13.7	13.4	13.5	13.2	13.2	14.0	13.7	13.7	14.1	14.4	13.8	13.8	14.1	13.9
	25	17.5	17.3	17.2	17.5	17.4	17.3	17.3	17.5	17.3	17.4	17.5	17.5	17.4	17.5	17.6	17.5
	26	18.8	18.7	18.7	18.9	18.9	19.0	18.6	18.9	18.7	18.7	19.0	19.1	19.3	19.3	19.3	19.1
	27	64.4	64.6	64.6	64.5	64.3	64.1	64.1	64.6	64.4	64.5	64.5	64.5	64.3	64.4	64.7	64.8
	28	180.8	180.0	180.0	176.6	176.4	176.4	176.4	176.4	176.5	176.4	176.3	176.4	176.6	176.6	176.4	176.4
	29	33.2	33.2	33.1	32.7	32.6	32.3	31.9	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.1	33.1
	30	23.2	23.8	23.8	23.5	23.6	23.4	24.1	24.1	24.1	24.1	24.1	24.1	24.3	24.3	24.2	24.2
23-COOCH$_3$				52.0	51.8	51.7	51.7	51.7		52.2	52.2			52.2	52.3		52.5
Glucose	1		105.1	105.4					105.0	104.8	105.0	105.0	105.0	105.1	105.1	105.0	105.0
	2		75.2	74.8					75.3	75.5	75.2	75.2	75.3	75.2	75.2	75.2	75.0
	3		78.2	78.3					78.1	77.9	78.0	78.1	78.0	78.1	78.1	78.2	78.2
	4		71.7	71.7					71.7	71.7	71.7	71.7	71.7	71.5	71.5	71.7	71.7
	5		78.2	78.3					78.1	77.9	78.0	78.1	78.0	78.1	78.1	78.2	78.2
	6		62.8	62.8					63.0	62.7	62.8	62.9	62.9	62.9	62.9	62.9	63.0
Fucose	1				96.1	95.7	94.7	94.6	94.8	94.8	94.6	94.9	94.8	94.8	94.8	95.0	95.2
	2				72.5	73.6	76.6	76.3	77.0	76.8	76.9	77.0	77.1	77.1	77.1	77.2	77.2
	3				75.7	74.1	73.6	73.5	73.4	74.9	74.3	80.4	80.4	80.6	80.6	79.8	79.8

	4	72.7	79.5	73.1	72.8	78.1	72.9	72.9	78.1	78.0	72.2	72.2	78.2	72.2
	5	71.1	71.0	72.0	72.1	70.9	71.3	71.7	70.5	70.8	71.5	71.5	70.7	71.9
	6	17.2	17.2	16.8	16.8	16.6	16.7	16.7	16.9	16.9	16.9	16.9	16.9	16.9
Rhamnose	1			101.2	101.0	101.6	101.0	100.9	101.8	101.8	101.6	101.6	101.9	101.7
	2			73.2	72.3	72.3	72.2	72.2	72.2	72.6	72.2	72.2	71.8	71.9
	3			73.2	71.4	71.9	71.2	71.7	71.8	71.8	71.5	71.5	82.1	82.0
	4			73.4	84.4	84.7	84.6	84.6	84.4	84.6	84.9	84.9	83.9	83.9
	5			69.6	68.0	68.4	68.2	68.1	68.0	68.0	68.1	68.2	68.8	68.5
	6			18.6	18.3	18.5	18.6	18.3	18.4	18.5	18.4	18.4	18.5	18.5
Xylose	1				106.9	106.7	106.5	106.7	106.6	106.7	106.7	106.7	104.8	104.8
	2				75.6	75.0	74.9	74.8	74.9	75.0	74.9	74.9	74.7	74.7
	3				77.9	75.6	75.9	75.5	75.5	75.6	75.2	75.2	75.2	75.0
	4				70.5	78.0	77.9	77.7	78.1	78.0	78.1	78.1	78.2	78.4
	5				67.0	65.0	65.1	64.8	64.9	65.0	64.8	64.8	64.7	64.8
Galactose	1					104.1	103.9	104.1	104.2	104.1	104.2	104.2	104.2	104.3
	2					72.1	72.2	72.2	72.1	72.1	72.1	72.1	71.8	71.9
	3					75.0	74.9	74.8	74.9	75.0	74.9	74.9	74.9	75.0
	4					70.1	70.1	69.9	70.1	70.2	70.0	70.0	70.1	70.2
	5					76.5	76.2	76.2	76.4	76.3	76.3	76.4	76.3	76.4
	6					62.3	62.2	62.1	62.3	62.4	62.1	62.1	62.3	62.4
Rhamnose	1								104.5	104.6	104.6	104.7	104.5	104.3
(Terminal)	2								72.2	72.6	72.2	72.2	72.4	72.2
	3								72.2	72.6	72.2	72.2	72.4	72.2
	4								73.6	73.6	73.5	73.5	73.3	72.7
	5								70.1	70.1	70.0	70.0	70.1	70.1
	6								18.4	18.5	18.4	18.4	18.5	18.5
Apiose	1												111.6	111.6
	2												77.7	77.7
	3												79.6	79.6
	4												65.0	65.0
	5												74.7	74.7
Cinnamoyl	1		167.0			167.4			167.0	167.0			167.0	
	2		116.6			117.9			116.6	116.1			116.0	
	3		145.4			145.7			145.4	145.4			145.5	
	1′		127.6			130.4			127.6	127.6			127.7	
	2′		130.3			107.5			130.3	130.3			130.3	
	3′		114.9			139.2			114.9	114.9			114.9	
	4′		162.1			154.9			162.1	162.1			162.1	
	5′		114.9			139.2			114.9	114.9			114.9	
	6′		130.3			107.5			130.3	130.3			130.3	
	3′-OCH_3					56.8								
	4′-OCH_3		55.5			60.7			55.5	55.5			55.3	
	5′-OCH_3					56.8								

a) Assignments may be reversed.

ester (**15**), $C_{75}H_{126}O_{29}$, respectively. According to the procedures described for onjisaponins A and B, comparative studies of compounds **9** and **15** were carried out, and the structure of the oligosaccharide moiety of **3** was concluded to be 2-*O*-[D-galactopyranosyl(1→4)-D-xylopyranosyl(1→4)-L-rhamnopyranosyl]-4-*O*-(3′,4′,5′-trimethoxycinnamoyl)-D-fucopyranose, which is identical with the oligosaccharide moiety of senegin II except for the cinnamoyl group. A comparison of CMR spectra of **12** with that of des-3′,4′-dimethoxycinnamoyl senegin II monomethyl ester[2] supported the identity of both compounds.

Therefore, the structure of onjisaponin E has been established as presenegenin-(3)-*O*-*β*-D-glucopyranosido-(28)-2-*O*-[*β*-D-galactopyranosyl(1→4)-*β*-D-xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl]-4-*O*-(3′,4′,5′-trimethoxycinnamoyl)-*β*-D-fucopyranoside, formulated as **3**.

In the course of the structure elucidation of onjisaponins, we used CMR spectrometry, and the chemical shifts and assignments of each carbon signal of onjisaponins and their derivatives are listed in Table I.[8]

Further investigation on saponins of polygalaceous plants are in progress.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot-stage type) and are uncorrected. The optical rotations were measured with a Yanagimoto OR-50 polarimeter. The UV spectra were recorded with a Hitachi EPS-3 spectrometer, IR spectra with a JASCO IR-A-2 unit, PMR spectra with a Hitachi R-22 (90 MHz) spectrometer, and CMR spectra with a JEOL PFT-100 or JNM-FX-100 FT NMR spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Gas liquid chromatography (GLC) was run on a Shimadzu GC-6A unit with a flame ionization detector. TLC was performed on Kieselgel H (Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating.

Properties of Onjisaponins A, B and E——Onjisaponin A (**1**): A white powder from EtOH, (mp 253—254°C (dec.)), $[\alpha]_D^{17}$ −18.4° (c=1.24, MeOH). UV λ_{max}^{EtOH} nm (log ε): 316 (4.19). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid). CMR (C_5D_5N) δ: 95.0 (J=163 Hz, fucose-C_1), 101.9 (J=172 Hz, rhamnose-C_1), 104.2 (J=158 Hz, galactose-C_1), 104.5 (J=172 Hz, rhamnose-C_1), 104.8 (J=160 Hz, xylose-C_1), 105.0 (J=160 Hz, glucose-C_1), 111.6 (J=173 Hz, apiose-C_1). *Anal.* Calcd for $C_{80}H_{120}O_{39}$: C, 56.33; H, 7.07. Found: C, 55.86; H, 7.25.

Onjisaponin B (**2**): A white powder from aq. EtOH, (mp 249—251°C (dec.)), $[\alpha]_D^{17}$ −10.2° (c=1.08, MeOH). UV λ_{max}^{EtOH} nm (log ε): 316 (4.17). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), (1635 (C=C), 1610, 1515 (benzenoid). CMR (C_5D_5N) δ: 94.9 (J=163 Hz, fucose-C_1), 101.8 (J=172 Hz, rhamnose-C_1), 104.2 (J=158 Hz, galactose-C_1), 104.5 (J=171 Hz, rhamnose-C_1), 105.0 (J=160 Hz, glucose-C_1), 106.6 (J=160 Hz, xylose-C_1). *Anal.* Calcd for $C_{75}H_{112}O_{35}$: C, 57.24; H, 7.17. Found: C, 56.77; H, 7.34.

Onjisaponin E (**3**): Colorless needles from aq. EtOH, mp 245—247°C (dec.), $[\alpha]_D^{17}$ −6.5° (c=1.00, MeOH). UV λ_{max}^{EtOH} nm (log ε): 232 (4.21), 312 (4.21). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), 1635 (C=C), 1580, 1500 (benzenoid). CMR (C_5D_5N) δ: 94.8 (J=163 Hz, fucose-C_1), 101.6 (J=173 Hz, rhamnose-C_1), 104.1 (J=158 Hz, galactose-C_1), 105.0 (J=160 Hz, glucose-C_1), 106.7 (J=161 Hz, xylose-C_1). *Anal.* Calcd for $C_{71}H_{106}O_{33}\cdot 4H_2O$: C, 54.67; H, 7.37. Found: C, 54.87; H, 7.25.

Hydrolysis of 1, 2 and 3 with 1 N KOH——Solution of **1**, **2** and **3** (200 mg each) in 1 N KOH (20 ml) were heated under an N_2 gas flow on a water bath for 2 h. The reaction mixtures were cooled at room temperature and neutralized with 1 N HCl. Each solution was repeatedly extracted with benzene and the organic layers were combined, washed with water, dried over Na_2SO_4 and evaporated to dryness. The residues were subjected to column chromatography on silica gel using hexane–acetone (3: 1 v/v), to afford 4-methoxycinnamic acid from **1** or **2**, and 3,4,5-trimethoxycinnamic acid from **3**.

4-Methoxycinnamic Acid: Colorless needles from aq. EtOH, mp 171—172°C. UV λ_{max}^{EtOH} nm (log ε): 224 (4.12), 292 (4.38). IR ν_{max}^{KBr} cm^{-1}: 1680 (–C=C–COOH), 1620, 1600, 1510 (benzenoid). PMR ($CDCl_3$) δ: 3.70 (3H, s, –OC<u>H</u>$_3$), 6.28 (1H, d, J=16 Hz, –CH=C<u>H</u>–COOH), 6.82 (2H, d, J=8 Hz, 3,5-H), 7.34 (2H, d, J=8 Hz, 2,6-H), 7.58 (1H, d, J=16 Hz, –C<u>H</u>=CH–COOH), 9.90 (1H, s, COO<u>H</u>). CMR (C_5D_5N) δ: 55.3 (q, –O<u>C</u>H$_3$), 114.7 (d, C-3 and C-5), 118.0 (d, –CH=<u>C</u>H–COOH), 127.9 (s, C-1), 130.1 (d, C-2 and C-6), 143.2 (d, –<u>C</u>H=CH–COOH), 161.5 (s, C-4), 169.5 (s, <u>C</u>OOH). *Anal.* Calcd for $C_{10}H_{10}O_3$: C, 67.40; H, 5.66. Found: C, 67.67; H, 5.78. This product was identical with an authentic sample.

3,4,5-Trimethoxycinnamic Acid: Colorless needles from aq. EtOH, mp 127—128°C. UV λ_{max}^{EtOH} nm (log ε): 223 (4.30), 285 (4.23). IR ν_{max}^{KBr} cm^{-1}: 1680 (–C=C–COOH), 1615, 1580, 1500 (benzenoid). PMR ($CDCl_3$) δ: 3.90 (9H, s, –OC<u>H</u>$_3$×3), 6.35 (1H, d, J=16 Hz, –CH=C<u>H</u>–COOH), 6.78 (2H, s, arom. H), 7.70 (1H, d, J=16 Hz, –C<u>H</u>=CH–COOH), 9.90 (1H, s, COO<u>H</u>), CMR (C_5D_5N) δ: 56.1 (q, –O<u>C</u>H$_3$×2), 60.6 (q, –O<u>C</u>H$_3$), 106.1 (d, C-2 and C-6), 119.8 (d, –CH=<u>C</u>H–COOH), 131.0 (s, C-1), 140.6 (s, C-4), 144.5 (d, –<u>C</u>H=CH–COOH), 154.1

(s, C-3 and C-5), 169.3 (s, COOH). *Anal.* Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.39; H, 5.89. This product was identical with an authentic sample.

After removal of the benzene-soluble fraction, each aqueous layer was repeatedly extracted with BuOH and the organic layers were combined, washed with water then evaporated to dryness. The residue was subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (7: 3: 1 v/v, lower phase), to afford colorless needles (**16**) from aq. EtOH, mp 299—300°C (dec.), $[\alpha]_D^{24}$ +40.9° (c=0.98, MeOH). PMR (C_5D_5N) δ: 0.89, 1.00, 1.04, 1.49, 1.91 (3H each, s, *tert.* $CH_3\times5$), 5.05 (1H, d, J=7 Hz, anomeric H), 5.82 (1H, m, olefinic H). *Anal.* Calcd for $C_{36}H_{56}O_{12}\cdot H_2O$: C, 61.87; H, 8.37. Found: C, 61.92; H, 8.50. This product was identical with an authentic sample of tenuifolin (TLC, IR and CMR spectra).

Hydrolysis of 1, 2 and 3 with 4 N HCl–Dioxane–Benzene (3: 1: 2 v/v)——Compounds **1**, **2** and **3** (10 mg each) were each refluxed with 4 N HCl–dioxane–benzene (3: 1: 2 v/v, 6 ml) on a water bath for 4 h. After removal of the organic solvent, the reaction mixture was extracted with Et_2O. The aqueous layer was neutralized with Amberlite MB-3 and evaporated to dryness *in vacuo*. The residue was examined by TLC (solvent, $CHCl_3$–MeOH–H_2O=7: 3: 0.5 v/v), PPC (Tōyō-Roshi No. 51: solvent, BuOH–EtOH–H_2O= 52: 32: 16;[9]) detection with aniline hydrogen phthalate), and GLC (column, 5% SE-52 on Chromosorb W, 3 mm×2 m; column temperature, 170°C; carrier gas, N_2 1.2 kg/cm²; sample, trimethylsilyl ether (TMS) derivatives). TLC *Rf* 0.33 (rhamnose), 0.30 (fucose), 0.26 (xylose), 0.16 (glucose), 0.14 (galactose). PPC *Rf* 0.48 (rhamnose), 0.42 (fucose), 0.35 (xylose), 0.25 (glucose), 0.23 (galactose). GLC t_R (min) 4.9, 6.5 (rhamnose), 5.9, 7.0 (fucose), 6.8, 8.5 (xylose), 13.5, 16.3 (galactose), 15.8, 24.6 (glucose).

Hydrolysis of 1 with 0.2 N HCl–Dioxane (1: 1 v/v)——Compound **1** (15 mg) was refluxed with 0.2 N HCl–dioxane (1: 1 v/v 8 ml) on a water bath for 20 min. The reaction mixture was diluted with water, neutralized with Amberlite MB-3 and evaporated to dryness *in vacuo*. The residue was examined by PPC (Tōyō-Roshi No. 51; solvent BuOH–EtOH–H_2O=52: 32: 16 v/v; detection with benzidine–trichloroacetic acid[10]) and GLC (the same conditions as described above). PPC *Rf* 0.44 (apiose); GLC t_R(min) 4.0, 4.3, 4.5, 5.0 (apiose).

Methylation of 1, 2 and 3 with CH_2N_2——Compounds **1**, **2** and **3** (400 mg each) were each dissolved in MeOH (50 ml) and treated with ethereal diazomethane. The reaction mixture was allowed to stand at room temperature for 12 h, then the excess reagent was decomposed with AcOH, and the solvent was evaporated off *in vacuo*. The residue was subjected to column chromatography on silica gel, using $CHCl_3$–MeOH–H_2O (7: 3: 0.5 v/v), to afford onjisaponin A monomethyl ester (**4**, 380 mg), onjisaponin B monomethyl ester (**5**, 380 mg) and onjisaponin E monomethyl ester (**6**, 310 mg). **4**: a white powder from EtOH, (mp 240—242°C (dec.)), $[\alpha]_D^{22}$ −9.9° (c=1.82, MeOH). IR ν_{max}^{KBr} cm⁻¹: 3400 (OH), 1730 (COOR), 1600, 1510 (benzenoid). *Anal.* Calcd for $C_{81}H_{122}O_{39}$: C, 56.57; H, 7.15. Found: C, 56.18; H, 7.25. **5**: a white powder from EtOH, (mp 241—244°C (dec.)), $[\alpha]_D^{17}$ −6.1° (c=1.80, MeOH). IR ν_{max}^{KBr} cm⁻¹: 3400 (OH), 1730 (COOR), 1600, 1510 (benzenoid). *Anal.* Calcd for $C_{76}H_{114}O_{35}$: C, 57.49; H, 7.24. Found: C, 57.16; H, 7.30. **6**: a white powder from EtOH, (mp 224—226°C (dec.)), $[\alpha]_D^{22}$ −1.4° (c=0.84, MeOH). IR ν_{max}^{KBr} cm⁻¹: 3400 (OH), 1730 (COOR), 1580, 1500 (benzenoid). *Anal.* Calcd for $C_{72}H_{108}O_{33}$: C, 57.59; H, 7.25. Found: C, 57.24; H, 7.12.

Methylation of 4, 5 and 6 by Kuhn's Method——Compounds **4** (186 mg), **5** (152 mg) and **6** (98 mg) were methylated by Kuhn's method. To a solution of each compound in dimethylformamide (DMF, 10 ml) was added 6 ml of CH_3I and 2 g of freshly prepared Ag_2O. The reaction mixture was stirred for 4 d at room temperature then filtered. The filtrate was diluted with water and extracted with $CHCl_3$ several times. The $CHCl_3$ extracts were combined, washed with water and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel, eluting with benzene–acetone 4: 1 v/v) to afford each *O*-methyl ether. Onjisaponin A octadeca-*O*-methyl ether monomethyl ester (**7**, 62 mg): a white powder from hexane, (mp 138—139°C), $[\alpha]_D^{28}$ −9.2° (c=1.02, $CHCl_3$). IR ν_{max}^{Nujol} cm⁻¹: 3550 (OH), 1730 (COOR), 1600, 1510 (benzenoid). PMR ($CDCl_3$) δ: 4.20 (1H, d, J=7 Hz, anomeric H), 4.27 (1H, d, J=7 Hz, anomeric H), 4.50 (1H, d, J=6 Hz, anomeric H), 5.00 (1H, s, anomeric H), 5.20 (2H, s, anomeric H×2), 5.50 (1H, d, J=8 Hz, anomeric H), 6.37 (1H, d, J=16 Hz, olefinic H), 6.89 (2H, d, J= 8 Hz, arom. H×2), 7.47 (2H, d, J=8 Hz, arom. H×2), 7.69 (1H, d, J=16 Hz, olefinic H), *Anal.* Calcd for $C_{99}H_{158}O_{39}$: C, 60.29; H, 8.08. Found: C, 59.94; H, 8.08. Onjisaponin B hexadeca-*O*-methyl ether monomethyl ester (**8**, 53 mg): a white powder from hexane, (mp 140—142°C), $[\alpha]_D^{28}$ −1.4° (c=1.80, $CHCl_3$), IR ν_{max}^{Nujol} cm⁻¹: 3550 (OH), 1730 (COOR), 1600, 1510 (benzenoid). PMR ($CDCl_3$) δ: 4.20 (1H, d, J=8 Hz, anomeric H), 4.28 (1H, d, J=8 Hz, anomeric H), 4.56 (1H, d, J=8 Hz, anomeric H), 5.04 (1H, s, anomeric H), 5.20 (1H, s, anomeric H), 5.50 (1H, d, J=8 Hz, anomeric H), 6.40 (1H, d, J=16 Hz, olefinic H), 6.91 (2H, d, J= 8 Hz, arom. H×2), 7.51 (2H, d, J=8 Hz, arom. H×2), 7.70 (1H, d, J=16 Hz, olefinic H). *Anal.* Calcd for $C_{92}H_{146}O_{35}$: C, 60.97; H, 8.12. Found: C, 60.77; H, 8.08. Onjisaponin E tetradeca-*O*-methyl ether monomethyl ester (**9**, 47 mg): a white powder from hexane, (mp 134—136°C), $[\alpha]_D^{22}$ +4.2° (c=2.18, $CHCl_3$). IR ν_{max}^{Nujol} cm⁻¹: 3550 (OH), 1730 (COOR), 1580, 1500 (benzenoid). PMR ($CDCl_3$) δ: 4.22 (1H, d, J=7 Hz, anomeric H), 4.28 (1H, d, J=7 Hz, anomeric H), 4.58 (1H, d, J=7 Hz, anomeric H), 5.18 (1H, s, anomeric H), 5.50 (1H, d, J=8 Hz, anomeric H), 6.48 (1H, d, J=16 Hz, olefinic H), 6.85 (2H, s, arom. H×2), 7.70 (1H, d, J=16 Hz, olefinic H). *Anal.* Calcd for $C_{86}H_{136}O_{33}$: C, 60.83; H, 8.07. Found: C, 60.59; H, 7.98.

Hydrolysis of 4, 5 and 6 with 1 N KOH (Formation of Tenuifolin Monomethyl Ester (17))——Compounds **4**, **5** and **6** (140 mg each) were each dissolved in 1 N KOH (20 ml) and each solution was heated under an N_2

gas flow on a water bath for 1 h. The reaction mixture was cooled at room temperature and neutralized with 1 N HCl. Each solution was extracted with BuOH and the organic layers were combined, washed with water and then evaporated to dryness *in vacuo*. The residues were subjected to column chromatography on silica gel, using $CHCl_3$–MeOH–H_2O (7: 3: 1 v/v, lower phase), to afford colorless needles (**17**) from AcOEt saturated with water, mp 230—231°C, $[\alpha]_D^{18}$ +61.7° (c=0.97, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), 1700 (COOH). PMR (C_5D_5N) δ: 0.89, 0.98, 1.00, 1.48, 1.90 (3H each, s, *tert.* C$\underline{H}_3\times5$), 3.80 (3H, s, COOC$\underline{H}_3$), 4.98 (1H, J=7 Hz, anomeric H), 5.82 (1H, m, olefinic H). *Anal.* Calcd for $C_{37}H_{58}O_{12}\cdot2H_2O$: C, 60.82; H, 8.49. Found: C, 60.74; H, 8.29. Each product was identical with an authentic sample of tenuifolin monomethyl ester (**17**) obtained from senegin II (mixed fusion and IR spectra).

Hydrolysis of 4, 5 and 6 with 0.5% KOH——Compounds **4** (630 mg), **5** (320 mg) and **6** (370 mg) were each dissolved in 0.5% KOH (250 ml) and the solution was allowed to stand for 48 h at room temperature. After neutralization with Amberlite MB-3 the solvent was evaporated off *in vacuo*. The residue was subjected to column chromatography on silica gel, using $CHCl_3$–MeOH–H_2O (7: 3: 0.5 v/v), to give a descinnamoyl derivative, **10** (480 mg) from **4**, **11** (320 mg) from **5**, and **12** (310 mg) from **6**. Des-4-methoxycinnamoylonjisaponin A monomethyl ester (**10**): a white powder from EtOH, (mp 234—237°C (dec.)), $[\alpha]_D^{22}$ −9.8° (c=1.33, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR). CMR (C_5D_5N) δ: 95.2 (fucose-C_1), 101.7 (rhamnose-C_1), 104.3 (galactose and rhamnose-C_1), 104.8 (xylose-C_1), 105.0 (glucose-C_1), 111.6 (apiose-C_1). *Anal.* Calcd for $C_{71}H_{114}O_{37}$: C, 54.67; H, 7.37. Found: C, 54.12; H, 7.48. Des-4-methoxycinnamoylonjisaponin B monomethyl ester (**11**): a white powder from EtOH, (mp 235—237°C (dec.)), $[\alpha]_D^{27}$ +2.0° (c=1.00, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), CMR (C_5D_5N) δ: 94.8 (fucose-C_1), 101.6 (rhamnose-C_1), 104.2 (galactose-C_1), 104.6 (rhamnose-C_1), 105.1 (glucose-C_1), 106.7 (xylose-C_1). *Anal.* Calcd for $C_{66}H_{106}O_{33}$: C, 55.53; H, 7.48. Found: C, 55.13; H, 7.59. Des-3,4,5-trimethoxycinnamoylonjisaponin E monomethyl ester (**12**): a white powder from EtOH, (mp 231—233°C (dec.)), $[\alpha]_D^{22}$ +11.0° (c=1.41, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR). CMR (C_5D_5N) δ: 94.8 (fucose-C_1), 101.0 (rhamnose-C_1), 103.9 (galactose-C_1), 104.8 (glucose-C_1), 106.5 (xylose-C_1). *Anal.* Calcd for $C_{60}H_{96}O_{29}$: C, 56.24; H, 7.55. Found: C, 55.88; H, 7.60.

Methylation of 10, 11 and 12 by Hakomori's Method——According to Hakomori's method, NaH (360 mg) was stirred with dimethylsulfoxide (DMSO, 9 ml) at 65°C for 1 h under an N_2 gas flow. A solution of **10** (400 mg), **11** (230 mg) or **12** (340 mg) in DMSO (4 ml) was then added to this reagent and the mixture was stirred for 30 min at room temperature under an N_2 gas flow. CH_3I (5 ml) was added to the solution and the reaction mixture was stirred at room temperature for 5 h. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic phase was washed with water, dried and concentrated. The residue was chromatographed on a column of silica gel with benzene–acetone (4: 1 v/v) to afford the descinnamoyl *O*-methylated derivative, **13** (280 mg) from **10**, **14** (170 mg) from **11**, and **15** (250 mg) from **12**. Des-4-methoxycinnamoylonjisaponin A nonadeca-*O*-methyl ether monomethyl ester (**13**): a white powder from hexane, (mp 136—138°C), $[\alpha]_D^{28}$ −12.7° (c=1.53, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3550 (OH), 1730 (COOR). PMR ($CDCl_3$) δ: 4.24 (1H, d, J=7 Hz, anomeric H), 4.34 (1H, d, J=7 Hz, anomeric H), 4.53 (1H, d, J=7 Hz, anomeric H), 5.04 (1H, s, anomeric H), 5.12 (1H, s, anomeric H), 5.22 (1H, s, anomeric H), 5.51 (1H, d, J=8 Hz, anomeric H), *Anal.* Calcd for $C_{90}H_{152}O_{37}$: C, 59.19; H, 8.39. Found: C, 58.93; H, 8.34. Des-4-methoxycinnamoylonjisaponin B heptadeca-*O*-methyl ether monomethyl ester (**14**): a white powder from hexane, (mp 124—126°C), $[\alpha]_D^{28}$ −1.5° (c=0.67, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3550 (OH), 1730 (COOR). PMR ($CDCl_3$) δ: 4.24 (1H, d, J=7 Hz, anomeric H), 4.31 (1H, d, J=7 Hz, anomeric H), 4.58 (1H, d, J=7 Hz, anomeric H), 5.07 (1H, s, anomeric H), 5.20 (1H, s, anomeric H), 5.49 (1H, d, J=8 Hz, anomeric H), *Anal.* Calcd for $C_{83}H_{140}O_{33}$: C, 59.84; H, 8.47. Found: C, 59.69; H, 8.25. Des-3,4,5-trimethoxycinnamoylonjisaponin E pentadeca-*O*-methyl ether monomethyl ester (**15**): a white powder from hexane, (mp 123—125°C), $[\alpha]_D^{28}$ +10.0° (c=0.70, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3550 (OH), 1730 (COOR), PMR ($CDCl_3$) δ: 4.20 (1H, d, J=7 Hz, anomeric H), 4.30 (1H, d, J=7 Hz, anomeric H), 4.64 (1H, d, J=7 Hz, anomeric H), 5.30 (1H, s, anomeric H), 5.40 (1H, d, J=8 Hz, anomeric H), *Anal.* Calcd for $C_{75}H_{126}O_{29}$: C, 60.38; H, 8.51. Found: C, 60.45; H, 8.67.

Methanolysis of 7, 8, 9, 13, 14 and 15 with Methanolic 2 N HCl——Compounds **7**, **8**, **9**, **13**, **14** and **15** (10 mg each) were each refluxed with methanolic 2 N HCl (3 ml) for 2 h. Each reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrates were concentrated *in vacuo* and the residues were examined by TLC and GLC. The results can be summarized as follows.

Compound **7**: Methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**i**), methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside (**ii**), methyl 2,3-di-*O*-methylxylopyranoside (**iii**), methyl 2,3,4-tri-*O*-methylrhamnopyranoside (**iv**), methyl fucopyranoside (**v**), methyl 2,3,4-tri-*O*-methylapiofuranoside (**vi**), methyl 2-*O*-methylrhamnopyranoside (**vii**). Compound **8**: **i**, **ii**, **iii**, **iv**, **v**, methyl 2,3-di-*O*-methylrhamnopyranoside (**viii**). Compound **9**: **i**, **ii**, **iii**, **viii**, methyl 3-*O*-methylfucopyranoside (**ix**). Compound **13**: **i**, **ii**, **iii**, **iv**, **vi**, **vii**, and methyl 4-*O*-methylfucopyranoside (**x**) Compound **14**: **i**, **ii**, **iii**, **iv**, **viii** and **x**. Compound **15**: **i**, **ii**, **iii**, **viii** and methyl 3,4-di-*O*-methylfucopyranoside (**xi**). TLC (solvent: benzene–acetone=2: 1 v/v) *Rf* 0.68 (**iv**), 0 62 (**vi**), 0.60, 0.54 (**i**), 0.54, 0.48 (**ii**), 0.39, 0.33 (**iii**), 0.34 (**viii**), 0.26 (**xi**), 0.17 (**vii**), 0.09 (**ix** and **x**), 0.01 (**v**). GLC (column, 5% NPGS on Shimalite W, 60—80 mesh, 3 mm×2 m; column temperature, 155°C; carrier gas, N_2 1.2 kg/cm^2). t_R(min) 2.0 (**iv**), 2.3 (**vi**), 5.2, 6.4 (**i**), 6.5 (**viii**), 6.8, 7.4 (**iii**), 7.4, 11.6 (**xi**), 8.1, 9.4 (**ii**), 12.6, 16.0 (**x**), 14.3 (**ix** and **vii**).

Each methanolysate of **7**, **8**, **9**, **13** and **14** was acetylated with acetic anhydride and pyridine. Each reaction mixture was concentrated and examined by GLC (column, 3% ECNSS-M Gas Chrom Q, 100—120 mesh, 3 mm×2 m; column temperature, 180°C; carrier gas, N_2 0.9 kg/cm²) t_R(min) 5.2, 10.6 (methyl 2,4-di-*O*-acetyl-3-*O*-methylfucopyranoside), 6.6 (methyl 3,4-di-*O*-acetyl-2-*O*-methylrhamnopyranoside), 7.3, 11.0 (methyl 2,3-di-*O*-acetyl-4-*O*-methylfucopyranoside), 12.2, 14.3 (methyl 2,3,4-tri-*O*-acetylfucopyranoside).

Reductive Cleavage of 13, 14 and 15 with Lithium Aluminum Hydride——A solution of compound **13** (650 mg) in dried tetrahydrofuran (THF) was refluxed with 280 mg of $LiAlH_4$ for 4 h. The excess $LiAlH_4$ was decomposed with AcOEt, and the reaction mixture was poured into a large amount of water. The aqueous solution was extracted with ether and then with $CHCl_3$. The ether solution was washed with water, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was subjected to column chromatography on silica gel, using benzene–acetone (3: 1 v/v), to afford compound **18** as a white powder from hexane, (mp 124—126°C), $[\alpha]_D^{22}$ +52.8° (c=0.79, $CHCl_3$). IR ν_{max}^{Nujol} cm⁻¹: 3460 (OH). PMR ($CDCl_3$) δ: [0.92 (6H), 0.96 (3H), 0.98 (3H), 1.25 (3H)] (s, *tert.* C$\underline{H}_3$×5), 3.24, 3.33, 3.37, 3.53, 3.67 (3H each, s, OC$\underline{H}_3$×5), 4.30 (1H, d, J=7 Hz, anomeric H), 5.50 (1H, m, olefinic H). On methanolysis with methanolic 2 N HCl, compound **18** gave **i**, which was identified by TLC and GLC. Compound **18** was identical with an authentic sample of olean-12-ene-27-*O*-methyl-2,23,28-trihydroxy-3β-(tetra-*O*-methyl)-glucopyranoside obtained from senegin II (IR, PMR and co-TLC (solvent: benzene–acetone=2: 1 v/v, *Rf* 0.48)).

The chloroform solution was washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel, using $CHCl_3$–MeOH (20: 1 v/v), to afford a tetradeca-*O*-methyl oligosaccharide (260 mg, **19**), syrup, $[\alpha]_D^{20}$ −71.5° (c=2.83, $CHCl_3$). IR ν_{max}^{Nujol} cm⁻¹: 3480 (OH). PMR (C_6D_6) δ: 1.38 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$), 1.44 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$), 1.71 (3H, broad, *sec.* C$\underline{H}_3$), [3.20 (6H), 3.28 (3H), 3.35 (3H), 3.40 (3H), 3.44 (9H), 3.48 (6H), 3.51 (3H), 3.54 (3H), 3.64 (3H), 3.72 (3H)] (s, OC$\underline{H}_3$×14), 4.26 (1H, d, J=8 Hz, anomeric H), 4.88 (1H, d, J=8 Hz, anomeric H), 5.34 (1H, s, anomeric H), 5.39 (1H, s, anomeric H), 5.50 (1H, s, anomeric H). *Anal.* Calcd for $C_{48}H_{88}O_{26}$: C, 53.32; H, 8.20. Found: C, 53.17; H, 8.34.

Compound **14** (420 mg) was treated with $LiAlH_4$ (140 mg) by the procedure described above to give **18** and a dodeca-*O*-methyl oligosaccharide (170 mg, **22**), syrup, $[\alpha]_D^{22}$ −72.4° (c=1.11, $CHCl_3$). IR ν_{max}^{Nujol} cm⁻¹: 3460 (OH). PMR (C_6D_6) δ: 1.32 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$), 1.40 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$), 1.62 (3H, broad, *sec.* C$\underline{H}_3$), [3.16 (3H), 3.27 (3H), 3.34 (3H), 3.42 (6H), 3.45 (9H), 3.47 (6H), 3.58 (3H), 3.68 (3H)] (s, OC$\underline{H}_3$×12), 4.24 (1H, d, J=7 Hz, anomeric H), 4.90 (1H, d, J=8 Hz, anomeric H), 5.18 (1H, s, anomeric H), 5.26 (1H, s, anomeric H). *Anal.* Calcd for $C_{41}H_{76}O_{22}$: C, 53.46; H, 8.32. Found: C, 53.58; H, 8.29.

Compound **15** (220 mg) was treated with $LiAlH_4$ (80 mg) by the procedure described above to give **18** and a deca-*O*-methyl oligosaccharide (70 mg, **23**), syrup, $[\alpha]_D^{20}$ −61.5° (c=2.84, $CHCl_3$). IR ν_{max}^{Nujol} cm⁻¹: 3460 (OH). PMR (C_6D_6) δ: 1.24 (3H, d, J=7 Hz, *sec.* C$\underline{H}_3$), 1.64 (3H, broad), [3.16 (3H), 3.27 (3H), 3.29 (3H), 3.32 (3H), 3.37 (3H), 3.41 (3H), 3.44 (6H), 3.55 (3H), 3.68 (3H)] (s, OC$\underline{H}_3$×10), 4.14 (1H, d, J=8 Hz, anomeric H), 4.19 (1H, d, J=6 Hz, anomeric H), 5.17 (1H, s, anomeric H). *Anal.* Calcd for $C_{33}H_{62}O_{18}$: C, 53.07; H, 8.37. Found: C, 52.91; H, 8.25.

Methanolysis of 19, 22 and 23 with Methanolic 2 N HCl——Compounds **19**, **22** and **23** (5 mg each) were each refluxed with methanolic 2 N HCl for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. Each filtrate was evaporated to dryness *in vacuo* and the residue was examined by TLC and GLC (the same conditions as described for the methanolysates of **7** and others). **19**: **ii**, **iii**, **iv**, **vi**, **vii** and 4-*O*-methylfucitol (**xii**). **22**: **ii**, **iii**, **viii**, **iv** and **xii**. **23**: **ii**, **iii**, **viii** and 3,4-di-*O*-methylfucitol (**xiii**). Each methanolysate of **19**, **22** and **23** was acetylated with acetic anhydride and pyridine and the reaction mixture was concentrated and examined by GLC. t_R(min) 16.3 (1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylfucitol), 23.9 (1,2,3,5-tetra-*O*-acetyl-4-*O*-methylfucitol).

Partial Methanolysis of 19 with Methanolic 0.1 N HCl——Compound **19** (240 mg) was refluxed with methanolic 0.1 N HCl (12 ml) for 3 h, then the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue was examined by TLC (solvent: $CHCl_3$–MeOH=9: 1 v/v). The residue showed two main spots (*Rf* 0.72 and 0.52) and the products corresponding to *Rf* 0.72 and 0.52 were isolated by column chromatography on silica gel using $CHCl_3$–MeOH=100: 1—20: 1 v/v). The former (*Rf* 0.72), a colorless oil (**vi**, 11 mg), $[\alpha]_D^{22}$ −73.2° (c=0.64, $CHCl_3$) lit.[11] $[\alpha]_D$ −79°. PMR ($CDCl_3$) δ: 3.37, 3.40, 3.46, 3.50 (3H each, s, OC$\underline{H}_3$×4), 3.64 (1H, d, J=2.5 Hz, $\underline{H}$–C–OR), 4.01 (2H, q, J=10 and 5 Hz, –C$\underline{H}_2$–OR), 4.94 (1H, d, J=2.5 Hz, anomeric H), was identical with an authentic sample of methyl 2,3,4-tri-*O*-methyl-β-D-apio-D-furanoside[12] obtained from apiin permethylate[13] (co-TLC, GLC and PMR spectra).

The latter (**20**, *Rf* 0.52) was a syrup (162 mg), $[\alpha]_D^{22}$ −65.2° (c=0.85, $CHCl_3$). PMR (C_6D_6) δ: 1.29 (3H, d, J=7 Hz, *sec.* C$\underline{H}_3$), 1.40 (3H), d, J=7 Hz, *sec.* C$\underline{H}_3$), 1.65 (3H, broad d, *sec.* C$\underline{H}_3$), [3.14 (3H), 3.27 (3H), 3.33 (3H), 3.34 (3H), 3.44 (6H), 3.47 (3H), 3.49 (6H), 3.57 (3H), 3.61 (3H)] (s, OC$\underline{H}_3$×11), 4.10 (1H, d, J=7 Hz, anomeric H), 4.60 (1H, d, J=7 Hz, anomeric H), 5.18 (1H, s, anomeric H), 5.22 (1H, s, anomeric H), *Anal.* Calcd for $C_{40}H_{74}O_{22}$: C, 52.97; H, 8.22. Found: C, 53.25; H, 8.35. Compound **20** was methanolyzed with methanolic 2 N HCl under reflux for 2 h to give **ii**, **iii**, **iv**, **viii** and **xii**.

Methylation of 20 by Hakomori's Method——Compound **20** (40 mg) was methylated by Hakomori's method and the reaction mixture was treated by the procedure described above to afford a per-*O*-methyl-

pentasaccharide (**21**, 32 mg), a syrup, $[\alpha]_D^{22}$ −57.9° (c=0.94, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH (nil). PMR (C_6D_6) δ: 1.22 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$), 1.40 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$), 1.66 (3H, broad d, J=4 Hz, *sec.* C$\underline{H}_3$), [3.08 (3H), 3.12 (3H), 3.22 (3H), 3.24 (3H), 3.34 (3H), 3.38 (3H), 3.44 (9H), 3.48 (9H), 3.55 (3H), 3.68 (3H)] (s, OC$\underline{H}_3$×14), 4.12 (1H, d, J=8 Hz, anomeric H), 4.93 (1H, d, J=8 Hz, anomeric H), 5.28 (2H, s, anomeric H×2). *Anal.* Calcd for $C_{43}H_{80}O_{22}$: C, 54.41; H, 8.50. Found: 54.33; H, 8.56. Compound **21** (5 mg) was methanolyzed with methanolic 2 N HCl (2 ml) under reflux for 2 h to afford **ii**, **iii**, **iv**, **vii** and 1,4,5-tri-*O*-methylfucitol (unidentified, TLC (solvent: benzene–acetone=2: 1 *Rf* 0.27)). Compound **21** was identified as the per-*O*-methylate of 2-*O*-[β-D-galactopyranosyl(1→4)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl]-3-*O*-α-L-rhamnopyranosyl-D-fucitol by TLC (solvent: benzene–acetone=2: 1 v/v, *Rf* 0.26), IR and PMR spectral comparisons with an authentic sample obtained from senegin III.

Partial Methanolysis of 23 with Methanolic 0.2 N HCl——Compound **23** (70 mg) was refluxed with methanolic 0.2 N HCl (20 ml) for 2 h and the reaction mixture was neutralized with Ag_2CO_3 then filtered. The filtrate was evaporated to dryness *in vacuo* and the residue was examined by TLC (solvent: $CHCl_3$–MeOH=9: 1 v/v); it showed two main spots (*Rf* 0.71 and 0.24). The residue was subjected to column chromatography on silica gel, using $CHCl_3$–MeOH (50: 1—10: 1 v/v), to afford compounds **24** (30 mg, *Rf* 0.71) and **25** (24 mg, *Rf* 0.24), Compound **24**: syrup, $[\alpha]_D^{22}$ +12.2° (c=0.54, $CHCl_3$). PMR (C_6D_6) δ: [3.38 (3H), 3.40 (3H), 3.51 (6H), 3.56 (3H), 3.58 (3H), 3.61 (3H)] (s, OC$\underline{H}_3$×7), 4.30 (1H, d, J=7 Hz, anomeric H), 4.73 (1H, d, J=4 Hz, anomeric H). Compound **24** gave **ii** and **iii** on methanolysis with 2 N HCl under reflux for 2 h. Compound **25**: a syrup, $[\alpha]_D^{22}$ −6.8° (c=0.73, $CHCl_3$). PMR (C_6D_6) δ: 1.21 (3H, d, J=7 Hz, *sec.* C$\underline{H}_3$), 1.43 (3H, broad d, J=5 Hz, *sec.* C$\underline{H}_3$), 3.20, 3.23, 3.25, 3.29 (3H each, s, OC$\underline{H}_3$×4), 5.17 (1H, s, anomeric H). Compound **25** gave **viii** and **xiii** on methanolysis with 2 N HCl under reflux for 2 h.

Preparation of Compound 30 for CMR Analysis——Onjisaponin F monomethyl ester[1] (1 g) in 95% MeOH (400 ml) was oxidized with 30 ml of aqueous solution of $NaIO_4$ (3 g). The mixture was kept overnight at 4°C with stirring. The precipitate was filtered off and the filtrate was concentrated *in vacuo* at below 50°C to remove MeOH. The concentrated solution was diluted with water and then extracted with BuOH. The BuOH solution was washed with water and evaporated to dryness. The residue was dissolved in 95% MeOH (150 ml) and $NaBH_4$ (1 g) was added to the solution at room temperature with stirring. The mixture was kept for 2 h with stirring, then neutralized with 5% AcOH. The solution was concentrated at below 50°C under reduced pressure to remove MeOH and the concentrated solution was extracted with BuOH. The BuOH solution was washed with water and evaporated to dryness *in vacuo*. The residue was refluxed with 0.05 N H_2SO_4 in 50% MeOH (150 ml) for 30 min on a water bath. The reaction mixture was neutralized with $NaHCO_3$ and then concentrated to remove MeOH. The concentrated aqueous solution was extracted with BuOH and the BuOH solution was washed with water. The BuOH solution was evaporated to dryness under reduced pressure and the residue was dissolved in 0.5% KOH (200 ml). The solution was allowed to stand for 48 h at room temperature, then neutralized with Amberlite IR-200, and evaporated to dryness *in vacuo*. The residue was subjected to column chromatography on silica gel, using $CHCl_3$–MeOH–H_2O (70: 20: 10 v/v, lower phase), to afford compound **30** (210 mg), a white powder from aq. EtOH, (mp 228—230°C), $[\alpha]_D^{28}$ +17.2° (c=1.09, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1740 (COOR), 1710 (COOR), 1630 (C=C). CMR (C_5D_5N) δ: 94.5 (fucose-C_1), 101.0 (rhamnose-C_1), 106.9 (xylose-C_1). *Anal.* Calcd for $C_{48}H_{76}O_{19}$: C, 60.23; H, 8.00. Found: C, 59.98; H, 8.13. Compound **30** (5 mg) was refluxed with 4 N HCl–dioxane–benzene (3: 1: 2 v/v, 3 ml) on a water bath for 4 h and the reaction mixture was treated in the same way as for **1**. The monosaccharides were identified as rhamnose, fucose and xylose by TLC and GLC.

Acknowledgement The authors are grateful to Prof. O. Tanaka and Assoc. Prof. R. Kasai of Hiroshima University for measurement of CMR spectra. Thanks are also due to Assoc. Prof. Y. Ida of this laboratory for useful discussions and technical assistance throughout this study, and to the staff of the Analytical Laboratory of this School, for elemental analysis and PMR spectral measurement. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged.

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