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Studies on the Constituents of the Root of *Polygala tenuifolia* Willdenow. I.[1] Isolation of Saponins and the Structures of Onjisaponins G and F

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Seven triterpenoidal saponins, onjisaponins A, B, C, D, E, F, and G, were isolated from the roots of *Polygala tenuifolia* Willdenow. Among these saponins, the structures of onjisaponins G (**1**) and F (**2**) 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-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl}-4-O-(3′,4′,5′-trimethoxycinnamoyl)-β-D-fucopyranoside and presenegenin-(3)-β-D-glucopyranosido-(28)-2-O-{[β-D-apio-D-furanosyl-(1→3)][α-L-arabinopyranosyl(1→3)-β-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl}-4-O-(3′,4′,5′-trimethoxycinnamoyl)-β-D-fucopyranoside, respectively.

Keywords——triterpenoidal saponin; onjisaponin G; onjisaponin F; tenuifolin; D-apiose; *Polygala tenuifolia*; polygalae radix; Polygalaceae

A crude drug "Yuan zhi" (遠志, Japanese name: Onji), the dried root of *Polygala tenuifolia* Willdenow (Polygalaceae), is a well known Chinese medicine used as a expectorant, tonic and sedative. The constituents of this drug have been investigated and the presence of polygalitol (1,5-anhydro-D-sorbitol),[2] N-acetyl-D-glucosamine,[2] glucose,[2] fructose,[2] an unidentified alkaloid,[3] 3,4,5-trimethoxycinnamic acid and three xanthone derivatives[4] has been reported. Formerly, Chou *et al.*[5] and Fujita *et al.*[6] reported the isolation of sapogenins from the extract of the same crude drug by hydrolysis with mineral acid, but the sapogenins were assumed to be artifacts. Afterwards, Pelletier *et al.*[7] reported that the basic hydrolysis of the saponin fraction of *P. tenuifolia* gave tenuifolin as the major prosapogenin whose structure was confirmed to be 2β,27-dihydroxy-23-carboxy-oleanolic acid 3β-O-glucoside (=presenegenin 3β-O-glucoside) (**11**). This paper describes the isolation of seven saponins named onjisaponins A, B, C, D, E, F and G, and the structure elucidation of onjisaponin G and F, which led to the assignment of the structures (**1**) and (**2**), respectively.

The seven new onjisaponins were isolated from the methanolic extract of the commercial cut roots of *P. tenuifolia* Willdenow as shown in Chart 1 and the general properties of onjisaponins A—G are described in the experimental section.

Onjisaponin G (**1**), a white powder, $C_{70}H_{104}O_{32}$, $[\alpha]_D^{17}$ −15.0° (methanol), contained 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 absorption maxima at 232 nm (log ε 4.22) and 312 nm (log ε 4.22), and the ^{13}C nuclear magnetic resonance (CMR) spectrum showed five anomeric carbon signals at δ 94.9, 101.3, 105.3, 105.3, and 111.6. On the other hand, the IR and UV spectra of onjisaponin F (**2**), a white powder, $C_{75}H_{112}O_{36}$, $[\alpha]_D^{17}$ −10.7° (methanol), showed it to have the same functional groups as **1**, and the CMR spectrum showed six anomeric carbon signals at δ 94.7, 101.8, 104.8, 105.0, 105.3, and 111.6.

On hydrolysis with 1 N KOH, **1** and **2** gave **11** (tenuifolin=presenegenin 3β-O-glucoside) and 3,4,5-trimethoxycinnamic acid. On hydrolysis with 4 N hydrogen chloride–dioxane–benzene (3:1:2 v/v) **1** afforded D-glucose, D-fucose, L-rhamnose and D-xylose, while **2** afforded L-arabinose in addition to the same sugars as **1**. Furthermore, **1** and **2** gave D-apiose on hydrolysis with 0.2 N hydrogen chloride–dioxane (1:1 v/v). Based on the above results, the compositions of **1** and **2** were established to be one mol each of D-fucose, L-rhamnose, D-xylose,

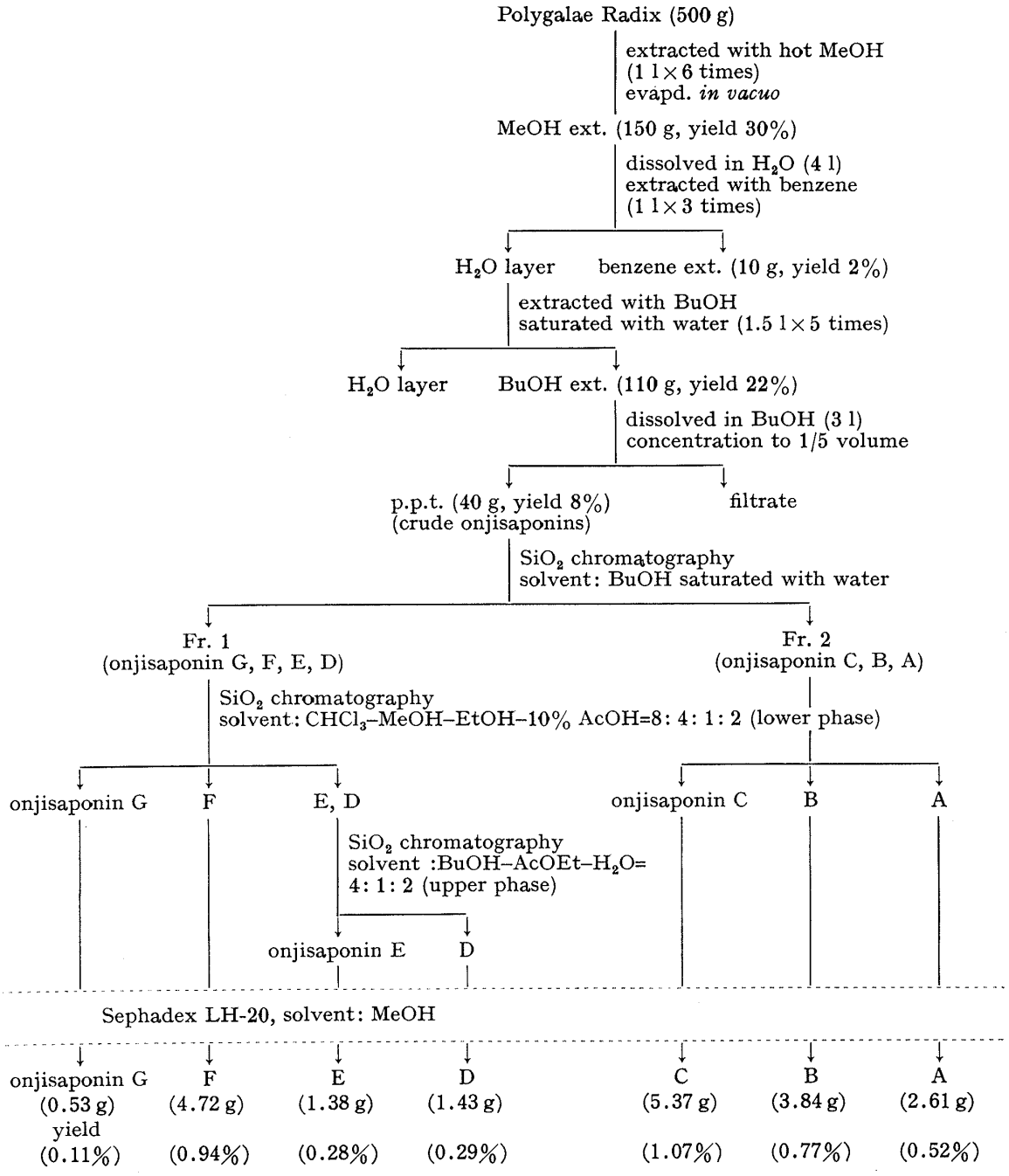


Chart 1. Extraction and Isolation of Onjisaponins

D-apiose, 3,4,5-trimethoxycinnamic acid and **11**, and D-fucose, L-rhamnose, D-xylose, D-apiose, L-arabinose, 3,4,5-trimethoxycinnamic acid and **11**, respectively.

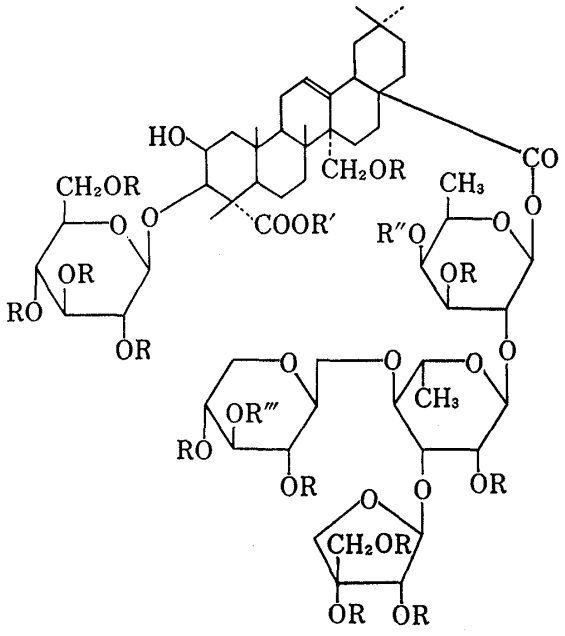
On methylation with diazomethane, **1** and **2** gave monomethyl esters (**3**), $C_{71}H_{106}O_{32}$, mp 234—236°C (dec.) and (**4**), $C_{76}H_{114}O_{36}$, mp 219—221°C (dec.), which were further methylated by Kuhn's method[8] to afford a trideca-O-methyl monomethyl ester (**5**), $C_{84}H_{132}O_{32}$, colorless needles, mp 129—131°C, and a pentadeca-O-methyl monomethyl ester (**6**), $C_{91}H_{144}O_{36}$, a white powder, mp 139—141°C, respectively.

As the IR spectra of **1** and **2** suggest the presence of two kinds of ester groups (1750 and 1730 cm^{-1}), alkali treatments of **3** and **4** were examined. When **3** and **4** were treated with 0.5% potassium hydroxide, des-3,4,5-trimethoxycinnamoyl onjisaponin G monomethyl ester (**7**), a white powder, $C_{59}H_{94}O_{28}$, $[\alpha]_D^{20}$ −8.6° (methanol), and des-3,4,5-trimethoxycinnamoyl

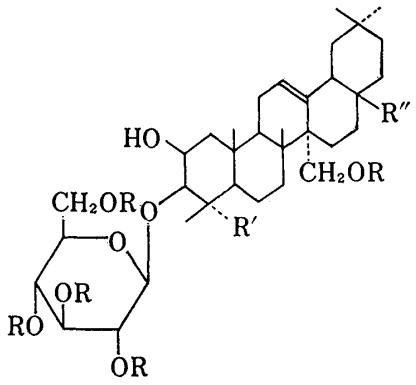
onjisaponin F monomethyl ester (**8**), a white powder, $C_{64}H_{102}O_{32}$, $[\alpha]_D^{20}$ −16.2° (methanol) were obtained and characterized by methylation according to Hakomori[9] to form des-3,4,5-trimethoxycinnamoyl onjisaponin G tetradeca-O-methyl ether monomethyl ester (**9**), $C_{73}H_{122}O_{28}$, colorless needles, mp 217—219°C, and des-3,4,5-trimethoxycinnamoyl onjisaponin F hexadeca-O-methyl ether monomethyl ester (**10**), $C_{80}H_{134}O_{32}$, colorless needles, mp 176—177°C, respectively.

Compounds **3** and **4** were hydrolyzed with 1 N potassium hydroxide under a nitrogen atmosphere to afford the same compound, $C_{37}H_{58}O_{12}\cdot H_2O$, $[\alpha]_D^{22}$ +63.0° (ethanol), colorless needles, mp 230—231°C, which was identified as tenuifolin 23-monomethyl ester (**12**) by comparison with an authentic sample obtained from senegin II[10] (mixed fusion and IR spectra). The formation of **12** from **3** and **4** suggests that of the two carboxyl groups the one at C-4 of presenegenin is present in the free form and the other at C-17 is in the ester form.

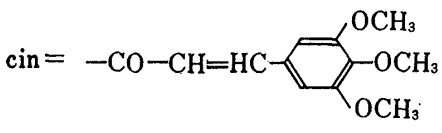
To confirm the location of 3,4,5-trimethoxycinnamic acid in onjisaponins G and F, comparative analyses of the methanolysates of **5**, **6**, **9** and **10** with methanolic 2 N hydrogen chloride were carried out. Methyl 2,3,4,6-tetra-O-methylglucopyranoside, methyl 2-O-methylrhamnopyranoside and methyl 2,3,4-tri-O-methylapioside were commonly detected in the methanolysates of compounds **5**, **6**, **9** and **10**. In addition, methyl 2,3,4-tri-O-methylxylopyranoside and methyl 3-O-methylfucopyranoside were obtained from **5**, while methyl 2,3,4-tri-O-methylxylopyranoside and methyl 3,4-di-O-methylfucopyranoside were obtained from **9**. Furthermore, methyl 3-O-methylfucopyranoside, methyl 2,4-di-O-methylxylopyranoside and methyl 2,3,4-tri-O-methylarabinopyranoside were detected in the methanolysate of **6**, while methyl 3,4-di-O-methylfucopyranoside, methyl 2,4-di-O-methylxylopyranoside and methyl 2,3,4-



1: R=R′=R‴=H, R″=cin
2: R=R′=H, R″=cin, R‴=arabinose
3: R=R‴=H, R′=CH_3, R″=cin
4: R=H, R′=CH_3, R″=cin, R‴=arabinose
5: R=R′=R‴=CH_3, R″=cin
6: R=R′=CH_3, R″=cin, R‴=arabinose
7: R=R″=R‴=H, R′=CH_3
8: R=R″=H, R′=CH_3, R‴=arabinose
9: R=R′=R″=R‴=CH_3
10: R=R′=R″=CH_3, R‴=arabinose



11: R=H, R′=R″=COOH
12: R=H, R′=$COOCH_3$, R″=COOH
13: R=CH_3, R′=R″=CH_2OH



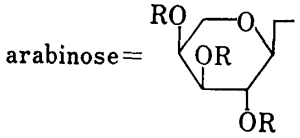


Chart 2

tri-O-methylarabinopyranoside were found in that of **10**. Consequently, the 3,4,5-trimethoxycinnamoyl groups in **1** and **2** are deduced to be located at the C-4 hydroxyl group of fucose, and the oligosaccharide moieties of **1** and **2** are suggested to be branched at the C-3 and C-4 hydroxyl groups of rhamnose.

On reduction with lithium aluminum hydride, compound **9** afforded compound **13**, $C_{41}H_{68}O_{10}$, and compound **14**, $C_{31}H_{58}O_{17}$, syrup, $[\alpha]_D^{22}$ −64.0° (chloroform), while compound **10** gave compound **15**, $C_{38}H_{70}O_{21}$, syrup, $[\alpha]_D^{22}$ −47.4° (chloroform) as well as compound **13**. The proton magnetic resonance (PMR) spectrum of **13** reveals the presence of five O-methyl groups (δ 3.24, 3.33, 3.37, 3.53 and 3.67 ppm), one anomeric proton (δ 4.30 (1H (d) J=7 Hz) and one vinyl proton (δ 5.50 multiplet). Based on the physical properties of **13** and the results of methanolysis, the structure of **13** is suggested to be olean-12-ene-27-O-methyl-2β,23,28-trihydroxy-3β-(tetra-O-methyl)-β-D-glucopyranoside, and it was shown to be identical with an authentic sample obtained from senegin II[10] by comparison of IR and NMR spectra. The

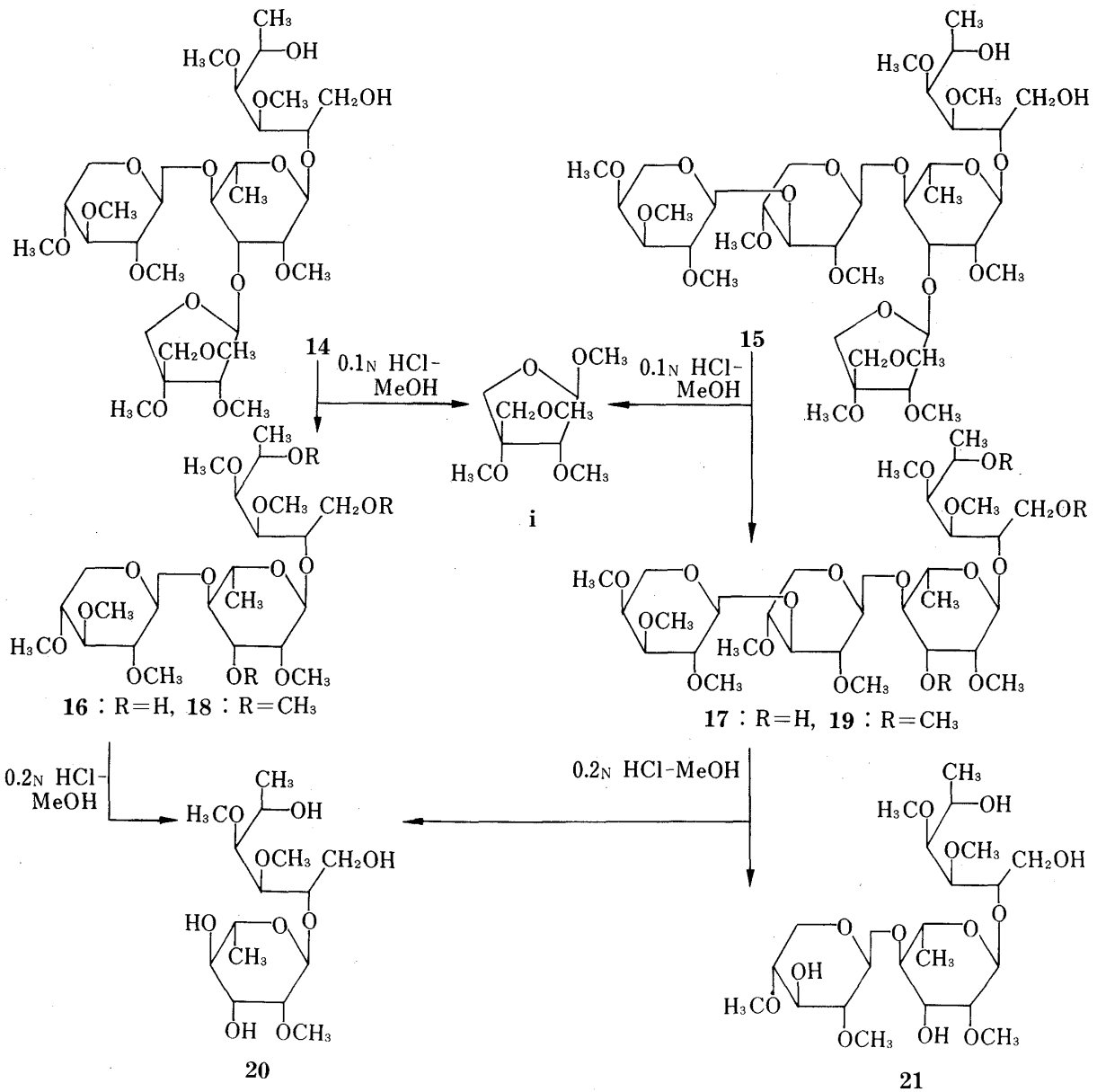


Chart 3

NMR spectrum of the other product, **14**, shows the presence of two secondary methyl groups (δ 1.27 3H(d) J=7 Hz; 1.62 3H(d) J=5 Hz), nine O-methyl groups (δ 3.22—3.65) and three anomeric protons (δ 4.76 1H(d) J=7 Hz; 5.20 1H(d) J=2 Hz; 5.50 1H(d) J=2 Hz), while that of **15** shows the presence of two secondary methyl groups (δ 1.28 3H (d) J=6 Hz; 1.70 3H (d) J=5 Hz), eleven O-methyl groups (δ 3.20—3.70) and four anomeric protons (δ 4.87 1H(d) J=8 Hz; 5.04 1H(d) J=6 Hz; 5.27 1H(d) J=2 Hz; 5.58 1H(d) J=2 Hz).

On methanolysis with 2 N hydrogen chloride, **14** and **15** gave methyl 2,3,4-tri-O-methylapioside, methyl 2-O-methylrhamnoside and 3,4-di-O-methylfucitol. In addition, methyl 2,3,4-tri-O-methylxyloside was detected from **14**, and methyl 2,4-di-O-methylxyloside and methyl 2,3,4-tri-O-methylarabinoside from **15**. The formation of 3,4-di-O-methylfucitol from **14** and **15** indicates that the oligosaccharide moieties of onjisaponins G and F link to the C-17 carboxyl group of presenegenin through an acetal hydroxyl group of fucose.

The sequence of the four monosaccharides of onjisaponin G was examined as follows. On treatment with methanolic 0.1 N hydrogen chloride by refluxing for 1 h, **14** gave methyl 2,3,4-tri-O-methyl-D-apio-D-furanoside and an O-methylated oligosaccharide (**16**) which gave 3,4-di-O-methylfucitol, methyl 2-O-methylrhamnoside and methyl 2,3,4-tri-O-methylxyloside upon methanolysis with methanolic 2 N hydrogen chloride. Oligosaccharide **16** was refluxed with methanolic 0.2 N hydrogen chloride to afford an O-methylated oligosaccharide (**20**) which gave 3,4-di-O-methylfucitol and methyl 2-O-methylrhamnoside on hydrolysis with methanolic 2 N hydrogen chloride.

After methylation of **16** by Hakomori's method, the resultant per-O-methylated oligosaccharide (**18**) was methanolyzed with methanolic 2 N hydrogen chloride to afford methyl 2,3-di-O-methylrhamnopyranoside, methyl 2,3,4-tri-O-methylxylopyranoside and 1,3,4,5-tetra-O-methylfucitol (unidentified). Consequently, the oligosaccharide of onjisaponin G was suggested to be 2-O-{[D-apio-D-furanosyl(1→3)] [D-xylopyranosyl(1→4)]-L-rhamnopyranosyl}-4-O-(3′,4′,-5′-trimethoxycinnamoyl)-D-fucopyranoside. The configuration of each monosaccharide of onjisaponin G was assigned from the value of coupling constant of each anomeric proton in the PMR spectrum and by comparison of the molecular optical rotations of the partial hydrolysis products (Table I). From these experimental data, the structure of onjisaponin G has been established to be presenegenin-(3)-O-β-D-glucopyranosido-(28)-2-O-{[β-D-apio-D-furanosyl(1→3)][β-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl}-4-O-(3′,4′,5′-trimethoxycinnamoyl)-β-D-fucopyranoside.

TABLE I

	Onjisaponin G (1)		Onjisaponin F (2)	
Apiose → rhamnose	$[M]_{D\cdot(14)}$—$[M]_{D\cdot(16)}$ −212°[a]	β	$[M]_{D\cdot(15)}$—$[M]_{D\cdot(17)}$ −184.5°[a]	β
Arabinose → xylose			$[M]_{D\cdot(17)}$—$[M]_{D\cdot(21)}$ +40°[e]	α
			NMR anomeric H of (**10**)	
			δ=4.75, J=5 Hz	α
Xylose → rhamnose	$[M]_{D\cdot(16)}$—$[M]_{D\cdot(20)}$ −131.6°[b]	β	$[M]_{D\cdot(21)}$—$[M]_{D\cdot(20)}$ −158.2°[b]	β
	NMR anomeric H of (**9**)		NMR anomeric H of (**10**)	
	δ=4.51, J=7 Hz	β	δ=4.54, J=8 Hz	β
Rhamnose → fucose	$[M]_{D\cdot(20)}$—$[M]_{D\cdot DMF}$[d] −106.3°[c]	α	$[M]_{D\cdot(20)}$—$[M]_{D\cdot DMF}$[d] −106.3°[c]	α
Fucose → aglycon	NMR anomeric H of (**9**)		NMR anomeric H of (**10**)	
	δ =5.42, J=8 Hz	β	δ =5.40, J=8 Hz	β

a) 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°.
b) Methyl 2,3,4-tri-O-methyl-α-D-xylopyranoside: $[M]_D$ +232.4°.
Methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside: $[M]_D$ −143.3°.
c) Methyl 2-O-methyl-α-L-rhamnopyranoside: $[M]_D$ −94.2°.
d) DMF=3,4-di-O-methyl-D-fucitol: $[M]_D$ 0°.
e) Methyl 2,3,4-tri-O-methyl-α-L-arabinopyranoside: $[M]_D$ +95.3°.
Methyl 2,3,4-tri-O-methyl-β-L-arabinopyranoside: $[M]_D$ +515.6°.

Furthermore, the structure of oligosaccharide **15**, obtained from onjisaponin F was established as follows. On refluxing with methanolic 0.1 N hydrogen chloride, **15** gave methyl 2,3,4-tri-O-methyl-D-apio-D-furanoside and an O-methylated oligosaccharide (**17**) which gave 3,4-di-O-methylfucitol, methyl 2-O-methylrhamnoside, methyl 2,4-di-O-methylxyloside and methyl 2,3,4-tri-O-methylarabinoside upon hydrolysis with methanolic 2 N hydrogen chloride. Oligosaccharide **17** was refluxed with methanolic 0.2 N hydrogen chloride to afford two kinds of O-methylated oligosaccharides (**20** and **21**). On methanolysis, oligosaccharide **20** gave 3,4-di-O-methylfucitol and methyl 2-O-methylrhamnoside, while oligosaccharide **21** gave 3,4-di-O-methylfucitol, methyl 2-O-methylrhamnoside and methyl 2,4-di-O-methylxyloside. Furthermore, oligosaccharide **17** was methylated by Hakomori's method to afford an undeca-O-methylated oligosaccharide (**19**) which was methanolyzed with methanolic 2 N hydrogen chloride to give methyl 2,3-di-O-methylrhamnoside, methyl 2,4-di-O-methylxyloside, methyl 2,3,4-tri-O-methylarabinoside and 1,3,4,5-tetra-O-methylfucitol (unidentified). Based on the above results the structure of the oligosaccharide of onjisaponin F was deduced to be 2-O-{[D-apio-D-furanosyl (1→3)] [L-arabinopyranosyl (1→3)-D-xylopyranosyl (1→4)]-L-rhamnopyranosyl}-4-O-(3′,4′,5′-trimethoxycinnamoyl)-D-fucopyranose. The configuration of each monosaccharide of onjisaponin F was assigned from the value of coupling constant of each anomeric proton in PMR spectrum and by comparison of the molecular optical rotations of the partial methanolysis products (Table I). Consequently, the structure of onjisaponin F has been established to be presenegenin-(3)-O-β-D-glucopyranosido-(28)-2-O-{[β-D-apio-D-furanosyl-(1→3)][α-L-arabinopyranosyl(1→3)-β-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl}-4-O-(3′,4′-5′-trimethoxycinnamoyl)-β-D-fucopyranoside.

Pharmacological and biological activities of onjisaponins will be reported in another paper.

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 spectrophotometer, IR spectra with a JASCO IRA-1 unit, PMR spectra with a Hitachi R-22 (90 MHz) spectrometer, and CMR spectra with JEOL JNM-FX-100 FT NMR spectrometer (25.15 MHz) equipped with a JEC-980B computer. Chemical shifts are given on a δ(ppm) scale with tetramethylsilane as an internal standard. 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.

Extraction and Isolation of Onjisaponins——Polygalae radices (dried roots of *Polygala tenuifolia* WILLDENOW) were crushed and treated as shown in Chart 1. The crude saponin fraction was subjected to column chromatography on silica gel with BuOH saturated with H_2O to separate fraction 1 (a mixture of onjisaponins D, E, F and G) and Fr. 2 (a mixture of onjisaponins A, B and C). Each fraction was rechromatographed on silica gel, eluting with $CHCl_3$–MeOH–EtOH–10% AcOH (8: 4: 1: 2 v/v, lower phase), to afford crude onjisaponins A, B, C, F, G and a mixture of onjisaponins D and E. A mixture of onjisaponins D and E was rechromatographed on silica gel, eluting with BuOH–AcOEt–H_2O (4: 1: 2 v/v, upper phase) to separate crude onjisaponins E and D. Crude onjisaponins A, B, C, D, E, F and G were each subjected to Sephadex LH-20 chromatography, eluting with MeOH, to afford chromatographically pure onjisaponins. The yield of each onjisaponin is shown in Chart 1.

Properties of Onjisaponins A, B, C, D, E, F and G——Onjisaponin A: A white powder from EtOH, (mp 253—254°C (dec.)), $[\alpha]_D^{17}$ −18.4° (c=1.24, MeOH), UV λ_{max}^{EtOH} nm: 316, IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid). *Anal.* Found: C, 54.70; H, 7.26.

Onjisaponin B: 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: 316, IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid). *Anal.* Found: C, 55.18; H, 7.22.

Onjisaponin C: A white powder from aq. EtOH, (mp 264—266°C (dec.)), $[\alpha]_D^{17}$ −19.3° (c=1.63, MeOH), UV λ_{max}^{EtOH} nm: 316, IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid). *Anal.* Found: C, 54.72; H, 7.36.

Onjisaponin D: Colorless needles from EtOH, mp 248—251°C (dec.), $[\alpha]_D^{17}$ −17.3° (c=1.27, MeOH), UV λ_{max}^{EtOH} nm: 316, IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid). *Anal.* Found: C, 54.76; H, 7.21.

Onjisaponin E: Colorless needles from aq. EtOH, mp 245—247°C (dec.), $[\alpha]_D^{17}$ −6.5° (c=1.00, MeOH),

UV λ_{max}^{EtOH} nm: 232, 312, IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1750, 1730 (COOR), 1710 (COOH), 1635 (C=C), 1580, 1500 (benzenoid). *Anal.* Found: C, 54.87; H, 7.25.

Onjisaponin F (**2**): A white powder from EtOH, (mp 246—249°C (dec.)), $[\alpha]_D^{17}$ −10.7° (c=1.15, MeOH), UV λ_{max}^{EtOH} nm (log ε): 232 (4.25), 312 (4.25), 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: 94.7, 101.8, 104.8, 105.0, 105.3, 111.6 (six anomeric carbons). *Anal.* Calcd for $C_{75}H_{112}O_{36}$: C, 56.66; H, 7.10. Found: C, 56.29; H, 7.20.

Onjisaponin G (**1**): A white powder from EtOH, (mp 245—247°C (dec.)), $[\alpha]_D^{17}$ −15.0° (c=1.33, MeOH), UV λ_{max}^{EtOH} nm (log ε): 232 (4.22), 312 (4.22), 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: 94.9, 101.3, 105.3, 105.3, 111.6 (five anomeric carbons). *Anal.* Calcd for $C_{70}H_{104}O_{32}$: C, 57.68; H, 7.19. Found: C, 57.07; H, 7.21.

Hydrolysis of 1 and 2 with 1 N KOH——A solution of **1** (200 mg) or **2** (200 mg) in 1 N KOH (15 ml) was heated under an N_2 gas flow at 95°C 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 give 3,4,5-trimethoxycinnamic acid, colorless needles from aq. EtOH, mp 127—128°C, UV λ_{max}^{EtOH} nm (log ε): 223 (4.31), 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>), *Anal.* Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.71; H, 6.02. 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 and then evaporated to dryness. 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 (**11**) from aq. EtOH, which were identical with an authentic sample of tenuifolin obtained from senegins. mp 299—300°C (dec.), $[\alpha]_D^{24}$ +42.3° (c=1.16, MeOH), PMR (C_5D_5N) δ: 0.88, 1.00, 1.03, 1.50, 1.90 (3H each, s, *tert* C<u>H</u>$_3$×5), 5.04 (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, 62.01; H, 8.43. A methanolic solution (8 ml) of **11** (52 mg) was treated with an excess of ethereal diazomethane and allowed to stand for 5 h. After the excess reagent had been decomposed with 5% AcOH, the solvent was removed under reduced pressure and the residue was crystallized from AcOEt to give colorless needles (36 mg), mp 269—271°C, $[\alpha]_D^{18}$ +39.4° (c= 1.21, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730, 1240 (COOR), *Anal.* Calcd for $C_{38}H_{60}O_{12}$: C, 64.38; H, 8.62. Found: C, 64.27; H, 8.62. The product was identical with an authentic sample of tenuifolin dimethyl ester (mixed fusion and IR spectra), which was further acetylated with pyridine and Ac_2O for 2 d at room temperature to afford a dimethyl ester pentaacetate of **11**, colorless needles from $CHCl_3$–hexane, mp 220—222°C, $[\alpha]_D^{23}$ +70.8° (c=1.13, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3570 (OH), 1760, 1740 (COOR), PMR ($CDCl_3$) δ: 0.70, 0.89, 0.95, 1.25, 1.34 (3H each, s, *tert* C<u>H</u>$_3$×5), [2.01 (3H), 2.03 (6H), 2.06 (3H), 2.09 (3H)] (s, OCOC<u>H</u>$_3$×5), 3.65, 3.73 (3H each, s, COOC<u>H</u>$_3$×2), 4.45 (1H, d, J=7 Hz, anomeric H), 5.59 (1H, m, olefinic H), *Anal.* Calcd for $C_{48}H_{70}O_{17}$: C, 62.73; H, 7.68. Found: C, 62.57; H, 7.70. This product was identical with an authentic sample of tenuifolin dimethyl ester pentaacetate (mixed fusion and IR spectra).

Hydrolysis of 1 and 2 with 4 N HCl–Dioxane–Benzene (3: 1: 2 v/v)——Compounds **1** and **2** (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;[11]) 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/cm^2; sample, tetramethylsilyl ether (TMS) derivatives). onjisaponin G (**1**): TLC *Rf* 0.33 (rhamnose), 0.30 (fucose), 0.26 (xylose), 0.16 (glucose). PPC *Rf* 0.48 (rhamnose), 0.42 (fucose), 0.35 (xylose), 0.25 (glucose). GLC t_R (min) 4.9, 6.5 (rhamnose), 5.9, 7.0 (fucose), 6.8, 8.5 (xylose), 15.8, 24.6 (glucose). Onjisaponin F (**2**): TLC *Rf* 0.33 (rhamnose), 0.30 (fucose), 0.26 (xylose), 0.22 (arabinose), 0.16 (glucose). PPC *Rf* 0.48 (rhamnose), 0.42 (fucose), 0.35 (xylose), 0.33 (arabinose), 0.25 (glucose). GLC t_R(min) 4.6, 5.3 (arabinose), 4.9, 6.5 (rhamnose), 5.9, 7.0 (fucose), 6.8, 8.5 (xylose), 15.8, 24.6 (glucose).

Compound **1** (480 mg) was refluxed with 4 N HCl–dioxane–benzene (3: 1: 2 v/v, 60 ml) on a water bath for 4 h. The reaction mixture was treated by the procedure described above. The residue was separated by column chromatography on silica gel, using $CHCl_3$–MeOH–H_2O (7: 3: 0.5 v/v), to afford L-rhamnose $[\alpha]_D^{22}$ +8.0° (c=1.37, H_2O), lit.[12] $[\alpha]_D$ +8.5°, D-fucose $[\alpha]_D^{22}$ +63.9° (c=1.22, H_2O), lit.[13] $[\alpha]_D$ +76.0° and D-xylose $[\alpha]_D^{22}$ +17.5° (c=1.14, H_2O), lit.[14] $[\alpha]_D$ +20.2°.

Hydrolysis of 1 and 2 with 0.2 N HCl–Dioxane (1: 1)——Compounds **1** and **2** (15 mg each) were each 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[15]) 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 and 2 with CH_2N_2——Compounds **1** and **2** (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 G monomethyl ester (**3**, 320 mg), and onjisaponin F monomethyl ester (**4**, 350 mg). **3**: a white powder from aq. EtOH, (mp 234—236°C (dec.)), $[\alpha]_D^{24}$ −16.2° (c=1.05, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), 1580, 1500 (benzenoid). *Anal.* Calcd for $C_{71}H_{106}O_{32}$: C, 57.95; H, 7.26. Found: C, 57.54; H, 7.24. **4**: a white powder from aq. EtOH, (mp 219—221°C (dec.)), $[\alpha]_D^{22}$ −11.9° (c=1.36, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), 1580, 1500 (benzenoid). *Anal.* Calcd for $C_{76}H_{114}O_{36}$: C, 56.92; H, 7.17. Found: C, 56.54; H, 7.26.

Methylation of 3 and 4 by Kuhn's Method——Compounds **3** and **4** were methylated by Kuhn's method. To a solution of **3** (220 mg) in dimethylformamide (DMF, 12 ml) was added 3 ml of CH_3I and 2 g of freshly prepared Ag_2O. The reaction mixture was stirred for 4 d at room temperature and filtered. The filtrate was diluted with water and extracted with $CHCl_3$ several times. The $CHCl_3$ solutions were combined, washed with water and dried over Na_2SO_4. The solvents were removed *in vacuo* and the residue was purified by column chromatography on silica gel, eluting with benzene–acetone (4: 1 v/v), to give a trideca-O-methyl monomethyl ester (**5**, 45 mg), which was recrystallized from MeOH to afford colorless needles, mp 129—131°C, $[\alpha]_D^{20}$ −6.7° (c=1.95, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3600 (OH), 1730 (COOR), 1580, 1500 (benzenoid), PMR ($CDCl_3$) δ: 0.80, 0.95, 1.01, 1.36, 1.47 (3H each, s, *tert* C$\underline{H}_3\times5$), 1.35 (6H, C$\underline{H}_3\times2$), [3.39 (3H), 3.45 (6H), 3.48 (6H), 3.51 (6H), 3.56 (3H), 3.59 (9H), 3.63 (3H), 3.67 (3H)] (s, OC$\underline{H}_3\times13$), 3.78 (3H, s, COOC$\underline{H}_3$), 3.94, 3.95, 3.98 (3H each, s, arom. OC$\underline{H}_3\times3$), 4.22 (1H, d, J=7 Hz, anomeric H), 4.55 (1H, d, J=7 Hz, anomeric H), 5.27 (2H, s, anomeric H×2), 5.30 (1H, m, $\underline{H}$–C–OCOR), 5.48 (1H, d, J=8 Hz, anomeric H), 5.60 (1H, m, olefinic H), 6.50 (1H, d, J=16 Hz, olefinic H), 6.88 (2H, s, arom. H×2), 7.74 (1H, d, J=16 Hz, olefinic H), *Anal.* Calcd for $C_{84}H_{132}O_{32}$: C, 61.00; H, 8.05. Found: C, 59.82; H, 8.20.

Compound **4** (310 mg) was methylated by the procedure described above to give a pentadeca-O-methyl monomethyl ester (**6**, 58 mg), which was precipitated from aq. MeOH to afford a white powder, (mp 139—141°C), $[\alpha]_D^{22}$ −4.3° (c=0.89, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3600 (OH), 1730 (COOR), 1580, 1500 (benzenoid), PMR ($CDCl_3$) δ: 0.78, 0.94, 0.99, 1.23, 1.42 (3H each, s, *tert* C$\underline{H}_3\times5$), *ca.* 1.28 (6H, C$\underline{H}_3\times2$), [3.32 (3H), 3.38 (3H), 3.40 (3H), 3.44 (9H), 3.51 (3H), 3.54 (12H), 3.56 (3H), 3.57 (3H), 3.59 (3H), 3.62 (3H)] (all s, OC$\underline{H}_3\times15$), 3.76 (3H, s, COOC$\underline{H}_3$), 3.90, 3.92, 3.95 (3H each, s, arom. OC$\underline{H}_3\times3$), 4.22 (1H, d, J=7 Hz, anomeric H), 4.55 (1H, d, J=8 Hz, anomeric H), 4.76 (1H, d, J=5 Hz, anomeric H), 5.24 (2H, s, anomeric H×2), 5.30 (1H, m, $\underline{H}$–C–OCOR), 5.50 (1H, d, J=8 Hz, anomeric H), 5.60 (1H, m, olefinic H), 6.47 (1H, d, J=16 Hz, olefinic H), 6.84 (2H, s, aromatic H×2), 7.68 (1H, d, J=16 Hz, olefinic H), *Anal.* Calcd for $C_{91}H_{144}O_{36}$: C, 60.25; H, 8.00. Found: C, 59.95; H, 8.28.

Hydrolysis of 3 and 4 with 1 N KOH (Formation of Tenuifolin Monomethyl Ester (12))——Compounds **3** and **4** (300 mg each) were each dissolved in 1 N KOH (50 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). The product was obtained as colorless needles (43 mg from **3** and 39 mg from **4**) from AcOEt saturated with water, mp 230—231°, $[\alpha]_D^{22}$ +63.0° (c=0.80, EtOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), 1700 (COOH), PMR (C_5D_5N) δ: 0.87, 0.98, 1.04, 1.48, 1.90 (3H each, s, *tert* C$\underline{H}_3\times5$), 3.80 (3H, s, COOC$\underline{H}_3$), 4.98 (1H, d, J=7 Hz, anomeric H), 5.80 (1H, m, olefinic H), *Anal.* Calcd for $C_{37}H_{58}O_{12}\cdot H_2O$: C, 62.34; H, 8.41. Found: C, 62.10; H, 8.59. Each product was identical with an authentic sample of tenuifolin monomethyl ester (**12**) obtained from senegin II (mixed fusion and IR spectra).

Hydrolysis of 3 and 4 with 0.5% KOH——Compound **3** (750 mg) was dissolved in 0.5% KOH (200 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 des-3,4,5-trimethoxycinnamoyl onjisaponin G monomethyl ester (**7**, 420 mg), a white powder from aq. EtOH, (mp 241—243°C (dec.)), $[\alpha]_D^{20}$ −8.6° (c=0.81, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), *Anal.* Calcd for $C_{59}H_{94}O_{28}$: C, 56.63; H, 7.57. Found: C, 56.31; H, 7.48.

Compound **4** (1.2 g) was hydrolyzed by the procedure described above to give des-3,4,5-trimethoxycinnamoyl onjisaponin F monomethyl ester (**8**, 870 mg), a white powder from aq. EtOH, (mp 247—248°C (dec.)), $[\alpha]_D^{22}$ −16.2° (c=1.73, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (COOR), *Anal.* Calcd for $C_{64}H_{102}O_{32}$: C, 55.56; H, 7.43. Found: C, 55.06; H, 7.24.

Methylation of 7 and 8 by Hakomori's Method——According to Hakomori's method, NaH (400 mg) was stirred with dimethylsulfoxide (DMSO, 8 ml) at 65°C for 1 h under an N_2 gas flow. A solution of **7** (370 mg) in DMSO (3 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 (3 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 des-3,4,5-trimethoxycinnamoyl onjisaponin G tetradeca-O-methyl monomethyl ester (**9**, 230 mg), colorless needles from MeOH, mp 217—219°C, $[\alpha]_D^{29}$ −1.5° (c=2.18, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3600 (OH), 1750, 1730, 1240 (COOR), PMR ($CDCl_3$) δ: 0.76, 0.90, 0.94, 1.20, 1.42 (3H each, s, *tert* CH_3×5), 1.23 (3H, d, J=6 Hz, *sec* CH_3), 1.28 (3H, d, J=6 Hz, *sec* CH_3), [3.27 (3H), 3.33 (3H), 3.37 (3H), 3.40 (3H), 3.42 (6H), 3.47 (3H), 3.49 (6H), 3.50 (3H), 3.54 (6H), 3.56 (3H), 3.60 (3H)] (s, OCH_3×14), 3.74 (3H, s, $COOCH_3$), 4.22 (1H, d, J=6 Hz, anomeric H), 4.51 (1H, d, J=7 Hz, anomeric H), 5.25 (2H, s, anomeric H×2), 4.52 (1H, d, J=8 Hz, anomeric H), 5.54 (1H, m, olefinic H). *Anal.* Calcd for $C_{73}H_{122}O_{28}$: C, 60.56; H, 8.49. Found: C, 60.30; H, 8.43.

Compound **8** (930 mg) was methylated by the procedure described above to give des-3,4,5-trimethoxycinnamoyl onjisaponin F hexadeca-O-methyl ether monomethyl ester (**10**, 580 mg), colorless needles from MeOH, mp 176—177°C, $[\alpha]_D^{20}$ +2.0° (c=1.00, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3600 (OH), 1730, 1240 (COOR), PMR ($CDCl_3$) δ: 0.78, 0.92, 0.97, 1.22, 1.44 (3H each, s, *tert* CH_3×5), *ca.* 1.28 (6H, CH_3×2), [3.31 (3H), 3.38 (3H), 3.40 (3H), 3.45 (3H), 3.48 (9H), 3.50 (3H), 3.54 (15H), 3.57 (3H), 3.60 (3H), 3.66 (3H)] (s, OCH_3×16), 3.79 (3H, s, $COOCH_3$), 4.20 (1H, d, J=6 Hz, anomeric H), 4.54 (1H, d, J=8 Hz, anomeric H), 4.75 (1H, d, J=5 Hz, anomeric H), 5.20 (2H, s, anomeric H×2), 5.45 (1H, d, J=8 Hz, anomeric H), 5.49 (1H, m, olefinic H), *Anal.* Calcd for $C_{80}H_{134}O_{32}$: C, 59.76; H, 8.40. Found: C, 59.82; H, 8.51.

Methanolysis of 5, 6, 9 and 10 with Methanolic 2 N HCl——Compounds **5**, **6**, **9** and **10** (20 mg each) were each refluxed with methanolic 2 N HCl (4 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 **5**: methyl 2,3,4-tri-O-methyl-D-apio-D-furanoside (**i**), methyl 2,3,4-tri-O-methyl-D-xylopyranoside (**ii**), methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside (**iii**), methyl 3-O-methyl-D-fucopyranoside (**iv**), and methyl 2-O-methyl-L-rhamnopyranoside (**v**). Compound **6**: (**i**), (**iii**), (**iv**), (**v**), methyl 2,3,4-tri-O-methyl-L-arabinopyranoside (**vi**), and methyl 2,4-di-O-methyl-D-xylopyranoside (**vii**). Compound **9**: (**i**), (**ii**), (**iii**), (**v**), and methyl 3,4-di-O-methyl-D-fucopyranoside (**viii**). Compound **10**: (**i**), (**iii**), (**v**), (**vi**), (**vii**), and (**viii**). TLC (solvent: benzene–acetone (2: 1 v/v): *Rf* 0.62 (**i**); 0.62, 0.58 (**ii**); 0.60, 0.54 (**iii**), 0.33 (**vi**), 0.26 (**viii**), 0.28, 0.21 (**vii**), 0.17 (**v**), 0.09 (**iv**). 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/cm²) t_R(min) 2.3 (**i**), 2.3, 2.8 (**ii**), 4.0 (**vi**), 5.2, 6.4 (**iii**), 5.9, 8.4 (**vii**), 7.4, 11.6 (**viii**), 14.3 (**iv**, **v**).

Each methanolysate of **5**, **6**, **9** and **10** 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-methyl-D-fucopyranoside), 6.6 (methyl 3,4-di-O-acetyl-2-O-methyl-L-rhamnopyranoside.

Compound **10** (310 mg) was methanolyzed with methanolic 2 N HCl (20 ml) for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue was heated with 2 N HCl (20 ml) on a boiling water bath for 2 h. The reaction mixture was neutralized with Amberlite MB-3 and evaporated to dryness *in vacuo*. The residue was examined by TLC (solvent: benzene–acetone=2: 1 v/v; *Rf* 0.45, 0.33, 0.29, 0.20, 0.17 and 0.11) and subjected to column chromatography on silica gel, eluting with $CHCl_3$–MeOH (50: 1 v/v), to afford 2,3,4-tri-O-methyl-L-arabinose (*Rf* 0.29), $[\alpha]_D^{18}$ +128.9° (c=1.35, H_2O), lit.[16] $[\alpha]_D$ +157°, 2,4-di-O-methyl-D-xylose (*Rf* 0.20), $[\alpha]_D^{19}$ +19.1° (c=1.15, H_2O), lit.[17] $[\alpha]_D$ +22°, 3,4-di-O-methyl-D-fucose (*Rf* 0.17), $[\alpha]_D$ +107.8° (c=1.41, H_2O), and 2-O-methyl-L-rhamnose (*Rf* 0.11), $[\alpha]_D^{19}$ +32.8° (c=1.28, H_2O), lit.[18] $[\alpha]_D$ +31°.

Reductive Cleavage of 9 and 10 with Lithium Aluminum Hydride——A solution of compound **9** (280 mg) in dried tetrahydrofuran (THF) was refluxed with 100 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 **13** as a white powder from hexane, (mp 124—126°C), $[\alpha]_D^{20}$ +50.0° (c=1.60, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3460 (OH), PMR ($CDCl_3$) δ: [0.90 (6H), 0.95 (3H), 0.98 (3H), 1.24 (3H)] (s, *tert* CH_3×5), 3.24, 3.33, 3.37, 3.53, 3.67, (3H each, s, OCH_3×5), 4.30 (1H, d, J=7 Hz, anomeric H), 5.50 (1H, m, olefinic H). Compound **13** 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 spectra and co-TLC (solvent: benzene–acetone=2: 1 v/v *Rf* 0.48)).

On methanolysis with methanolic 2 N HCl, compound **13** gave **iii**, which was identified by TLC and GLC.

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 nona-O-methyl oligosaccharide (**14**, 150 mg), syrup, $[\alpha]_D^{28}$ −64.0° (c=2.25, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), PMR (C_6D_6) δ: 1.27 (3H, d, J=7 Hz, *sec* CH_3), 1.62 (3H, d, J=5 Hz, *sec* CH_3), [3.22 (6H), 3.38 (6H), 3.44 (3H), 3.47 (3H), 3.52 (3H), 3.57 (3H), 3.65 (3H)] (s, OCH_3×9), 4.76 (1H, d, J=7 Hz, anomeric H), 5.20 (1H, d, J=2 Hz, anomeric H), 5.50 (1H, d, J=2 Hz, anomeric H), *Anal.* Calcd for $C_{31}H_{58}O_{17}$: C, 52.98; H, 8.32. Found: C, 53.11; H, 8.54.

Compound **10** (685 mg) was treated with $LiAlH_4$ by the procedure described above to give **13** and an

undeca-O-methyl oligosaccharide (322 mg, **16**), syrup, $[\alpha]_D^{22}$ −47.4° (c=1.25, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), PMR (C_6D_6) δ: 1.28 (3H, d, J=6 Hz, *sec* C$\underline{H}_3$), 1.70 (3H, d, J=5 Hz, *sec* C$\underline{H}_3$), [3.20 (6H), 3.36 (6H), 3.42 (3H), 3.45 (3H), 3.49 (3H), 3.54 (3H), 3.60 (3H), 3.62 (3H), 3.70 (3H)] (s, OC$\underline{H}_3$×11), 4.87 (1H, d, J= 8 Hz, anomeric H), 5.04 (1H, d, J=6 Hz, anomeric H), 5.27 (1H, d, J=2 Hz, anomeric H), 5.58 (1H, d, J=2 Hz, anomeric H). *Anal.* Calcd for $C_{38}H_{70}O_{21}$: C, 52.89; H, 8.18. Found: C, 52.63; H, 8.22.

Methanolysis of 14 and 15 with Methanolic 2 N HCl——Compounds **14** and **15** (6 mg each) were each refluxed with methanolic 2 N HCl for 2 h, and the reaction mixtures were neutralized with Ag_2CO_3 and filtered. Each filtrate was evaporated to dryness *in vacuo* and the residue was examined by TLC and GLC. **14**: **i**, **ii**, **v** and 3,4-di-O-methyl-D-fucitol (**ix**). **15**: **i**, **v**, **vi**, **vii** and **ix**. Isolation and identification of compound **ix** is described later.

Partial Methanolysis of 14 with Methanolic 0.1 N HCl——Compound **14** (220 mg) was refluxed with methanolic 0.1 N HCl (10 ml) for 1 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); it showed two main spots (*Rf* 0.72 and 0.49). The products corresponding to *Rf* 0.72 and 0.49 were isolated by column chromatography on silica gel using $CHCl_3$–MeOH=100: 1 v/v. The former (*Rf* 0.72), colorless oil (18 mg), $[\alpha]_D^{19}$ −67.9° (c=0.82, $CHCl_3$) lit.[19] $[\alpha]_D$ −79°, PMR ($CDCl_3$) δ: 3.40, 3.42, 3.46, 3.50 (3H each, s, OC$\underline{H}_3$×4), 3.62 (1H, d, J=2.5 Hz, $\underline{H}$–C–OR), 4.01 (2H, q, J=10 and 5 Hz, –C$\underline{H}_2$–OR), 4.97 (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 (**i**)[20] obtained from apiin permethylate[21] (co-TLC, GLC and PMR spectra).

The latter (**16**, *Rf* 0.49), syrup (63 mg), $[\alpha]_D^{27}$ −45.0° (c=0.33, $CHCl_3$), PMR (C_6D_6) δ: 1.16 (3H, d, J= 6 Hz, *sec* C$\underline{H}_3$), 1.67 (3H, d, J=6 Hz, *sec* C$\underline{H}_3$), [3.07 (3H), 3.19 (3H), 3.25(3H), 3.46 (3H), 3.49 (6H)] (s, OC$\underline{H}_3$×6), 4.62 (1H, d, J=6 Hz, anomeric H), 5.20 (1H, s, anomeric H), gave **ii**, **v** and **ix** on methanolysis with 2 N HCl under reflux for 2 h.

Partial Methanolysis of 16 with Methanolic 0.2 N HCl——Compound **16** (45 mg) was refluxed with methanolic 0.2 N HCl for 2 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) to reveal the presence of **ii**, **v**, **ix**, **20** (*Rf* 0.16), and another compound (*Rf* 0.40). The residue was subjected to column chromatography on silica gel, eluting with $CHCl_3$–MeOH (100: 1 v/v), to afford the compounds corresponding to the spots of *Rf* 0.40, 0.20 (**ix**) and 0.16.

The compound with *Rf* 0.40 was considered to be a disaccharide composed of 2,3,4-tri-O-methyl-D-xylopyranose and **v** based on the results of methanolysis with methanolic 2 N HCl under reflux for 2 h.

Compound **ix** was obtained as colorless needles from a mixture of acetone–hexane, mp 96—97°C, $[\alpha]_D^{24}$ 0° (c=0.35, $CHCl_3$), PMR ($CDCl_3$) δ: 1.34 (3H, d, J=6 Hz, *sec* C$\underline{H}_3$), 3.54 (6H, s, OC$\underline{H}_3$×2). It was identified as 3,4-di-O-methyl-D-fucitol by mixed fusion with an authentic sample. Acetylation of 3,4-di-O-methyl-D-fucitol afforded the 1,2,5-tri-O-acetyl derivative of **ix**, which was identical with an authentic sample as judged by GLC (column, 3% ECNSS-M on Gas Chrom Q 100—120 mesh (3 mm×2 m); column temperature, 180°C; carrier gas, N_2 0.9 kg/cm^2); t_R(min) 16.3 (1,2,5-tri-O-acetyl-3,4-di-O-methyl-D-fucitol).

Compound **20** (*Rf* 0.16) was obtained as a syrup, $[\alpha]_D^{24}$ −30.0° (c=0.86, $CHCl_3$), PMR (C_6D_6) δ: 1.16 (3H, d, J=6 Hz, *sec* C$\underline{H}_3$), 1.38 (3H, d, J=6 Hz, *sec* C$\underline{H}_3$), 3.11, 3.18, 3.23 (3H each, s, OC$\underline{H}_3$×3), 5.12 (1H, d, J=2 Hz, anomeric H), which was methanolyzed with methanolic 2 N HCl under reflux for 2 h to afford **v** and **ix**.

Methylation of 16 by Hakomori's Method——Compound **16** (8 mg) was methylated by Hakomori's method as described above. The product was isolated by preparative TLC (solvent: benzene–acetone=2: 1 v/v, *Rf* 0.33) to afford a per-O-methyltrisaccharide (**18**, 6 mg), a colorless oil, IR ν_{max}^{Nujol} cm^{-1}: OH (nil), PMR (C_6D_6) δ: 1.16 (3H, d, J=6 Hz, *sec* C$\underline{H}_3$), 1.74 (3H, d, J=5 Hz, *sec* C$\underline{H}_3$), [3.11 (3H), 3.13 (3H), 3.15 (3H), 3.34 (3H), 3.38 (6H), 3.40 (3H), 3.56 (3H), 3.60 (3H)] (s, OC$\underline{H}_3$×9), 4.95 (1H, d, J=7 Hz, anomeric H), 5.37 (1H, d, J=2 Hz, anomeric H). Compound **18** was methanolyzed with methanolic 2 N HCl under reflux for 2 h to give **ii**, methyl 2,3-di-O-methyl-L-rhamnopyranoside (**x**) and 1,3,4,5-tetra-O-methylfucitol (**xi**), which were identified by TLC (solvent: benzene–acetone=2: 1 v/v, *Rf* 0.62, 0.58 (**ii**), 0.43 (**x**) and 0.40 (**xi**)) and GLC (the same conditions as described for the methanolysates of **5**, t_R(min) 2.3, 2.8 (**ii**), 6.4 (**xi**) and 6.8 (**x**)).

Partial Methanolysis of 15 with Methanolic 0.1 N HCl——Compound **15** (280 mg) was refluxed with methanolic 0.1 N HCl (20 ml) for 1 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) to show two main spots. The residue was subjected to column chromatography on silica gel, using $CHCl_3$–MeOH=100: 1 v/v, to afford **i** and a tetrasaccharide (**17**). Compound **i**: a colorless oil (22 mg), $[\alpha]_D^{18}$ −66.1° (c=1.42, $CHCl_3$), PMR ($CDCl_3$) δ: 3.39, 3.40, 3.45, 3.49 (3H each, s, OC$\underline{H}_3$×4), 3.60 (1H, d, J=2.5 Hz, $\underline{H}$–C–OR), 4.00 (2H, q, J=10 and 5 Hz, –C$\underline{H}_2$–OR), 4.90 (1H, d, J=2.5 Hz, anomeric H), which was identical with an authentic sample of methyl 2,3,4-tri-O-methyl-β-D-apio-D-furanoside (co-TLC, GLC and PMR). Compound **17**: syrup (86 mg), $[\alpha]_D^{27}$ −32.6° (c=1.22, $CHCl_3$), PMR (C_6D_6) δ: 1.21 (3H, d, J= 6 Hz, *sec* C$\underline{H}_3$), 1.67 (3H, d, J=5 Hz, *sec* C$\underline{H}_3$), [3.16 (3H), 3.22 (3H), 3.33 (6H), 3.39 (3H), 3.42 (3H), 3.50 (6H)] (s, OC$\underline{H}_3$×8), 4.70 (1H, d, J=6 Hz, anomeric H), 4.85 (1H, d, J=6 Hz, anomeric H), 5.18 (1H, s,

anomeric H). Compound **17** was methanolyzed with methanolic 2 N HCl under reflux for 2 h to afford **v**, **vi**, **vii** and **ix**.

Partial Methanolysis of 17 with Methanolic 0.2 N HCl——Compound **17** (156 mg) was refluxed with methanolic 0.2 N HCl (20 ml) for 2 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 subjected to column chromatography on silica gel, using $CHCl_3$–MeOH=100: 1 v/v, to afford **20** and an O-methylated trisaccharide (**21**). Compound **21**: syrup, $[\alpha]_D^{27}$ −51.4° (c=0.30, $CHCl_3$), TLC (solvent: $CHCl_3$–MeOH=9: 1 v/v) *Rf* 0.35, PMR (C_6D_6) δ: 1.16 (3H, d, J=6 Hz, *sec* CH_3), 1.61 (3H, d, J=6 Hz, *sec* CH_3), 3.05, 3.19, 3.22, 3.42, 3.53 (3H each, s, $OCH_3\times5$), 4.60 (1H, d, J=7 Hz, anomeric H), 5.12 (1H, d, J=2 Hz, anomeric H). Compound **21** was methanolyzed with methanolic 2 N HCl under reflux for 2 h to give **v**, **vii** and **ix**.

Methylation of 17 by Hakomori's Method——Compound **17** (12 mg) was methylated by Hakomori's method as described above. The product was isolated by preparative TLC (solvent: benzene–acetone= 2: 1 v/v, *Rf* 0.17) to afford an undeca-O-methyltetrasaccharide (**19**, 9 mg,) colorless oil, IR ν_{max}^{Nujol} cm^{-1}: OH (nil), PMR (C_6D_6) δ: 1.20 (3H, d, J=6 Hz, *sec* CH_3), 1.67 (3H, d, J=5 Hz, *sec* CH_3), [3.18 (3H), 3.19 (3H), 3.20 (3H), 3.32 (3H), 3.38 (6H), 3.41 (9H), 3.56 (3H), 3.64 (3H)] (s, $OCH_3\times11$), 4.93 (1H, d, J=8 Hz, anomeric H), 4.98 (1H, d, J=6 Hz, anomeric H), 5.30 (1H, s, anomeric H). Compound **19** was methanolyzed with methanolic 2 N HCl under reflux for 2 h to give **vi**, **vii**, **x** and **xi**.

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References and Notes

1) A part of this work was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1979.
2) K. Takiura and S. Honda, *Yakugaku Zasshi*, **84**, 1223 (1964).
3) J.H. Kim, *Yakhak Hoeji*, **8**, 59 (1964).
4) H. Ito, H. Taniguchi, T. Kita, Y. Matsuki, E. Tachikawa, and T. Fujita, *Phytochem.*, **16**, 1614 (1977).
5) T.Q. Chou, J.H. Chu, and P.F. Mei, *J. Am. Pharm. Assoc.*, **36**, 241 (1947).
6) M. Fujita and H. Itokawa, *Chem. Pharm. Bull.*, **9**, 1006 (1961).
7) S.W. Pelletier, S. Nakamura, and R. Soman, *Tetrahedron*, **27**, 4417 (1971).
8) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
9) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
10) Y. Tsukitani, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.*, **21**, 791 (1973).
11) J. Mendicino and J.M. Picken, *J. Biol. Chem.*, **240**, 9797 (1965).
12) M.L. Wolfrom and A. Thompson, *Methods Carbohyd. Chem.*, **1**, 202 (1962).
13) O. Th. Schmidt, *Methods Carbohyd. Chem.*, **1**, 193 (1962).
14) R.L. Whistler and J.N. BeMiller, *Methods Carbohyd. Chem.*, **1**, 88 (1962).
15) J.S.D. Bacon and J. Edelman, *Biochem. J.*, **48**, 114 (1951).
16) H.T. Neher and W.N. Lewis, *J. Am. Chem. Soc.*, **53**, 4411 (1931).
17) E.L. Hirst, E.G.V. Percival, and C.B. Wylam, *J. Chem. Soc.*, **1954**, 189.
18) H.B. MacPhillamy and R.C. Elderfield, *J. Org. Chem.*, **4**, 150 (1939).
19) D.H. Ball, F.H. Bissett, I.L. Klundt, and L. Long, Jr., *Carbohyd. Res.*, **17**, 165 (1971).
20) A. Tada, Y. Kaneiwa, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.*, **23**, 2965 (1975).
21) R.K. Hulgalkar, J.K.N. Jones, and M.B. Perry, *Can. J. Chem.*, **43**, 2085 (1965).