[Chem. Pharm. Bull. 21(7)1564—1574(1973)]

UDC 547.597'457.1.02 : 581.192 : 615.322.011.5

Studies on the Constituents of Senegae Radix. III.[1] The Structures of Senegin-III and -IV, Saponins from *Polygala senega* Linne var. *latifolia* Torry et Gray.

Yasumasa Tsukitani[2a] and Junzo Shoji[2]

School of Pharmaceutical Sciences, Showa University[2]

(Received April 2, 1973)

The chemical structures of senegin-III(Ia), $C_{75}H_{112}O_{35}$, $[\alpha]_D^{20}$ −6.6° (MeOH) and senegin-IV(Ib), $C_{81}H_{122}O_{39}$, $[\alpha]_D^{20}$ −20.2° (MeOH), which were isolated from Senegae Radix (root of *Polygata senega* Linn var. *latifolia* Torry et Gray), were established to be presenegenin-(3)-[β-D-glucopyranosyl]-(28)-{2-[β-D-galactopyranosyl($1_{gal}\rightarrow4_{xyl}$)-β-D-xylopyranosyl($1_{xyl}\rightarrow4_{rham}$)-α-L-rhamnopyranosyl]-3-(α-L-rhamnopyranosyl)-4-(4′-methoxycinnamoyl)-β-D-fucopyranoside} and presenegenin-(3)-[β-D-glucopyranosy-l]-(28)-{2-[4-[β-D-galactopyranosyl-($1_{gal}\rightarrow4_{xyl}$)-β-D-xylopyranosyl]-3-[α-L-rhamnopyranosyl]-α-L-rhamnopyranosyl]-3-(α-L-rhamnopyranosyl)-4-(4′-methoxycinnamoyl)-β-D-fucopyranoside}, on the basis of physical data of both compounds and several of their derivatives and degradation products.

As we reported in the previous papers,[3a,b] senegin-III and -IV are saponins of senegae radix (root of *Polygala senega* Linne var. *latifolia* Torry et Gray (Polygalaceae)). The chemical structure of main saponin of this crude drug, namely senegin-II, has been established to be presenegenin-(3)-[β-D-glucopyranosyl]-(28)-[β-D-galactopyranosyl($1_{gal}\rightarrow4_{xyl}$)-β-D-xylopyranosyl-($1_{xyl}\rightarrow4_{rham}$)-α-L-rhamnopyranosyl ($1_{rham}\rightarrow2_{fuc}$)-4-(3′,4′-dimethoxycinnamoyl)-β-D-fucopyranoside].[1] The present paper deals with the structure elucidations of senegin-III and -IV which lead to the assignments of the structure (Ia) and (Ib), respectively.

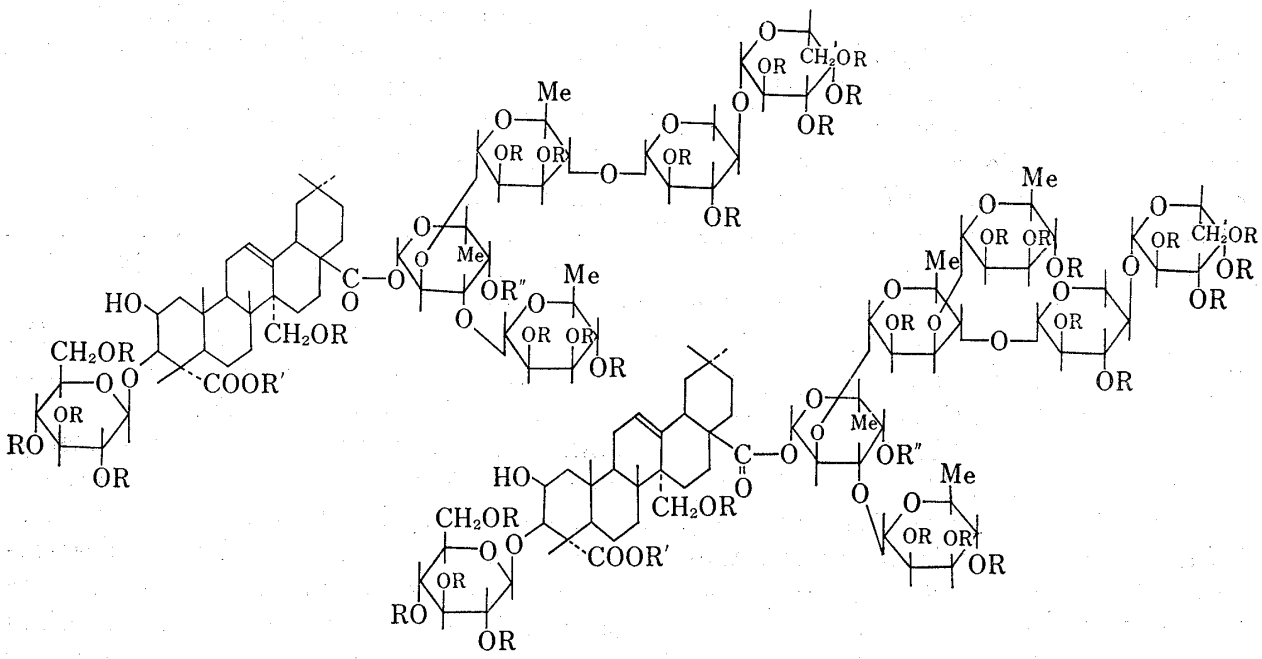
Senegin-III(Ia), $C_{75}H_{112}O_{35}\cdot3H_2O$, $[\alpha]_D^{20}$ −6.6° (methanol), a white powder, is composed of one mole each of presenegenin, 4-methoxycinnamic acid, glucose, galactose, fucose, xylose and two moles of rhamnose. The infrared (IR) spectrum of Ia indicates the presence of hydroxyl groups, two ester groups, carboxylic group, double bond and benzenoid system, while the ultraviolet (UV) spectrum suggests the presence of 4-methoxycinnamoyl ester.

On the other hand, senegin-IV (Ib), $C_{81}H_{122}O_{39}\cdot3H_2O$,[4] $[\alpha]_D^{20}$ −20.2° (methanol), a colorless crystalline powder, was found to be composed of one mole each of presenegeinn, 4-methoxycinnamic acid, glucose, galactose, fucose, xylose and three moles of rhamnose. The IR and UV spectra of Ib reveal the presence of the same functional groups as those of Ia.

On methylation with diazomethane, Ia and Ib gave the monomethyl esters (IIa, IIb), which were further methylated by the Kuhn's method[5] to afford the hexadeca-O-methyl monomethyl ester (IIIa), $C_{92}H_{146}O_{35}$, as a white powder and the octadeca-O-methyl monomethyl ester (IIIb), $C_{100}H_{160}O_{39}$, as a white powder, respectively.

As IR absorption bands of Ia and Ib suggest the presence of two kinds of ester groups (1750 and 1730 cm^{-1}), the alkali treatments of IIa and IIb were examined. When IIa and IIb were treated with 0.5% potassium hydroxide, des-4-methoxycinnamoyl senegin-III monomethyl ester (IVa), a white powder, $C_{66}H_{106}O_{33}\cdot3H_2O$, $[\alpha]_D^{20}$ +4° (water), and des-4-methoxy-

1) Part II: Y. Tsukitani, S. Kawanishi and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **21**, 791 (1973).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo*; *a*) Present adress: *Iyakushigen Institute for Medical Research, Nukuikitamachi-3-chome, Koganei-shi, Tokyo.*
3) *a*) J. Shoji, S. Kawanishi and Y. Tsukitani, *Yakugaku Zasshi*, **91**, 198 (1971); *b*) J. Shoji and Y. Tsukitani, *Chem. Pharm. Bull.* (Tokyo), **20**, 424 (1972).
4) The molecular formula, $C_{75}H_{112}O_{35}$, reported in the previous paper[3a] is erroneous and it was revised in this paper.
5) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).



Ia: R=R′=H, R″= -CO-CH=CH-⟨⟩-OMe
Ib: R=R′=H, R″= -CO-CH=CH-⟨⟩-OMe
IIa: R=H, R′=Me, R″= -CO-CH=CH-⟨⟩-OMe
IIb: R=H, R′=Me, R″= -CO-CH=CH-⟨⟩-OMe
IIIa: R=R′=Me, R″= -CO-CH=CH-⟨⟩-OMe
IIIb: R=R′=Me, R″= -CO-CH=CH-⟨⟩-OMe
IVa: R=R″=H, R′=Me
IVb: R=R″=H, R′=Me
Va: R=R″=Ac, R′=Me
Vb: R=R″=Ac, R′=Me
VIa: R=R′=R″=Me
VIb: R=R′=R″=Me

Chart 1

cinnamoyl senegin-IV monomethyl ester (IVb), a white powder, $C_{72}H_{116}O_{37}\cdot 2H_2O$, $[\alpha]_D^{20}$ −7.2° (water) were formed and charaterized by acetylation with acetic anhydride and pyridine to form the heptadecaacetate (Va), $C_{100}H_{140}O_{50}$, $[\alpha]_D^{20}$ +5° (chloroform) and the nonadeca acetate (Vb), $C_{110}H_{154}O_{56}\cdot H_2O$, respectively. Furthermore, compound IVa and IVb were methylated by the Hakomori's method[6] to give des-4-methoxycinnamoyl senegin-III heptadeca-O-methyl ether monomethyl ester (VIa), $C_{83}H_{140}O_{33}$, as a white powder and des-4-methoxycinnamoyl senegin-IV nonadeca-O-methyl ether monomethyl ester (VIb), $C_{91}H_{154}O_{37}$, as a white powder. Compound IIa and IIb were hydrolysed with 1N potassium hydroxide under nitrogen atmosphere to afford the same compound (VII), $C_{37}H_{58}O_{12}\cdot 2H_2O$, $[\alpha]_{20}^{D}$ +60° (ethanol), colorless needles, mp 230—231°, which were acetylated to from compound VIII, $C_{47}H_{68}O_{17}$, as colorless needles. The IR spectrum of VIII shows the presence of hydroxyl groups (3560 cm^{-1}) and ester groups (1740 cm^{-1} with inflection at 1760 cm^{-1}). The compound VII and its pentaacetate (VIII) were identified with authentic samples of presenegenin 3-O-glucoside monomethyl ester and its pentaacetate obtained from senegin-II[3a,b] by mixed fusion and comparison of IR spectra, respectively. The formation of VII from IIa and IIb suggests that among the two carboxyl groups the one at C-4 of presenegenin is present in free form and the other at C-17 in ester form.

To confirm the location of 4-methoxycinnamic acid in senegin-III and -IV, the comparative analyses of the methanolysates of IIIa, b and VIa, b with methanolic 3N hydrogen chloride were carried out. Methyl 2,3,4,6-tetra-O-methylglucoside, methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside and methyl 2,3,4-tri-O-methylrhamnoside

6) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

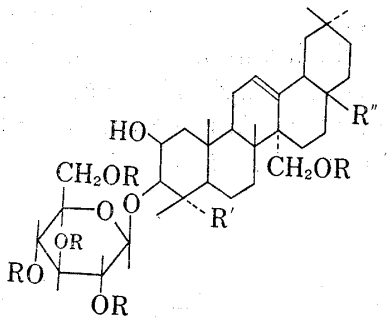
were commonly detected from the each methanolysate of compound IIIa, IIIb, VIa, and VIb. Besides, methyl fucoside and methyl 2,3-di-O-methylrhamnoside were obtained from IIIa, while methyl 2,3-di-O-methylrhamnoside and methyl 4-O-methylfucoside from VIa. Furthermore, methyl 2-O-methylrhamnoside and methyl fucoside were detected in the methanolysate of IIIb, while methyl 2-O-methylrhamnoside and methyl 4-O-methylfucoside in that of VIb. Consequently, 4-methoxycinnamoyl groups in Ia and Ib are deduced to be located at C–4 hydroxyl group of fucose, and the oligosaccharide moieties of Ia and Ib are suggested to be branched at C–2 and C–3 hydroxyl groups of fucose. Besides, the oligosaccharide moiety of Ib is deduced to be branched at C–3 and C–4 hydroxyl groups of rhamnose.

On reduction with lithium aluminium hydride, compound IIIa afforded compound IX, $C_{41}H_{68}O_{10}$ and compound Xa, $C_{40}H_{74}O_{22}\cdot H_2O$, $[\alpha]_D^{20}$ −75° (chloroform), while compound IIIb gave compound Xb, $C_{48}H_{88}O_{26}$, $[\alpha]_D^{20}$ −70° (chloroform) besides compound IX. The nuclear magnetic resonance (NMR) spectrum of compound IX reveals the presence of five O-methyl groups (δ=3.2, 3.28, 3.3, 3.48, and 3.59 ppm), one anomeric proton (δ=4.2, 1H(d) J=7 Hz) and one vinyl proton (δ=5.4, multiplet). Based on the physical properties of compound IX and its methanolysis experiment, the structure of IX has been suggested to be olean-12-ene-27-O-methyl-2β,23,28-trihydroxy-3β-(tetra-O-methyl)-β-D-glucopyranoside which was identified with an authentic sample obtained from senegin-II by mixed fusion and comparison of IR and NMR spectra. The other product, Xa, shows the presence of three secondary methyl groups (δ=1.30 3H(d) J=6 Hz, δ=1.33 3H(d) J=6 Hz, δ=1.55 3H(d) broad), eleven O-methyl groups (δ=3.13—3.65) and four anomeric protons (δ=4.13 1H(d) J=7 Hz, δ=4.30 1H(d) J=7 Hz, δ=5.20 2H(s)) in NMR spectrum, while Xb shows the presence of four secondary methyl groups (δ=1.28 3H(d) J=6 Hz, δ=1.35 3H(d) J=6 Hz, δ=1.36 3H(d) J=6 Hz, δ=1.55(3H(d) broad), thirteen O-methylgroups (δ=3.15—3.55) and five anomeric protons (δ=4.18 1H(d) J=7 Hz, δ=4.75 1H(d) J=7 Hz, δ=5.22 2H(broad s),δ= 5.40 1H(s)). On methanolysis with 3N hydrogen chloride, compound Xa and Xb gave methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3,4-tri-O-methylrhamnoside, methyl 2,3-di-O-methylxyloside and fucitol. Besides, methyl 2,3-di-O-methylrhamnoside was detected from Xa and methyl 2-O-methylrhamnoside from Xb. The formation of fucitol from compound X indicates that the oligosaccharide moieties of senegin-III and -IV link to C–17 carboxyl group of presenegenin through an acetal hydroxyl group of fucose.

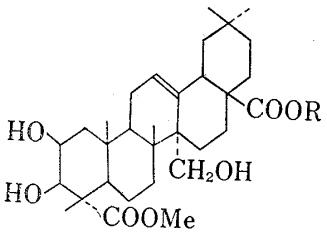
The sequence of five monosaccharides of senegin-III and six monosaccharides of senegin-IV were examined as follows. On treatment with methanolic 0.2N hydrogen chloride at room temperature, VIa gave a per-O-methylpentasaccharide (XI), which was isolated by preparative thin-layer chromatography (TLC). The compound XI refluxed with methanolic 1N hydrogen chloride for 1 hr and the reaction mixture was purified by preparative TLC to afford an O-methyltrisaccharide (XIIa), *Rf* 0.23 (solvent A), which afforded methyl 4-O-methylfucoside, methyl 2,3-di-O-methylrhamnoside and methyl 2,3,4-tri-O-methylrhamnoside by methanolysis with methanolic 3N hydrogen chloride. After methylation by the Hokomori's method the resulted per-O-methyltrisaccharide of XIIa was methanolyzed with methanolic 3N hydrogen chloride to afford methyl 4-O-methylfucoside and methyl 2,3,4-tri-O-methylrhamnoside.

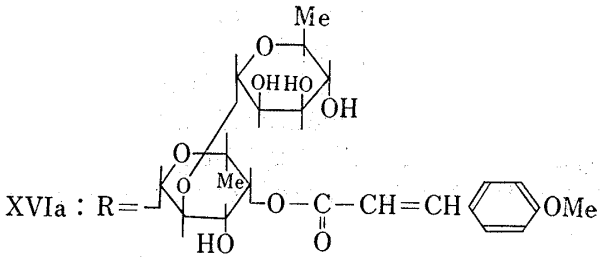
Accordingly, the compound XIIa was suggested to be methyl 2-(2,3-di-O-methylrhamnopyranosyl)-3-(2,3,4-tri-O-methylrhamnopyranosyl)-4-O-methylfucopyranoside or methyl 2-(2,3,4-tri-O-methylrhamnopyranosyl)-3-(2,3-di-O-methylrhamnopyranosyl)-4-O-methylfucopyranoside. Furthermore, compound XIIa was treated with methanolic 3N hydrogen chloride at room temperature to give O-methyldisaccharide (XIIIa) which was composed of one mole each of 2,3-di-O-methylrhamnose and methyl 4-O-methylfucoside. The compound XIIIa was methylated by the Hakomori's method to form per-O-methylate which was methanolyzed to afford methyl 3,4-di-O-methylfucoside and methyl 2,3,4-tri-O-methylrhamnoside. Consequently, the compound XIIa was established to be methyl 2-(2,3-di-O-methylrhamno-

pyranosyl)-3-(2,3,4-tri-O-methylrhamnopyranosyl)-4-O-methylfucopyranoside and the structure of XI was deduced to be methyl (3)-[2,3,4-tri-O-methylrhamnopyranosyl]-(2)-[2,3,4,6-tetra-O-methylgalactopyranosyl($1_{gal}\rightarrow4_{xyl}$)-2,3-di-O-methylxylopyranosyl($1_{xyl}\rightarrow4_{rham}$)-2,3-di-O-methylrhamnopyranosyl]fucopyranoside formulated as XI.

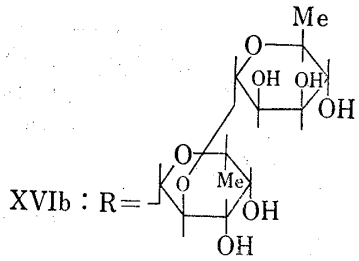


VII : R=H, R′=COOMe, R″=COOH
VIII : R=Ac, R′=COOMe, R″=COOH
IX : R=Me, R′= R″=$-CH_2OH$

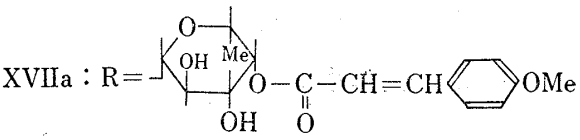




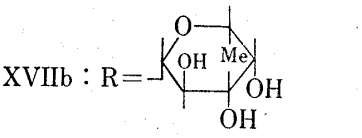
XVIa : R=



XVIb : R=



XVIIa : R=



XVIIb : R=

Chart 2

From the results of the foregoing experiments the structure of senegin-III was suggested to be presenegenin-(3)-[β-D-glucopyranosyl]-(28)-{2-[D-galactopyranosyl($1_{gal}\rightarrow4_{xyl}$)-D-xylopyranosyl($1_{xyl}\rightarrow4_{rham}$)-L-rhamnopyranosyl]-3-(L-rhamnopyranosyl)-4-(4′-methoxycinnamoyl)-D-fucopyranoside}.

On the other hand, the sequence of six monosaccharides of senegin-IV was determined as follows. On refluxing with methanolic 0.3N hydrogen chloride for 1 hr, compound VIb gave several kinds of partially O-methylated monosaccharide and oligosaccharide. Trisaccharide XIIb and disaccharide XIIIb were isolated by preparative TLC. The compound XIIb was composed of 2,3,4-tri-O-methylrhamnoside, 2-O-methylrhamnoside and 4-O-methylfucoside, while the compound XIIIb was composed of 2-O-methylrhamnoside and 4-O-methylfucoside. After methylation by the Hakomori's method, per-O-methyl ethers of compound XIIb and XIIIb were methanolyzed. Methyl 2,3,4-tri-O-methylrhamnoside and methyl 4-O-methylfucoside were detected from per-O-methylate of XIIb, while methyl 2,3,4-tri-O-methylrhamnoside and methyl 3,4-di-O-methylfucoside from that of XIIIb. The foregoing experiments concluded that compound XIIb and XIIIb were methyl 2-(2-O-methylrhamnopyranosyl)-3-(2,3,4-tri-O-methylrhamnopyranosyl)-4-O-methylfucopyranoside and methyl 2-(2-O-methylrhamnopyranosyl)-4-O-methylfucopyranoside, respectively.

Furthermore, compound Xb was refluxed with methanolic 0.1N hydrogen chloride to afford three oligosaccharides (XIV, XV, Xc) besides the partially O-methylated monosaccharides. Compound XIV is a trisaccharide which gave methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside and methyl 2-O-methylrhamnoside by methanolysis,

while compound XV is a tetrasaccharide, which afforded methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2-O-methylrhamnoside and fucitol by refluxing with methanolic 3N hydrogen chloride. The third oligisaccharide, namely compound Xc, gave methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and fucitol on methanolysis. The components of monosaccharides of Xc were the same as that of Xb, but the *Rf* value of Xc (0.48, solvent B) was different from that of Xb (0.55, solvent B). Then Xc was methylated according to the Hakomori's method to form Xd, which was methanolyzed to afford methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and partially O-methylated fucitol. Consequently, the structure of hexasaccharide is deduced to be depicted as Xb shown in Chart 2.

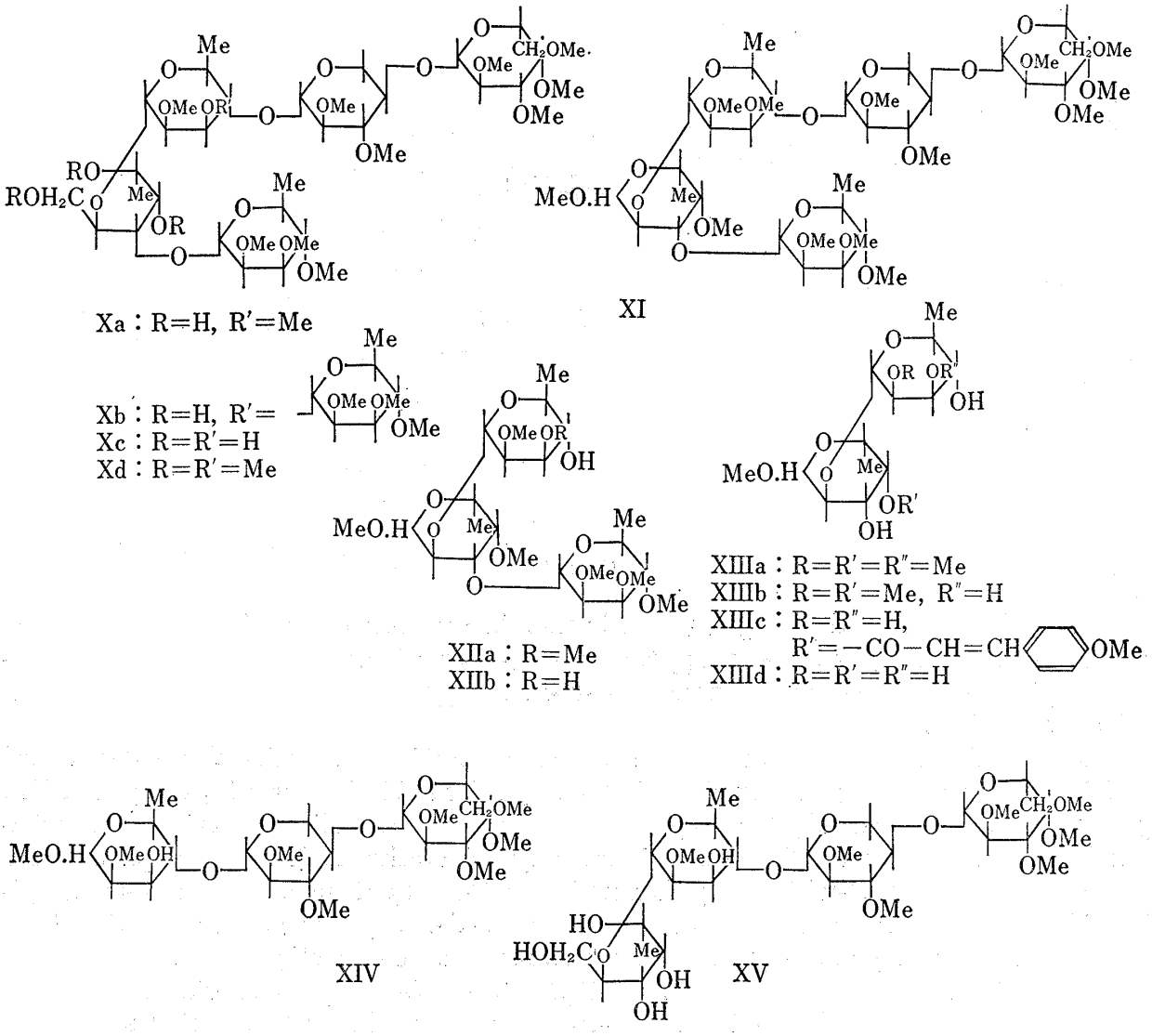


Chart 3

To support the partial structure of the oligosaccharide moiety of senegin-IV the following additional experiment was carried out. The oxidation of senegin-IV monomethyl ester, IIb, with sodium metaperiodate followed by reduction with sodium borohydride and hydrolysis with 0.05N hydrogen chloride gave a white powder, XVIa, $C_{53}H_{76}O_{17}$, $[\alpha]_D^{20}$ +30.8° (ethanol), which was composed of presenegenin, fucose, rhamnose and 4-methoxycinnamic acid. This product, XVIa, was treated with 0.5% potassium hydroxide to afford $C_{43}H_{68}O_{15}$,

Table I. Molecular Rotations of Senegin-II, Senegin-III and Senegin-IV

Des-3,4-dimethoxycinnamoyl senegin-II methyl ester (glu-presenegenin-fuc-rham-xyl-gal)	$[M]_{D-I}$ +217°
Des-4-methoxycinnamoyl senegin-III methyle sterr (glu-presneegenin-fuc-rham-xyl-gal) rham	$[M]_{D-II}$ +57°
Des-4-methoxycinnamoyl senegin-IV methyl ester (glu-presenegenin-fuc-rham-xyl-gal) rharm rham	$[M]_{D-III}$ −113°
$[M]_{D-II}-[M]_{D-I} = -160°$	
$[M]_{D-III}-[M]_{D-II} = -170°$	
$[M]_{D-III}-[M]_{D-I} = -330°$	
Methyl α-rhamnopyranoside	$[M]_D = -111°$
Methyl β-rhamnopyranoside	$[M]_D = +170°$

XVIb, $[\alpha]_D^{20}$ +37.7° (ethanol), as colorless needles. The compound XVIb was composed of presenegenin, fucose and rhamnose, and the NMR spectrum suggested that the configuration of fucose might be β-form based on the coupling constant of the anomeric proton (δ=6.05 1H(d) J=10 Hz). The formation of compound XVIa from IIb by oxidation with sodium metaperiodate supports the partial structure of IIb.

As the results of foregoing experiments the structure of sugar moiety of senegin-IV was established to be 2-{3-[rhamnopyranosyl($1_{rham}\rightarrow3_{rham}$)]-4-[galactopyranosyl($1_{gal}\rightarrow4_{xyl}$)-xylopyranosyl($1_{xyl}\rightarrow4_{rham}$)]-rhamnopyranosyl}-3-[rhamnopyranosyl($1_{rham}\rightarrow3_{fuc}$)]-4-(4′-methoxycinnamoyl)-fucopyranoside.

The configuration of each monosaccharide of senegin-III was assigned as follows. The configuration of galactose and xylose were assigned to be all β form from the value of coupling constants (J=7 Hz) in NMR spectrum of compound Xa, while that of L-rhamnose of main chain was assigned to be α form as follows. On partial methanolysis with 0.03N sulfuric acid containing small amount of dioxane, senegin-III monomethyl ester (IIa) gave a product which was assumed to be 2-(L-rhamnopyranosyl)-4-(4′-methoxycinnamoyl)-D-fucose (XIIIc). This product was hydrolyzed with 0.5% potassium hydroxide to afford XIIId, 2-(α-L-rhamnopyranosyl)-D-fucose, mp 145°, which was identified with an authentic sample obtained from senegin-II monomethyl ester by partition paper chromatography (PPC) and mixed fusion. The configuration of the D-fucose attached to the C-17 carboxyl group of presenegenin was assigned to be β form as follows. Oxidation of senegin-III monomethyl ester (IIa) with sodium metaperiodate followed by reduction with sodium borohydride and by hydrolysis with 0.05N hydrogen chloride gave compound XVIIa. Further hydrolysis with 0.5% potassium hydroxide gave presenengenin D-fucopyranoside monomethyl ester (XVIIb), $C_{37}H_{58}O_{11}\cdot H_2O$, $[\alpha]_D^{20}$ +71.3° (ethanol), whose NMR spectrum showed the signal of anomeric proton (δ=5.55 1H(d) J=10 Hz). Finally, the configuration of the branched L-rhamnose was assigned to be α form from the comparison of molecular optical rotation of des-3,4-dimethoxycinnamoyl senegin-II monomethyl ester ($[M]_D$ +217°), des-4-methoxycinnamoyl senegin-III monomethyl ester ($[M]_D$ +57°) and methyl α-L-rhamnopyranoside ($[M_D]$ −111°). From these experimental data, the structure of senegin-III has been established to be presenegenin-(3)-[β-D-glucopyranosyl]-(28)-{2-[β-D-galactopyranosyl($1_{gal}\rightarrow4_{xyl}$)-β-D-xylopyranosyl($1_{xyl}\rightarrow4_{rham}$)-α-L-rhamnopyranosyl]-3-(α-L-rhamnopyranosyl)-4-(4′-methoxycinnanoyl)-β-D-fucopyranoside formulated as Ia.

On the other hand, the configuration of each monosaccharide of senegin-IV was assigned as follows. The comparison of molecular optical rotation of compound XVIb and presenegenin fucoside monomethyl ester which was obtained from senegin-III monomethyl ester by oxidation with sodium metaperiodate revealed that the configuration of rhamnose should be assigned to be α form. Furthermore, the configurations of xylose and galactose were assigned to be

β form from the analyses of NMR coupling constants of their anomeric protons. Based on the Klyne's rule, the difference of molecular optical rotation ($[M]_D$ −330.8°) between decinnamoyl senegin II monomethyl ester ($[M]_D$ +217.6°) and decinnamoyl senegin-IV monomethyl ester ($[M]_D$ −113.2°) suggests that the configurations of two rhamnose attached to the C-3 hydroxyl group of rhamnose in main chain and C-3 hydroxyl group of fucose were assumed to be all α forms. (Table I).

Consequently, the structure of senegin-IV has established to be presenegenin-(3)-[β-D-glucopyranosyl]-(28)-{2-[4-β-D-galactopyranosyl($1_{gal}\rightarrow 4_{xyl}$)-$\beta$-D-xylopyranosyl]-3-[$\alpha$-L-rhamnopyranosyl]-$\alpha$-L-rhamnopyranosyl]-3-($\alpha$-L-rhamnopyranosyl)-4-(4′-methoxycinnamoyl)-β-D-fucopyranoside}.

The study on the senegin-I and other minor glycosides is under way.

Experimental

All melting points were taken on a Yanagimoto Micro Melting Point Apparatus and uncorrected. IR absorption spectra were obtained with a Hitachi Model 215. NMR spectra were obtained with a Hitachi Model R-20 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in δ and the solvent used are indicated. Gas chromatograph used was a Japan Electron Co., JGC Model 810 with hydrogen flame ionization detector. Molecular weight was determined using a Hitachi Perkin-Elmer molecular weight apparatus Model 115.

The *Rf* values were determined by thin-layer chromatography on silica gel H using solvent A: hexane–acetone (1: 1), solvent B: $CHCl_3$–MeOH (5: 1), solvent C: $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase) and spots were detected by spraying 10% H_2SO_4 followed by heating.

Senegin-III(Ia)——Senegin-III was purified by repeated reprecipitation from EtOH to afford a colorless crystalline powder, mp 247—248° (decomp.), $[\alpha]_D^{20}$ −6.6° (c=2.0 MeOH), *Anal.* Calcd. for $C_{75}H_{112}O_{35}\cdot 3H_2O$: C, 55.35; H, 7.37. Found: C, 55.53; H, 7.10. UV λ_{max}^{EtOH} mμ (log ε): 315(4.30), Mol. wt. (osmotic vapor pressure method in MeOH) Calcd. for $C_{75}H_{112}O_{35}$: 1602; Found: 1482. IR ν_{max}^{Nujol} cm^{-1}: 3500—3300 (OH), 1750(COOR), 1730(COOR), 1710(COOH), 1635(C=C), 1610, 1515(benzenoid). H. I. (haemolytic index): 25773.

Senegin-IV(Ib)——Senegin-IV was purified by reprecipitation from EtOH to afford a colorless crystalline powder, mp 249—250° (decomp.), $[\alpha]_D^{20}$ −20.2° (c=2.0 MeOH), *Anal.* Calcd. for $C_{81}H_{122}O_{39}\cdot 3H_2O$: C, 54.82, H, 7.22. Found: C, 54.92; H, 7.14. Mol. wt. (osmotic vapor pressure method in MeOH) Calcd. for $C_{81}H_{122}O_{39}$ 1718, Found: 1620. IR ν_{max}^{Nujol} cm^{-1}: 3500—3300 (OH), 1750, 1730(COOR), 1710(COOH), 1635 (C=C), 1610, 1515 (benzenoid). UV λ_{max}^{EtOH} mμ (log ε): 315 (4.30). H. I. (haemolytic index): 22910.

Senegin-III Monomethyl Ester(IIa) and Senegin-IV Monomethyl Ester(IIb)——i) Senegin-III(1 g) and senegin-IV (1 g) were dissolved in MeOH (30 ml) and treated with ethereal diazomethane in a refrigerator overnight, respectively. The excess reagent was decomposed with AcOH and the reaction mixture was evaporated to dryness. The residue was purified by column chromatography on silica gel using solvent C to afford hygroscopic senegin-III monomethyl ester (IIa, 600 mg) and senegin-IV monomethyl ester (IIb, 600 mg) which were characterised by methylation.

ii) Senegin-III monomethyl ester (IIa) and senegin-IV monomethylester (IIb) were also obtained from the BuOH soluble fraction of the methanol extract of senegae radix reported in the previous paper by the same methylation as described above. The reaction mixture was treated as usual and the crude product was separated by repeated column chromatography on silica gel eluted with $CHCl_3$–MeOH–H_2O (7: 3: 1, the lower phase). The products were identified with authentic samples of senegin-III monomethyl ester (IIa) and senegin-IV monomethyl ester (IIb) which were obtained by the method (i). The materials used for this study were supplied by method (ii), because the separation of esteric derivatives of crude saponins was easier than that of free saponin mixture.

Senegin-III Hexadeca-O-methyl Monomethyl Ester (IIIa) and Senegin-IV Octadeca-O-methyl Monomethyl Ester (IIIb)——IIa and IIb were methylated by the Kuhn's method, respectively. To the solutions of IIa (3 g) and IIb (3 g) in dimethylformamide (60 ml) were added 20 ml of CH_3I and 20 g of freshly prepared Ag_2O. The reaction mixture were stirred for 60 hr at room temperature and filtered. To the filtrates were added CH_3I (20 ml) and Ag_2O (20 g). The reaction mixturs were kept for 50 hr with stirring. After filtration, the reaction mixtures were poured into a large amount of water and the products were extracted with $CHCl_3$ for several times. The $CHCl_3$ solutions were combined, washed with water and dried over Na_2SO_4. The solvents were removed *in vacuo* to give a yellow powder. The each residue was purified by column chromatography on silica gel eluted with ethyl acetate to afford a white powder reprecipitated from hexane.

Senegin-III Hexadeca-O-methyl Monomethyl Ester: (mp 142—143°), IR ν_{max}^{Nujol} cm^{-1}: 3500(OH), 1710, 1730 (1750 shoulder, COOR), 1630 (C=C), 1600, 1500 (benzeniod), 1250, 1100 (C–O–C). *Anal.* Calcd. for $C_{92}H_{146}O_{35}$: C, 60.99; H, 8.06. Found: C, 60.76; H, 8.23.

Senegin-IV Octadeca-O-methyl Monomethyl Ester: (mp 141—143°), $[\alpha]_D^{20}$ −8° (c=1.25 $CHCl_3$), *Anal.* Calcd. for $C_{100}H_{160}O_{39}$: C, 60.48; H 8.06. Found: C, 60.28; H 7.94. IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1750, 1730, 1710 (COOR), 1630 (C=C), 1600, 1510 (benzenoid), 1250, 1070 (broad, C–O–C).

Des-4-methoxycinnamoylsenegin-III Monomethyl Ester(IVa) and Des-4-methoxycinnammoylsenegin-IV Monomethyl Ester (IVb)——Senegin-III monomethyl ester (IIa, 1 g) and senegin-IV monomethyl ester (IIb, 1 g) were dissolved in 0.5% KOH (300 ml) respectively and the solutions were allowed to stand obernight. After neutralization with ion exchanged resin (Amberlite IR-MB) the solvent was evaporated *in vacuo*. The residues were purified on silica gel column eluted with solvent C to give des-4-methoxycinnamoylsenegin-III monomethyl ester (IVa, 500 mg) and des-4-methoxycinnamoylsenegin-IV monomethyl ester (IVb, 600 mg) reprecipitated from EtOH.

IVa: (mp 230—233° (decomp.)), $[\alpha]_D^{20}$ +4° (c=2.0 H_2O). *Anal.* Calcd. for $C_{66}H_{106}O_{33}\cdot 3H_2O$: C, 53.51; H, 7.56. Found: C, 53.34; H, 7.46.

IVb: (mp 236—238° (decomp.)), $[\alpha]_D^{20}$ −7.2° (c=2.8 H_2O). *Anal.* Calcd. for $C_{72}H_{116}O_{37}\cdot 2H_2O$: C, 53.73; H 7.46. Found: C, 53.70; H, 7.58.

Des-4-methoxycinnamoylsenegin-III Monomethyl Ester Heptadecaacetate (Va) and Des-4-methoxycinnamoylsenegin-IV Monomethyl Ester Nonadecaacetate (Vb)——IVa and IVb were acetylated with acetic anhydride and pyridine, respectively. The reaction mixtures were worked up as usual and the products were purified with EtOH to give a heptadecaacetate (Va) and a nonadecaacetate (Vb) as a white powder.

Va: (mp 172—174°), $[\alpha]_D^{20}$+5° (c=1.9 $CHCl_3$), *Anal.* Calcd. for $C_{100}H_{140}O_{50}$: C, 56.08; H 6.54. Found: C, 55.96; H, 6.50.

Vb: (mp 174—176°), $[\alpha]_D^{20}$ 0° (c=1.0 $CHCl_3$). *Anal.* Calcd. for $C_{110}H_{154}O_{56}\cdot H_2O$: C, 55.27: H, 6.53. Found: C, 55.11; H 6.40.

Des-4-methoxycinnamoylsenegin-III Heptadeca-O-methyl Ether Monomethyl Ester (VIa) and Des-4-methoxycinnamoylsenegin-IV Nonadeca-O-methyl Ether Monomethyl Ester (VIb)——According to the Hakomori's method, NaH (200 mg) was warmed with dimethylsulfoxide (DMSO, 5 ml) at 65° for 1 hr with stirring under N_2 gas flow. To this reagent solutions of IVa (1.0 g) and IVb (1.0 g) in DMSO (5 ml) were added respectively and the mixtures were allowed to stand for 15 min with stirring under N_2 gas flow. Then CH_3I (3 ml) was added to each solution and the mixture was allowed to stand at room temperature for 2 hr with stirring. After dilution with water, the mixtures were extracted with $CHCl_3$ and the organic layers were washed with water, dried over Na_2SO_4 and evaporated. The each residue was chromatographed on silica gel column and eluted with ethyl acetate to give VIa and VIb, respectively.

VIa: A white powder from hexane, (mp 134—136°). *Anal.* Calcd. for $C_{83}H_{140}O_{33}$: C, 59.85; H, 8.41. Found: C, 59.69; H, 8.25. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1730 (1750 shoulder COOR), 1620 (C=C), 1250, 1100 (C–O–C).

VIb: A white powder (380 mg), (mp 134—136°), $[\alpha]_D^{20}$ −6.8° (c=1.47 $CHCl_3$). *Anal.* Calcd. for $C_{91}H_{154}O_{37}$: C, 59.41; H 8.37. Found: C, 59.18; H, 8.24. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1730 (1750 shoulder, COOR), 1620 (C=C), 1250, 1100 (C–O–C).

Hydrolysis of Senegin-III Monomethyl Ester (IIa) and Senegin-IV Monomethyl Ester (IIb) with 1N KOH (Formation of Tenuifolin Monomethyl Ester (VII))——Compound IIa (500 mg) and compound IIb (500 mg) were dissolved in 1N KOH (50 ml) respectively and each solution was heated under N_2 gas flow on a water bath for 1 hr. The reaction mixtures were cooled at room temperature and adjusted to pH 2.0—3.0 with 1N HCl. Each solution was extracted twice with *n*-BuOH and the organic layers were combied, washed with water and then evaporated to dryness. The residues were submitted to chromatography on silica gel using ethyl acetate saturated with water to give colorless needles, which were identified with an authentic sample of tenuifolin monomethyl ester obtained from senegin-II by mixed fusion and comparison of IR spectra. mp 230—231°, IR ν_{max}^{Nujol} cm^{-1}: 3500—3400 (OH), 1725 (COOR), 1690 (COOH), 1630 (C=C). *Anal.* Calcd. for $C_{37}H_{58}O_{12}\cdot 2H_2O$: C, 60.82; H, 8.49. Found: C, 60.48; H, 8.16.

Acetylation of Compound VII (Formation of Tenuifolin Monomethyl Ester Pentaacetate (VIII))——The compound VII was acetylated by the same method as described in the previous paper. The product was recrystallized from methanol to give a pentaacetate as colorless needles, mp 216—217°, IR ν_{max}^{Nujol} cm^{-1}: 3560 (OH), 1740, 1725 (1760 shoulder, COOR), 1690 (COOH), 1250 (C–O–C). The compound VIII was identified with an authentic sample of tenuifolin monomethyl ester pentaacetate obtained from senegin-II by mixed fusion and comparison of IR spectra.

Methanolysis of Compound IIIa, IIIb, VIa, and VIb with Methanolic 3N HCl——Compound IIIa, IIIb, VIa, and VIb (100 mg) were refluxed with methanolic 3N HCl (5 ml) for 2 hr, respectively. The reaction mixture were neutralized with Ag_2CO_3 and filtered. The filtrates were evaporated *in vacuo* and the residues were fractionated by column chromatography on silica gel with $CHCl_3$–MeOH (50: 1—20: 1). Furthermore, each fraction was separated by preparative TLC using solvent A to afford six O-methylated sugars, which were summarized as follows.

Compound IIIa: Methyl 2,3,4,6-tetra-O-methylglucoside, methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and methyl fucoside.

Compound IIIb: Methyl 2,3,4,6-tetra-O-methylglucoside, methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and methyl fucoside.

Compound VIa: Methyl 2,3,4,6-tetra-O-methylglucoside, methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and methyl 4-O-methylfucoside.

Compound VIb: Methyl 2,3,4,6-tetra-O-methylglucoside, methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and methyl 4-O-methylfucoside.

TLC i) Solvent A: *Rf* 0.68, 0.59 (α- and β-methyl 2,3,4,6-tetra-O-methylglucoside); 0.57, 0.51 (α- and β-methyl 2,3,4,6-tetra-O-methylgalactoside); 0.48, 0.41 (α- and β-methyl 2,3-di-O-methylxyloside); 0.68, 0.59 (α- and β-methyl 2,3,4,-tri-O-methylrhamnoside); 0.47 (methyl 2,3-di-O-methylrhamnoside); 0.29, 0.22 (α- and β-methylrhamnoside); 0.17 (methyl 4-O-methylfucoside).

ii) Solvent B: *Rf* 0.79 (methyl 2,3,4,6-tetra-O-methylglucoside); 0.78 (methyl 2,3,4,6-tetra-O-methylgalactoside); 0.64, 0.59 (methyl 2,3-di-O-methylxyloside); 0.77 (methyl 2,3,4-tri-O-methylrhamnoside); 0.41, 0.37 (α- and β-methyl 4-O-methylfucoside); 0.45, 0.40 (methyl 2-O-methylrhamnoside); 0.64 (methyl 2,3-di-O-methylrhamnoside).

iii) Solvent C: *Rf* 0.5 (methyl fucoside).

GLC column, 1,4-butandiol succinate (5%) on Shimalite W (60—80 mesh), 3 mm × 2 m, column temperature 175°, carrier gas N_2 50 ml/min. t_R(min) 5.0, 7.0 (α- and β-methyl 2,3,4,6-tetra-O-methylglucoside); 8.4 (methyl 2,3,4,6-tetra-O-methylgalactoside); 6.0, 8.2 (α- and β-methyl 2,3-di-O-methylxyloside); 15.0 (methyl 4-O-methylfucoside); 2.3, 3.3 (α- and β-methyl 2,3,4-tri-O-methylrhamnoside); 7.0 (methyl 2,3-di-O-methylrhamnoside); 13.7 (methyl 2-O-methylrhamnoside).

Reductive Cleavage of IIIa and IIIb with Lithium Aluminium Hydride——To a solution of compound IIIa and IIIb (500 mg) in absolute ether (40 ml) was added 200 mg of $LiAlH_4$ respectively and each reaction mixture was refluxed for 2 hr. After the excess $LiAlH_4$ was decomposed with ethyl acetate the reaction mixture was poured into a large amount of water and 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 *in vacuo*. The residue was purified by chromatography on silica gel eluted with $CHCl_3$ or hexane–acetone (10: 1) to afford compound IX as a white powder from hexane, (mp 125°), *Anal.* Calcd. for $C_{41}H_{68}O_{10}$: C, 68.30; H, 9.51. Found: C, 67.61; H, 9.66. IR ν_{max}^{Nujol} cm^{-1}: 3500—3400 (OH), 1630 (C=C), 1090 (C–O–C). The compound IX was identified with olean-12-ene-27-O-methyl-2,23,28-trihydroxy-3β-(tetra-O-methyl)glucopyranoside by comparing the melting point, IR spectra and NMR spectra.

Both $CHCl_3$ solutions were washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The residues were chromatographed on silica gel column using $CHCl_3$–MeOH (50: 1) and the eluate was purified by reprecipitation from hexane to give a white powder, Xa (mp 68—70°) and Xb (mp 67—68°), respectively.

Xa: $[\alpha]_D^{20}$ −75° (c=2.2 $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{74}O_{22}\cdot H_2O$: C, 51.94; H, 8.22. Found: C, 51.64; H, 8.22. NMR (in benzene) δ: 1.30 (3H(d) J=6 Hz –ĊH–C$\underline{H}_3$), 1.33 (3H(d) J=6 Hz –ĊH–C$\underline{H}_3$), 1.55 (3H(d) broad –ĊH–C$\underline{H}_3$), 3.13 (3H × 2(s) –OC$\underline{H}_3$), 3.27 (3H × 2(s) –OC$\underline{H}_3$), 3.33 (3H(s) –OC$\underline{H}_3$), 3.45 (3H(s) –OC$\underline{H}_3$), 3.46 (3H(s) –OC$\underline{H}_3$), 3.41 (3H(s) –OC$\underline{H}_3$), 3.50 (3H(s) –OC$\underline{H}_3$), 3.65 (3H(s) –OC$\underline{H}_3$), 4.13 (1H(d) J=7 Hz anomeric $\underline{H}$), 4.3 (1H(d) J=7 Hz anomeric $\underline{H}$), 5.2 (1H × 2(s) anomeric $\underline{H}$).

Xb: $[\alpha]_D^{20}$ −70° (c=3.0 $CHCl_3$). *Anal.* Calcd. for $C_{48}H_{88}O_{26}$: C, 53.33; H, 8.14. Found: C, 53.54; H, 7.88. NMR (in benzene) δ: 1.28 (3H(d) J=6 Hz –ĊH–C$\underline{H}_3$), 1.35 (3H(d) J=6 Hz –ĊH–C$\underline{H}_3$), 1.36 (3H,(d) J=6 Hz –ĊH–C$\underline{H}_3$), 1.55 (3H(d) broad –ĊH–C$\underline{H}_3$), 3.15 (3H(s) × 2 –OC$\underline{H}_3$), 3.25 (3H(s) –OC$\underline{H}_3$), 3.27 (3H(s) –OC$\underline{H}_3$), 3.37 (3H(s) –OC$\underline{H}_3$), 3.38 (3H(s) –OC$\underline{H}_3$), 3.45 (3H(s) × 2 –OC$\underline{H}_3$), 3.47 (3H(s) –OC$\underline{H}_3$), 3.50 (3H(s) × 2 –OC$\underline{H}_3$), 3.55 (3H(s) –OC$\underline{H}_3$), 3.62 (3H(s) –OC$\underline{H}_3$), 4.18 (1H(d) J=7 Hz anomeric $\underline{H}$), 4.75 (1H(d) J=7 Hz anomeric $\underline{H}$), 5.22 (1H(s) × 2 anomeric $\underline{H}$), 5.40 (1H(s) anomeric $\underline{H}$).

Methanolysis of Xa and Xb with Methanolic 3N HCl——Compound Xa and Xb were refluxed with methanolic 3N HCl for 2 hr respectively and the reaction mixtures were neutralized with Ag_2CO_3 and filtered. Each filtrate was evaporated *in vacuo* and the residue was examined by TLC (Solvent B) and GLC. Methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3,4-tri-O-methylrhamnoside, methyl 2,3-di-O-methylrhamnoside and fucitol were detected from the methanolysate of Xa, while methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3,4-tri-O-methylrhamnoside, methyl 2-O-methylrhamnoside and fucitol from that of Xb.

Partial Methanolysis of VIa with Methanolic 2N HCl——The solution of VIa (100 mg) in methanolic 2N HCl was allowed to stand overnight at room temperature. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was examined by TLC (Solvent A) to show the main three spots (*Rf* 0.56, 0.44 (VIa), 0.30 (XI)). The products corresponding to *Rf* 0.56 and 0.30 were isolated by preparative TLC using the solvent A. The former (*Rf* 0.56) was suggested to be composed of 2,3,4,6-tetra-O-methylglucose and partially methylated presenegenin by methanolysis with methanolic 3N HCl, while the latter, compound XI (*Rf* 0.30) gave methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and methyl 4-O-methylfucoside on methanolysis.

Partial Methanolysis of XI with Methanolic 1N HCl——The compound XI was refluxed with methanolic 1N HCl for 1 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was examined by TLC (Solvent A) to reveal the formation of compound XIIa (*Rf* 0.23) besides the partially O-methylated monosaccharides. The compound XIIa was isolated by preparative TLC using solvent A as a colorless oil. This product, XIIa, was also obtainable from VIa by meth anolysis with methanolic 1N HCl refluxing for 1 hr. Methyl 2,3-di-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and methyl 4-O-methylfucoside were identified from the methanolysate of XIIa with methanolic 3N HCl refluxing for 2 hr by TLC and GLC.

Permethylation of XIIa by the Hakomori's Method——According to the Hakomori's method. compound XIIa was methylated and the reaction mixture was treated as usual. The product was isolated by preparative TLC using solvent A (*Rf* 0.38) and methanolyzed with methanolic 3N HCl refluxing for 2 hr to afford methyl 2,3,4-tri-O-methylrhamnoside and methyl 4-O-methylfucoside, which were identified with authentic samples by TLC and GLC.

Partial Methanolysis of XIIa with Methanolic 3N HCl——The compound XIIa was dissolved in methanolic 3N HCl and left at room temperature overnight. The reaction mixture was treated as described above and examined by TLC (Solvent A) to show the formation of compound XIIIa (*Rf* 0.19) accompanied with three partially O-methylated monosaccharides. Methyl 2,3-di-O-methylrhamnoside and methyl 4-O-methylfucoside were detected in the methanolysate obtained by refluxing XIIIa with methanolic 3N HCl for 2 hr by TLC and GLC.

Permethylation of XIIIa by the Hakomori's Method——According to the Hakomori's method, compound XIIIa was methylated and the reaction mixture was treated as usual. The product was isolated by preparative TLC using solvent A (*Rf* 0.61) and methanolyzed with methanolic 3N HCl refluxing for 2 hr to afford methyl 2,3,4-tri-O-methylrhamnoside and methyl 3,4-di-O-methylfucoside, which were identified with authentic samples by TLC and GLC.

Partial Methanolysis of VIb with Methanolic 0.3N HCl——The solution of VIb (100 mg) in methanolic 0.3N HCl was refluxed for 2 hr and the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was examined by TLC to show the main two spots, compound XIIb, *Rf* 0.19 (Solvent A), 0.52 (Solvent B) and compound XIIIb, *Rf* 0.07 (Solvent A), 0.19 (Solvent B), besides the partially O-methylated monosaccharides. Compound XIIb and XIIIb were isolated by preparative TLC using the solvent B. Compound XIIb was suggested to be a trisaccharide composed of 4-O-methylfucose, 2-O-methylrhamnoside and 2,3,4-tri-O-methylrhamnoside by methanolysis with methanolic 3N HCl refluxing for 2 hr, while compound XII was deduced to be a disaccharide composed of 4-O-methylfucose and 2-O-methylrhamnose from the result of methanolysis with methanolic 3N HCl refluxing for 2 hr.

Permethylation of XIIb and XIIIb by the Hakomori's Method——According to the Hakomori's method, both XIIb and XIIIb were methylated and the reaction mixtures were treated as usual, respectively. The permethylates were purified by preparative TLC using solvent A. The permethyl ether of XIIb (*Rf* 0.62) afforded methyl 4-O-methylfucoside and methyl 2,3,4-tri-O-methylrhamnoside, while that of XIIIb (*Rf* 0.61) gave methyl 3,4-di-O-methylfucoside and methyl 2,3,4-tri-O-methylrhamnoside on methanolysis with methanolic 3N HCl refluxing for 2 hr.

Partial Methanolysis of Xb with Methanolic 0.1N HCl——The solution of Xb (64 mg) in methanolic 0.1N HCl was refluxed for 30 min and the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was examined by TLC (Solvent A and B) to recognize the three compounds, namely XIV *Rf* 0.35 (A), 0.78 (B), XV *Rf* 0.30 (B) and Xc *Rf* 0.48 (B) besides the partially O-methylated monosaccharides. The products were submitted to the silica gel column chromatography (3 mm × 50 cm) eluted with $CHCl_3$–MeOH (100: 1—50: 10) and three oligosaccharides (XIV, XV, and Xc) were isolated. On methanolysis with methanolic 3N HCl refluxing for 2 hr, compound Xc afforded methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside and methyl 2-O-methylrhamnoside, while compound XV gave methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2-O-methylrhamnoside and fucitol. By the same methanolysis compound Xc gave the same partially O-methylated monosaccoharides as compound Xb, but the *Rf* value of Xb, *Rf* 0.55 (B), was different from that of Xc.

Per-O-methylation of Xc by the Hokomori's Method——According to the Hokamori's method, compound Xc was methylated and the reaction mixture was treated as usual. The product (Xd) was isolated by preparative TLC using solvent A (*Rf* 0.38). Compound Xd was methanolysed with methanolic 3N HCl refluxing for 2 hr to afford methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside, methyl 2,3,4-tri-O-methylrhamnoside and partial O-methylfucitol.

Compound Xd was identified with an authentic sample of per-O-methylpentasaccharide obtained from per-O-methylsenegin-III by TLC (Solvent A *Rf* 0.38).

Oxidative Degradation of IIa and IIb with $NaIO_4$——To the solutions of IIa and IIb (1 g) in 95% MeOH (300 ml) a solution of $NaIO_4$ (3 g) in H_2O (30 ml) was added, respectively. The reaction mixtures were kept overnight at 4° with stirring. The resulting precipitates were filtered off and the filtrates were evaporated *in vacuo* at 50° to remove MeOH. The residual solutions were diluted with water and extracted with BuOH. The each BuOH solution was washed with water and evaporated *in vacuo*. The residues were dis-

solved in 95% MeOH (100 ml) and then added $NaBH_4$ (1 g) in portionwise at room temperature with stirring. After the mixture was stirred for 1 hr, the reaction mixture was neutralized with 5% AcOH. The solution was evaporated *in vacuo* at 50° to remove MeOH and the residue was extracted with BuOH. The BuOH solution was washed with water and evaporated *in vacuo*. Each residue was refluxed with 0.05N H_2SO_4 in 50% MeOH (100 ml) for 30 min on a water bath. Both reaction mixtures were neutralized with $NaHCO_3$ and concentrated *in vacuo* to remove MeOH.

The residual solutions were extracted with BuOH and the BuOH solutions were washed with water and the solvent was evaporated *in vacuo*. The residues were purified by column chromatography on silica gel using $CHCl_3$–MeOH (50: 1) to afford XVIIa (140 mg) from IIa and XVIa (170 mg) from IIb as a white powder. Compound XVIIa was characterized by basic hydrolysis described later.

XVIa: (mp 173—176° (decomp.)), $[\alpha]_D^{20}$ +30.8° (c=1.3 EtOH), *Anal.* Calcd. for $C_{53}H_{76}O_{17}\cdot 1/2H_2O$: C, 64.04; H, 7.75. Found: C, 64.17; H, 7.46. IR ν_{max}^{Nujol} cm^{-1}: 3500—3300 (OH), 1740, 1720, 1700 (COOR), 1630 (C=C), 1600, 1510 (benzenoid), 1250, 1150, 1050 (C–O–C).

Hydrolysis of XVIIa with 1N KOH——Compound XVIIa was heated with 1N KOH on a water bath. The reaction mixture was treated as usual and 4-methoxycinnamic acid, fucose and presenegenin monomethyl ester were chracterized by TLC, PPC and GLC.

Hydrolysis of XVIIa and XVIa with 0.5% KOH in 50% EtOH——Compound XVIIa and XVIa (100 mg) were dissolved in alcoholic 0.5% KOH (50 ml) respectively and the solutions were allowed to stand overnight at room temperature. After neutralization with ion exchange resin (Amberlite IR–MB) the solvent was evaporated *in vacuo*. The residue from XVIIa was purified by precipitation from aqueous EtOH to give presenegeninfucoside monomethyl ester (XVIIb) as a white powder which was identified with an authentic sample by comparison of IR and NMR spectra. (mp 198—201° (decomp.)), $[\alpha]_D^{20}$ +71.3° (c=1.55 EtOH), *Anal.* Calcd. for $C_{37}H_{58}O_{11}\cdot H_2O$: C, 63.79; H, 8.62. Found: C, 63.32; H, 8.19. IR ν_{max}^{Nujol} cm^{-1}: 3300 (broad OH), 1710—1750 (COOR), 1640 (C=C), 1610, 1060 (broad, C–O–C). NMR (in $CDCl_3$) δ: 3.60 (3H(s) –CO-OC<u>H</u>$_3$), 5.55 (1H(d) J=10 Hz, anomeric <u>H</u>), 5.87 (1H(m) –Ċ=C<u>H</u>–).

The residue from XVIa was recrystallized from aqueous alcohol to give colorless needles (40 mg), XVIb, mp 217—219°, $[\alpha]_D^{20}$ +37.7° (c=1.14 EtOH), *Anal.* Calcd. for $C_{43}H_{68}O_{15}\cdot 1/2H_2O$: C, 61.94; H, 8.28. Found: C, 61.66; H, 8.36. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1740, 1710 (COOR), 1630 (C=C). 1250, 1060 (C–O–C). NMR (in pyridine) δ: 3.55 (3H(s) –COOC<u>H</u>$_3$), 4.88 (1H(d) J=2 Hz anomeric <u>H</u>), 6.05 (1H(d) J=10 Hz anomeric <u>H</u>), 5.84 (1H(m) –Ċ=C<u>H</u>–).

Partial Hydrolysis of IIa with 0.03N H_2SO_4——The solution of compound IIa (1 g) in 0.03N H_2SO_4 (200 ml) contained small amount of dioxane was refluxed on a water bath for 1 hr. The reaction mixture was cooled and extracted twice with BuOH. The BuOH solution was washed with water and evaporated *in vacuo* to give a pale yellow powder. The powder was dissolved in 0.5% KOH–50% EtOH and the solution was allowed to stand overnight. The solution was neutrlized with 1N HCl and extracted with BuOH. The aqueous layer was treated with ion exchange resin (Amberlite IR–MB) and evaporated *in vacuo*. The residue was submitted to silica gel column chromatography eluted with $CHCl_3$–MeOH–H_2O (7: 3: 1, the lower layer) to give colorless needles (XIIId, 5 mg), which were recrystallized from acetone. The product XIIId was identified with 2-(α-L-rhamnopyranosyl)-D-fucose by PPC (*Rf* 0.34, paper: Toyo-Roshi No. 51, solvent: AcOEt–pyridine–H_2O (2: 1: 2 the upper layer) and mixed fusion (mp 145°).

Futhermore, compound XIIId was methylated by the Hakomori's method and the product was purified by preparative TLC using solvent A (*Rf* 0.62) to give per-O-methylated bioside which gave methyl 3,4-di-O-methylfucoside and methyl 2,3,4-tri-O-methylrhamnoside by refluxing with methanolic 3N HCl for 2 hr.

Acknowledgement The authors express their gratitude to Dr. J.H. Westwood, Institute of Cancer Research, Royal Cancer Hospital, Prof. W. Keller, Eidgenössische Technische Hochschule, Zürich and Prof. T. Kawasaki, University of Kyushu, for their kind supply of the authentic samples. Thanks are also due to Assist. Prof. S. Kawanishi and Mr. S. Sakuma, the members of our laboratory, for their co-operation in this work, to the members of Analytical Laboratory of Showa University for elemental analyses.