Chem. Pharm. Bull.
33(2) 464—478 (1985)

Studies on the Constituents of *Momordica cochinchinensis* SPRENG. I. Isolation and Characterization of the Seed Saponins, Momordica Saponins I and II[1)]

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(Received March 8, 1984)

The structures of momordica saponins I and II, the seed saponins of *Momordica cochinchinensis* SPRENG. (Cucurbitaceae) have been elucidated on the basis of spectral and chemical evidence as the 3-*O*-*β*-D-galactopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→3)]-*β*-D-glucuronopyranosido-28-*O*-*β*-D-xylopyranosyl(1→3)-*β*-D-glucopyranosyl(1→3)-[*β*-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranosides of gypsogenin and quillaic acid, respectively.

I and II are contained in the seed kernels in a carboxylate form.

Keywords——*Momordica cochinchinensis*; Cucurbitaceae; triterpene saponin; momordica saponin I; momordica saponin II; bisdesmoside; glucuronide saponin; gypsogenin; quillaic acid

Momordica cochinchinensis SPRENG. is a perennial climber which belongs to the Cucurbitaceae. It ranges from China to the Moluccas, and has been used in traditional medicines in East and Southeast Asia. The seeds are considered to have cooling and resolvent properties, and are used as a remedy for fluxes, liver and spleen disorders, hemorrhoids, wounds, bruises, swelling and pus.[2,3)] The root is an expectorant and is also claimed to be a resolvent of furuncles, abscesses, buboes, mumps and edema of the legs.[2)]

Chemical constituents of this plant have been investigated by several investigators; oleanolic acid glycoside (named momordin) was isolated from the root,[4)] and α-spinasterol,[5)] momordic acid (1-oxo-oleanolic acid)[6)] and a saponin[7)] were isolated from the seeds. Kubota *et al.*[7)] reported the pharmacological action of the seed saponin. In the same report, they suggested it to be a gypsogenin glycoside, but the details have not yet been published.

This study was commenced in an attempt to elucidate the structures of the saponins contained in this plant, and this paper deals with the isolation and characterization of two seed saponins, momordica saponins I and II.

The defatted seed kernels were extracted and fractionated according to the procedure shown in Chart 1 to give a crude saponin, "momordica saponin" (yield; *ca.* 1%). It showed a single spot on a silica gel thin-layer chromatography (TLC) plate, but was separated into two on a reversed-phase TLC plate. The less polar saponin was designated as momordica saponin I (I), and the other as momordica saponin II (II). The two saponins were separated in a thin-layer chromatographically homogeneous state through a LiChroprep RP-18 column. They showed broad infrared (IR) absorption bands at 3500, 1730 and 1610 cm^{-1} which indicate that the two are ester glycosides having a carboxylate group. "Momordica saponin" was methanolyzed in a sealed tube to give a sapogenin fraction and a methyl glycoside fraction.

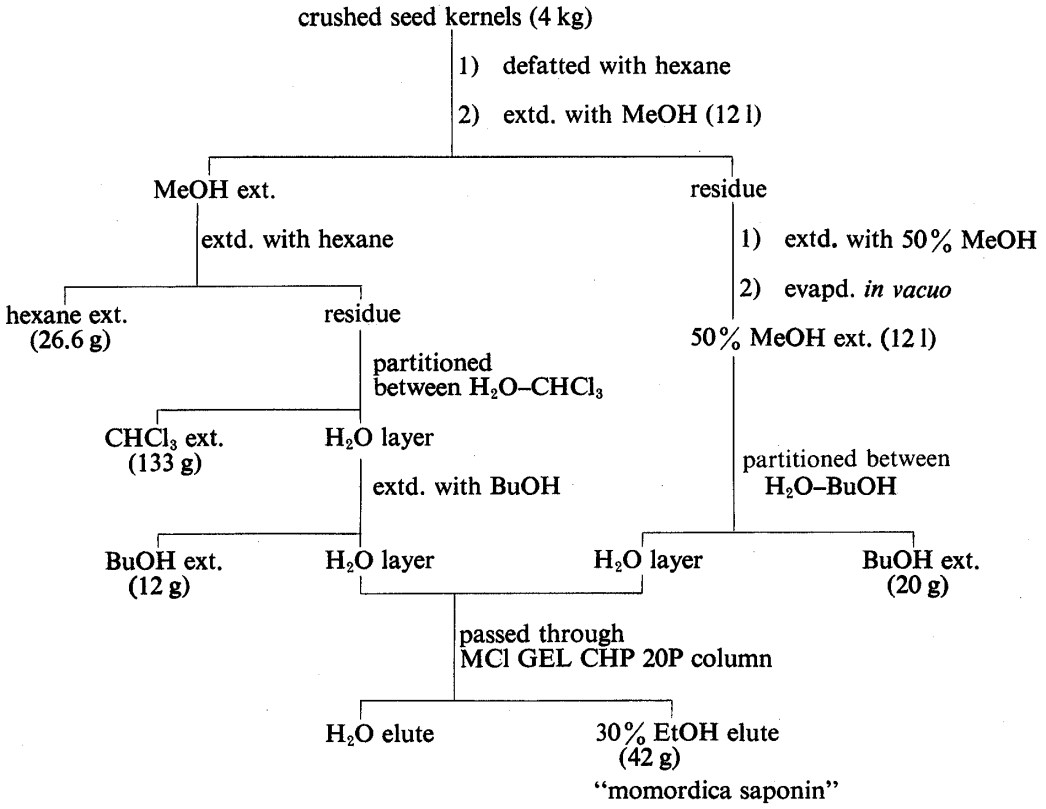


Chart 1. Extraction and Fractionation

The complicated TLC pattern of the former implied instability of the aglycone moiety to the drastic acidic conditions. The methyl glycoside fraction was acetylated and subjected to column chromatography, and the acetylated methyl glycosides of α-L-rhamnopyranose, α-D-fucopyranose, β-D-fucofuranose,[8] α-D-xylopyranose, β-D-galactopyranose, β-D-galactofuranose,[8] β-D-glucopyranose and α-D-glucuronic acid methyl ester were isolated in a pure state. The derivatives of α-D-glucopyranose and α-D-galactopyranose, β-D-xylopyranose (more than 90% purity) and β-L-rhamnopyranose were obtained as mixtures.[9]

Momordica saponins I and II were methanolyzed under the same conditions as employed for the "momordica saponin," and the methyl glycosides were checked by gas chromatography (GC) after conversion to their acetates. L-Rhamnose, D-fucose, D-xylose, D-galactose, D-glucose and D-glucuronic acid were confirmed as the component monosaccharides of both I and II.

Under the milder methanolysis conditions, "momordica saponin" gave two degradation products (III and IV), methyl oligosaccharide (V) and two saponin methyl esters (VI and VII). Under the same conditions, I gave III, V and VI, while II provided IV, V and VII.

The molecular formula of III was determined as $C_{49}H_{76}O_{19}\cdot 4H_2O$ on the basis of elementary analysis and the molecular ion mass number (*m/z* 991 $[M+Na]^+$, 969 $[M+1]^+$) in the field desorption mass (FD-MS) spectrum. The proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra suggested III to be a triterpene trioside having a formyl group, a carboxyl group and a carbomethoxyl group (Table I). III was methanolyzed to give methyl pyranosides of methyl glucuronate, rhamnose and galactose together with a triterpene monoside (VIII) and a sapogenin (IX), $C_{30}H_{46}O_4$. IX was identified as gypsogenin after conversion to the methyl ester acetate (X), $C_{33}H_{50}O_5$, by comparison of its ^{13}C-NMR data (Table I) with those reported.[11] The ^{13}C-NMR signal (180.0 s) of the carboxyl carbon of III is, therefore, attributable to C_{28} of the aglycone moiety,

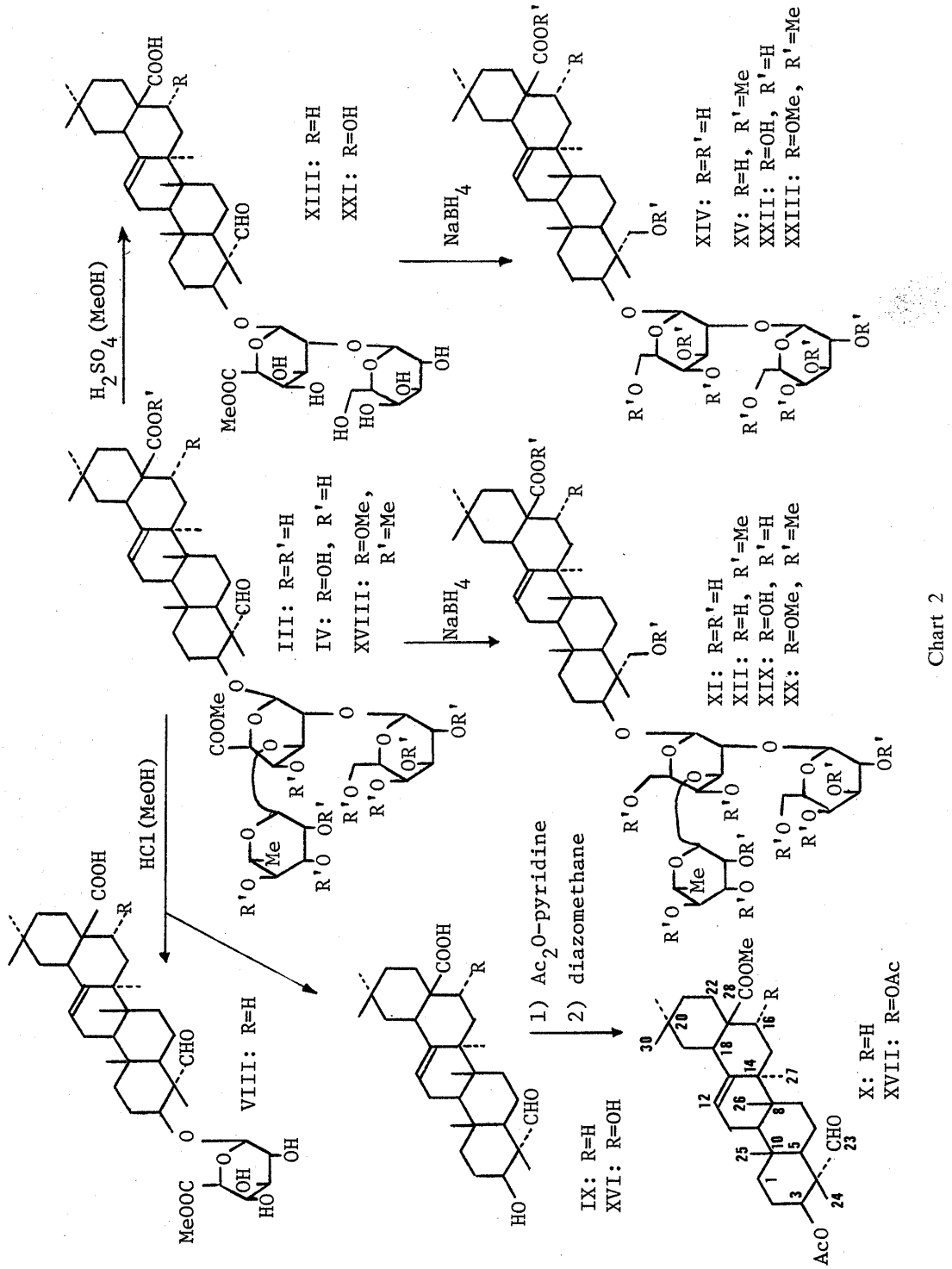


Chart 2

TABLE I. ^{13}C-NMR Chemical Shifts[10]

Compds.	III	IV	VIII	XI	XIX	IX	XVI	X	XVII
Solvent				Pyridine-d_5				$CDCl_3$	
C-1	38.0	38.1	37.8	38.6	38.6	38.4	38.5	37.8	37.9
C-2	25.2	25.3	25.0	25.3	25.3	27.0	26.9	22.5	22.5
C-3	84.2[a]	84.1[a]	81.9	83.1	83.3	71.6	71.6	73.4	73.3
C-4	54.9	55.0	55.2	43.4	43.4	56.2	56.2	54.3	54.2
C-5	48.7	48.8	47.7[a]	48.0[a]	48.5[a]	47.9	47.8	47.8	47.9
C-6	20.4	20.5	20.2	18.4	18.5	21.0	21.0	20.5	20.4
C-7	33.3	32.8	33.1	33.3	32.6	33.1	32.8	32.3	32.2[a]
C-8	39.9	40.1	39.8	39.7	39.9	40.0	40.1	39.6	39.8
C-9	47.8	47.0	47.5[a]	48.3[a]	48.3[a]	47.6	47.1	47.6	46.7
C-10	36.2	36.2	35.9	36.9	37.0	36.1	36.1	35.8	35.8
C-11	23.8	23.6	23.6	23.8	23.8	23.8	23.8	23.0	23.2
C-12	122.1	121.9	121.9	123.8	123.7	122.1	121.8	121.8	123.1
C-13	144.7	145.1	144.6	144.8	145.1	144.8	145.3	143.9	142.0
C-14	42.1	42.0	42.0	42.1	42.1	42.2	42.1	41.7	41.1
C-15	28.2	36.1[b]	28.0	28.3	36.2	28.2	35.9[a]	27.7	32.3[a]
C-16	23.8	74.6	23.6	23.8	74.7	23.8	74.7	23.3	76.1
C-17	46.5	48.8	46.4	46.6	48.9	46.5	49.0	46.7	47.5
C-18	42.1	41.4	41.7	41.9	41.4	41.9	41.5	41.3	40.4
C-19	46.5	47.1	46.4	46.2	47.3	46.4	47.3	45.8	46.1
C-20	30.9	31.0	30.8	30.9	31.0	30.9	30.9	30.7	30.4
C-21	34.2	36.2[b]	34.0	34.0	36.2	34.2	36.1[a]	33.8	35.0
C-22	32.5	31.0	32.2	32.8	31.0	32.5	30.9	32.0	30.9
C-23	210.1	210.0	206.5	65.1	65.1	207.0	207.0	204.4	204.3
C-24	11.1	11.1	10.2	13.5	13.6	9.6	9.6	9.5	9.5
C-25	15.5	15.7	15.4	16.0	16.1	15.7	15.3	15.6	15.7
C-26	17.2	17.4	17.1	17.4	17.5	17.3	17.4	16.8	16.8
C-27	26.1	27.1	25.9	26.1	27.2	26.1	27.2	25.9	26.4
C-28	180.0	180.0	180.0	180.1	180.0	180.0	180.8	178.1	175.9
C-29	33.3	33.3	33.1	33.3	33.3	33.3	33.4	33.1	33.1
C-30	23.8	24.7	23.6	23.8	24.8	23.8	25.0	23.6	24.2
$COO\underline{C}H_3$								51.5	52.1
$OCO\underline{C}H_3$								21.0	21.0
									22.0
$O\underline{C}OCH_3$								170.2	169.9
									170.2
			Sugar moiety						
	18.3	18.4	72.7	18.4	18.5				
	61.6	61.6	74.7	61.6	61.6				
	69.8	69.9	76.9	62.3	62.3				
	70.3	70.2	77.4	69.6	69.6				
	70.9	71.0	105.0	70.0	70.0				
	72.2	72.3	170.3	70.6	70.6				
	72.5	72.7		72.2	72.2				
	72.9	72.9		72.5	72.6				
	73.6	73.7		73.2	73.2				
	75.0	75.1		73.6	73.8				
	76.6	76.6		74.9	74.9				
	78.3	78.3		76.4	76.5				
	84.7[a]	84.5[a]		77.7	77.7				
	103.1	103.1		78.8	78.8				
	103.7	103.7		87.8	87.8				
	104.3	104.4		103.2	103.2				
	170.2	170.2		103.7	103.8				
				104.2	104.2				
$COO\underline{C}H_3$	52.1	52.1	51.8						

a, *b*) Values in any column may be reversed.

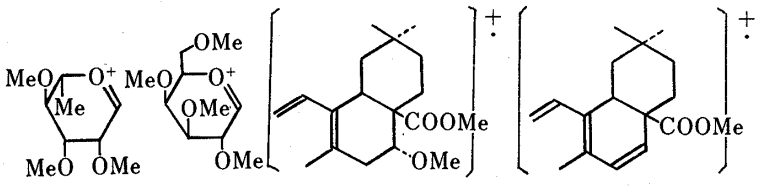
and consequently the carbomethoxyl group should be in the sugar moiety. VIII was concluded to be a 3-*O*-6′-*O*-methyl-*β*-D-glucuronopyranoside of gypsogenin by examination of the NMR spectra.

When III was treated with $NaBH_4$, and the reduction product (XI) was hydrolyzed, L-rhamnose, D-galactose, D-glucose and hederagenin were identified on TLC. The permethylate (XII) of XI gave methyl pyranosides of 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-galactose and 4,6-di-*O*-methyl-D-glucose on methanolysis.

Under milder methanolysis conditions, III gave a bioside (XIII). Treatment of XIII with $NaBH_4$ provided a reduction product (XIV). Methanolysis of the permethylate (XV) of XIV gave methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-D-galactose and 3,4,6-tri-*O*-methyl-D-glucose. From these results, it is apparent that the D-galactopyranosyl and L-rhamnopyranosyl groups are linked to the C_2- and C_3-hydroxyl groups, respectively, of the 6-*O*-methyl-D-glucuronopyranosyl residue. The configuration of the sugar linkages was determined as *β* for the 6-*O*-methyl-D-glucuronopyranosyl and D-galactopyranosyl groups from the ^{1}H-NMR coupling constants of the anomeric protons of XV. As regards the L-rhamnopyranosyl moiety, the configuration was determined as *α* from the ^{1}H-NMR signal pattern of the anomeric proton of XII and the molecular rotation difference (−76.5°) between XI and XIV.

On the basis of the above-mentioned results and spectral evidence, III was concluded to be the 3-*O*-*β*-D-galactopyranosyl(1→2)-[*α*-L-rhamnopyranosyl(1→3)]-6′-*O*-methyl-*β*-D-glucuronopyranoside of gypsogenin.

IV was obtained as colorless needles from MeOH, and the molecular formula was determined as $C_{49}H_{76}O_{20}\cdot 3H_2O$ based on elementary analysis and FD-MS (*m/z* 1007 $[M+Na]^+$). The general features of the NMR spectra were similar to those of III, strongly suggesting that IV is a similar triterpene glycoside having the same sugar moiety as III. The aglycone (XVI), $C_{30}H_{46}O_5$, was obtained by methanolysis of IV under mild conditions and it was identified as quillaic acid by comparing the ^{13}C-NMR spectrum of its methyl ester acetate (XVII) with that reported.[11] The FD-MS spectrum of the permethylate (XVIII) of IV showed a molecular ion peak at *m/z* 1124, and the electron-impact mass (EI-MS) spectrum showed pyronium ions at *m/z* 189 (i) and 219 (ii) which were derived from the fully methylated terminal methylpentose and hexose moieties, respectively. Prominent fragment ions at *m/z* 292 (iii) and 260 (iv) originated from the aglycone moiety. Therefore, it follows that the sugar



(i) (ii) (iii) (iv)

m/z 189 *m/z* 219 *m/z* 292 *m/z* 260

Chart 3

moiety in IV is attached to the hydroxyl group at C_3 of quillaic acid. The structure of the sugar moiety was determined to be the same as that of III by the same procedure as employed for III. From these experimental and spectral data, the degradation product IV of II is proposed to be the 3-*O*-*β*-D-galactopyranosyl(1→2)-[*α*-L-rhamnopyranosyl(1→3)]-6′-*O*-methyl-*β*-D-glucuronopyranoside of quillaic acid.

The methyl oligosaccharide (V) exhibited a molecular ion peak at *m/z* 773 $[M+Na]^+$ in the FD-MS spectrum, and showed in the ^{1}H-NMR spectrum the signals of methoxyl protons

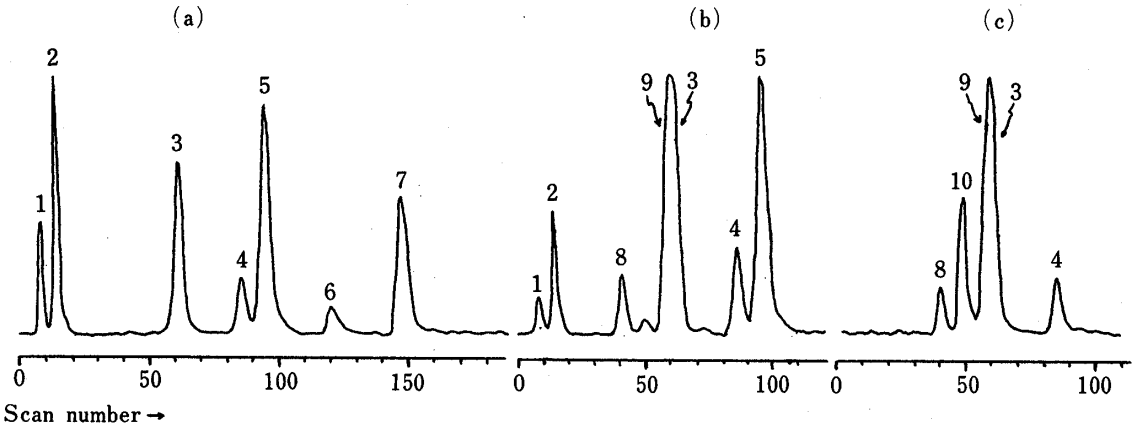


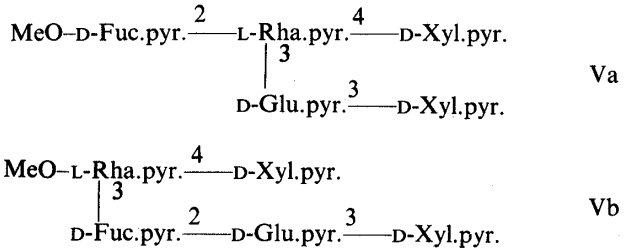
Fig. 1. Reconstructed Total GC-CI-MS Chromatograms of the Acetates of Methanolysis Products of XXIV (a), XXVII (b) and XXVIII (c)

Peak identification: acetylated methyl pyranosides of 1, 2,3,4-tri-OMe-β-D-xyl.; 2, α-anomer of 1; 3, 3,4-di-OMe-α-D-fuc.; 4, β-anomer of 3; 5, 2-OMe-α-L-rha.; 6, 2,4,6-tri-OMe-β-D-glu.; 7, α-anomer of 6; 8, 2,3,4,6-tetra-OMe-β-D-glu.; 9, α-anomer of 8; 10, 2,4-di-OMe-α-L-rha. If the mass spectrum of the peak top scan did not coincide with that of any of the authentic samples, the peak was judged to be unhomogeneous, and the components were identified by measuring the mass spectra of parts of the ascending and descending slopes of the peak and comparing them with the spectra of the authentic samples.

Conditions for GC: column, 3% OV-17 on Chromosorb WAW DMCS; 1 m glass column; column temperature, 130→190 °C (3 °C/min); carrier gas, He 20 ml/min.

Conditions for CI-MS: reagent gas, NH_3; scan number, 1—260 (t_R 1.20→17.90); mass range, m/z 100→350; scan speed, 4 s (m/z 1→1000); ion source temperature, 250 °C; ionization voltage, 150 eV; box current, 200 μA; ion accelerating voltage, 3.5 kV.

at δ 3.39 (3H, s) and of the two methyl groups adjacent to the methine groups at δ 1.4—1.7. The ^{13}C-NMR spectrum exhibited five anomeric carbon signals at around δ 100—106. The methanolysate of V was checked by GC after acetylation to reveal the presence of L-rhamnose, D-fucose, D-xylose and D-glucose as the component monosaccharides. The permethylate (XXIV) of V was methanolyzed and the product was acetylated. The acetates of methylated monosaccharides were examined by the combination of GC and chemical ionization mass (CI-MS) spectrometry. The results are shown in Fig. 1a. The acetates of methyl pyranosides of 2,3,4-tri-*O*-methyl-α- and -β-D-xyloses, 3,4-di-*O*-methyl-α-D-fucose, 2-*O*-methyl-α-L-rhamnose and 2,4,6-tri-*O*-methyl-α- and -β-D-glucoses were identified by comparison of the t_R values and CI-MS patterns with those of authentic samples.[14] These data indicate that V is a methyl pentaoside branched at the L-rhamnopyranosyl unit and has D-xylopyranosyl groups at two terminals. Methanolysis of XXIV under milder conditions gave two degradation products (XXV and XXVI). The less polar one (XXV) showed in the ^{1}H-NMR spectrum four anomeric proton signals, and the permethylate (XXVII) of XXV gave, on methanolysis, methyl pyranosides of 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-D-fucose and 2-*O*-methyl-L-rhamnose (Fig. 1b). The other degradation product (XXVI) showed three anomeric proton signals, and the permethylate (XXVIII) of XXVI gave methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-D-fucose and 2,4-di-*O*-methyl-L-rhamnose (Fig. 1c). From these results, two possible sugar sequences (Va and Vb) can be deduced for V.



Methanolysis of V under mild conditions afforded a methyl trioside (XXIX), which gave methyl α-D-fucopyranoside, L-rhamnose and D-glucose on enzymatic hydrolysis with cellulase. Consequently, the sugar sequence of V was concluded as shown in Va.

In order to determine the configurations of sugar linkages, the ^{1}H-NMR spectra and molecular rotations of XXIV and its degradation products were examined. XXVI showed three pairs of doublet signals assignable to anomeric protons (δ 4.60, d, J=8 Hz; 4.77, d, J=3 Hz; 5.01, d, J=2 Hz) (Fig. 2a). On the assumption that D-glucose and D-fucose are in the sterically preferred Cl conformation, and L-rhamnose unit is in the lC conformation, the two anomeric proton signals with the smaller J-values can be reasonably attributed to the anomeric protons of L-rhamnopyranosyl and methyl α-D-fucopyranosyl moieties, and the doublet with J=8 Hz at δ 4.60 is, consequently, ascribed to the anomeric proton of D-glucopyranoside with a β-configuration. A clue to the configuraion of the L-rhamnopyranosyl unit was provided by calculation of the contribution of the L-rhamnopyranosyl group to the molecular rotation of XXIX. The difference between the molecular rotation of XXIX ($[M]_D$ +62.7°) and the sum of those of methyl β-D-glucopyranoside ($[M]_D$ −66.3°) and methyl α-D-fucopyranoside ($[M]_D$ +355.5°) was −226.5°. The negative value of the difference suggests that the L-rhamnopyranosyl group retains an α-configuration ($[M]_D$ of methyl α-L-rhamnopyranoside, −109.3°; that of the β-anomer, +130.3°).

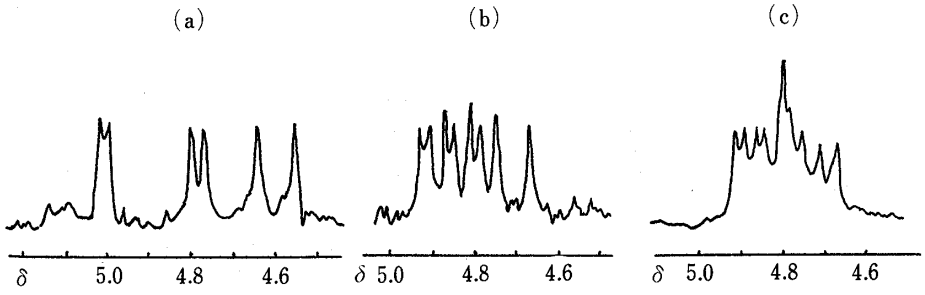
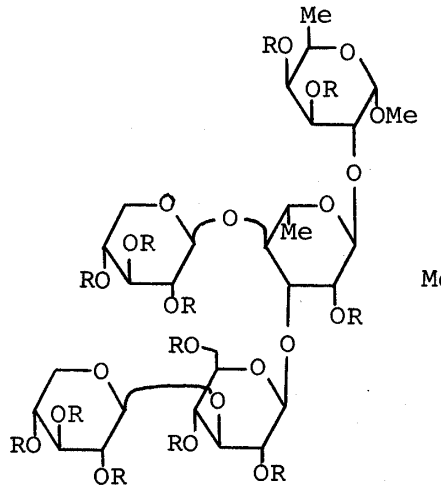


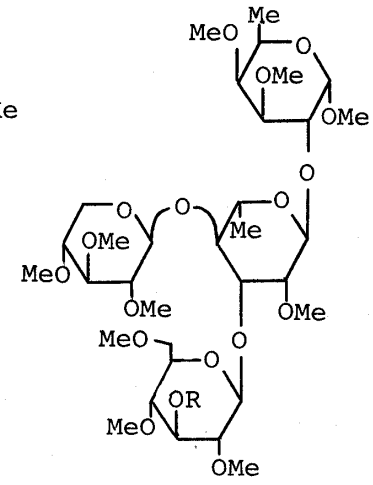
Fig. 2. ^{1}H-NMR Spectra of Anomeric Protons of XXVI (a), XXV (b) and XXIV (c)

XXIV and XXV showed congested patterns of anomeric proton signals (Fig. 2c and 2b) and it was difficult to identify unambiguously the anomeric proton signals of the D-xylopyranosyl groups of XXIV and XXV. The difference (−227.5°) between the molecular rotations of XXV ($[M]_D$ −162.9°) and XXVI ($[M]_D$ +114.6°), and the difference (−103.3°) between the molecular rotations of XXIV ($[M]_D$ −266.2°) and XXV ($[M]_D$ −162.9°) suggested that the two D-xylopyranosyl groups are in β-configuration ($[M]_D$ of methyl 2,3,4-tri-*O*-methyl-α-D-xylopyranoside, +232.4°;[15] β-anomer, −143.3°[15]). Thus, V was tentatively determined to be methyl β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-[β-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl(1→2)-α-D-fucopyranoside.

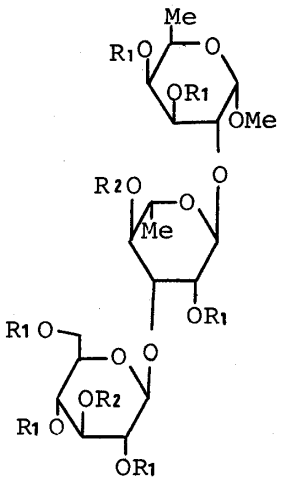
The aqueous solutions of I and II are neutral, but when each solution was passed through the cation-exchange resin, the effluents (I′ and II′) showed an acidic reaction. I and II were converted into the corresponding methyl esters (XXX and XXXI, respectively) by passing them through the cation-exchange resin followed by treatment with diazomethane.[16] XXX and XXXI showed the same *Rf* values and coloration on the thin-layer chromatogram as those of VI and VII, respectively. XXX exhibited an $[M+Na]^+$ ion peak at m/z 1709 in the fast atom bombardment mass (FAB-MS) spectrum and its ^{13}C-NMR spectrum showed at least eight anomeric carbon signals. Alkaline hydrolysis of I followed by neutralization and treatment with diazomethane gave a trioside dimethyl ester (XXXII), identical with the methyl ester of III.



V : R=H
XXIV : R=Me

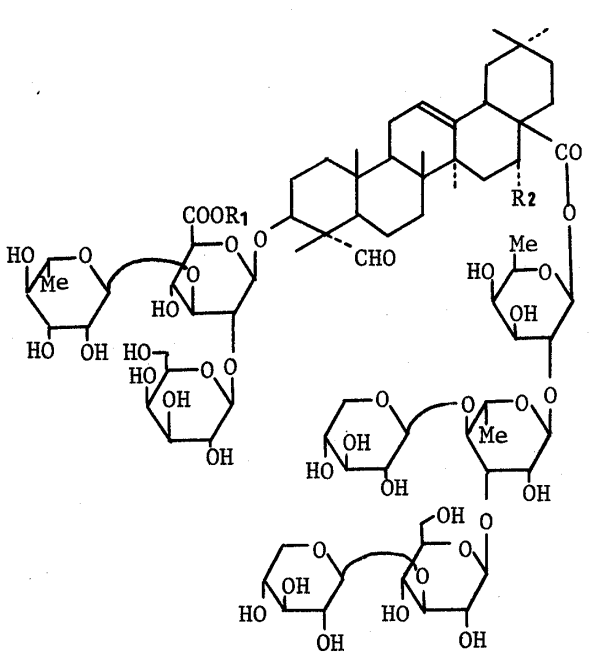


XXV : R=H
XXVII : R=Me

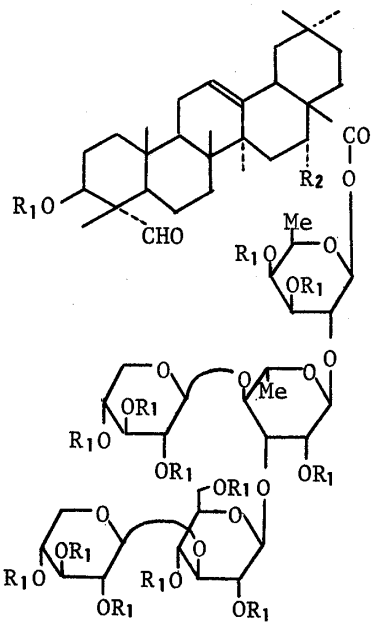


XXVI : R_1=Me, R_2=H
XXVIII : R_1=R_2=Me
XXIX : R_1=R_2=H

Chart 4



I' : R_1= R_2=H
II' : R_1=H, R_2=OH
VI=XXX: R_1=Me, R_2=H
VII=XXXI : R_1=Me, R_2=OH



XXXIII : R_1= Ac, R_2= H
XXXIV : R_1= R_2= H
XXXVI : R_1= Ac, R_2= OAc
XXXVII : R_1= H, R_2= OH

Chart 5

Kitagawa *et al.*[17] and Lindberg *et al.*[18] have developed methods for selective cleavage of the uronide linkage of glucuronide saponins and polysaccharides. I was treated with a hot mixture of acetic anhydride and triethylamine according to Lindberg's method and an ester

glycoside acetate (XXXIII) free from the triose moiety was obtained in a good yield. The deacetylation product (XXXIV) showed ^{13}C-NMR signals of five anomeric carbons, and the FD-MS spectrum showed an $[M+Na]^+$ ion signal at *m/z* 1211. Further treatment with 0.5 N HCl–MeOH at room temperature afforded a methyl pentaoside (V) and gypsogenin. The configuration of the D-fucopyranosyl group attached to the carboxyl group of gypsogenin was judged to be β from the *J*-value (8 Hz) of the anomeric proton signal in the downfield region (δ 6.06) of the ^{1}H-NMR spectrum of XXXIII.

Accordingly, I′ is gypsogenin 3-*O*-β-D-galactopyranosyl(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucuronopyranosido-28-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-[β-D-xylopyranosyl(1→4)]-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside.

The structure of II was determined in the same way as for I. The FAB-MS spectrum of the methyl ester (XXXI) of II′ showed an $[M+Na]^+$ ion at *m/z* 1725. Alkaline hydrolysis of II followed by neutralization and methylation with diazomethane furnished a trioside dimethyl ester (XXXV), which was identical with the methyl ester of IV. Treatment of II with a hot mixture of acetic anhydride and triethylamine gave an ester glycoside acetate (XXXVI). The FD-MS spectrum of the deacetylation product (XXXVII) showed an $[M+Na]^+$ ion peak at *m/z* 1227. Methanolysis of XXXVII with 0.5 N HCl–MeOH at room temperature gave a methyl pentaoside (V) and quillaic acid. The configurations of the component monosaccharides of XXXVII were regarded as the same as those of the corresponding monosaccharides of XXXIV since the anomeric carbon signals in the ^{13}C-NMR spectrum of XXXVII are almost superimposable on those of XXXIV.

Consequently, II′ is a quillaic acid 3,28-*O*-bisdesmoside having the same oligosaccharide moieties as those of I′. I and II are contained in the seed kernels in a carboxylate form.

Experimental[19]

Extraction and Fractionation——Coarsely crushed seed kernels (4.1 kg) were percolated with hexane (10 l). The kernels were air-dried and powdered finely. The powder was percolated with hexane (5 l), and then with MeOH (12 l). The MeOH was evaporated off *in vacuo* to give a resinous material, which was treated with hexane to remove remaining fatty oil. The hexane-insoluble material was suspended in water (3 l) and extracted with $CHCl_3$ and then with *n*-BuOH to give the $CHCl_3$ extract (133 g) and the BuOH extract (12 g). The aqueous layer was set aside. The powder after extraction with MeOH was percolated with 50% MeOH (12 l). The MeOH was evaporated off and the aqueous layer was concentrated to *ca.* 3 l, and then extracted with BuOH. The aqueous layer was combined with that from the MeOH extraction, and concentrated to 3 l. The aqueous solution (500 ml) was applied to a column of MCI Gel CHP 20P (300 ml) and eluted first with H_2O (1 l) and then with 30% EtOH (1 l). "Momordica saponin" was eluted with 30% EtOH (total yield: 42 g from 4.1 kg seed kernels).

Isolation of Momordica Saponins I and II——"Momordica saponin" (1 g) was chromatographed on a LiChroprep RP-18 column (100 g) using 50% MeOH as an eluting solvent. Separation of the two saponins was checked by TLC on an RP-18 plate (65% MeOH). Repeated column chromatography gave thin-layer chromatographically homogeneous momordica saponins I (I) (985 mg) and II (II) (1.1 g) from 5 g of the crude saponin. I: A white powder from MeOH. mp 241—244 °C (dec.). $[\alpha]_D^{19}$ −14.8° (*c*=0.7, MeOH–H_2O (1:2)). IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (OH), 2850 (CH_3, CH_2), 1730 (–COOR), 1610 (–COO^-), 1200—1000 (C–O–). *Anal.* Found: C, 49.41; H, 6.93. II: A white powder from MeOH. mp 252—255 °C (dec.). $[\alpha]_D^{19}$ −28.5° (*c*=0.67, MeOH–H_2O (1:2)). IR ν_{max}^{KBr} cm^{-1}: same as that of I. *Anal.* Found: C, 48.33; H, 6.57.

Methanolysis of "Momordica Saponin," Identification of the Component Sugars——"Momordica saponin" (1 g) was dissolved in 2 N HCl (MeOH) (5 ml) and heated in a sealed tube for 1 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was bubbled through with H_2S and concentrated. The residue was chromatographed over silica gel (30 g) ($CHCl_3$–MeOH–H_2O (32:8:1)) for rough separation to give the aglycone fraction (328 mg) and the methyl glycoside fraction (680 mg). The latter fraction was repeatedly chromatographed on silica gel (100 times the weight of materials) using 5% MeOH in $CHCl_3$ and $CHCl_3$–MeOH–H_2O (32:4:0.1, 32:8:1). Each fraction which showed a single spot on TLC was acetylated with Ac_2O–pyridine mixture at room temperature. The product was purified by passing it through a silica gel column (hexane–AcOEt (4:1, 2:1)). The ^{1}H-NMR spectrum of each fraction was taken and compared with that of the appropriate authentic sample. The fully acetylated methyl glycosides of the following sugars were identified:[20] β-D-fucofuranose ($[\alpha]_D^{16}$ −62.0° (*c*=0.4, $CHCl_3$)), 6-*O*-methyl-α-D-glucuronopyranose ($[\alpha]_D^{22}$ +106.0° (*c*=0.65, $CHCl_3$)), α-L-rhamnopyranose ($[\alpha]_D^{22}$ −52.4° (*c*=3.1, $CHCl_3$)), α-D-

fucopyranose ($[\alpha]_D^{22}+102.6°$ ($c=0.7$, $CHCl_3$)), α-D-xylopyranose ($[\alpha]_D^{22}+100.5°$ ($c=1.05$, $CHCl_3$)), β-D-galactofuranose ($[\alpha]_D^{16}-38.0°$ ($c=0.2$, $CHCl_3$)), β-D-glucopyranose ($[\alpha]_D^{22}-8.4°$ ($c=0.7$, $CHCl_3$)), β-D-galactopyranose ($[\alpha]_D^{22}-9.2°$ ($c=0.7$, $CHCl_3$)). Methyl tetra-*O*-acetyl-α-D-glucopyranoside and methyl tetra-*O*-acetyl-α-D-galactopyranoside were obtained as a mixture. Methyl tri-*O*-acetyl-β-D-xylopyranoside was contaminated with a small quantity (less than 10%) of methyl tri-*O*-acetyl-β-L-rhamnopyranoside.

Methanolysis of Momordica Saponins I and II, Identification of Component Sugars by GC——I and II (10 mg each) were methanolyzed in the same way as described for "momordica saponin." The acetylation products of the methanolysates were checked by GC (2% ECNSS-M on Chromosorb WAW DMCS (60—80 mesh); 2 m × 3 mm i.d. glass column; column temperature, 195 °C; carrier gas, He 30 ml/min). The products from I and II gave the same gas chromatograms. t_R values and identification of each peak are as follows:[21] 3.27 (methyl tri-OAc-α-L-rhamnopyranoside), 3.72 (methyl tri-OAc-α-D-fucopyranoside), 4.51 (methyl tri-OAc-α-D-xylopyranoside), 5.47 (methyl tri-OAc-β-D-xylopyranoside), 10.8,[22] 13.8,[22] 15.8 (6-*O*-methyl-tetra-OAc-α-D-glucuronopyranoside), 16.9 (methyl tetra-OAc-α-D-galactopyranoside), 18.9 (methyl tetra-OAc-α-D-glucopyranoside), 22.7 (methyl tetra-OAc-β-D-glucopyranoside), 23.8 (methyl tetra-OAc-β-D-galactopyranoside).

Partial Degradation of "Momordica Saponin," Isolation of III, IV, V, VI and VII——"Momordica saponin" (20 g) was suspended in 0.5 N HCl (MeOH) (200 ml) and stirred for 2 h at room temperature. The methanolysate (19 g) showed 4 major spots on silica gel TLC ($CHCl_3$–MeOH–H_2O (25 : 17 : 3)), the lowest spot of which was separated into two spots on an RP-18 plate (80% MeOH). The methanolysate was subjected to column chromatography on silica gel (200 g). Elution with $CHCl_3$–MeOH–H_2O (32 : 8 : 1) gave III (2.0 g), IV (2.0 g), and $CHCl_3$–MeOH (1 : 1) eluted a mixture (7.5 g) of V, VI and VII. The effluent with $CHCl_3$–MeOH (1 : 1) was repeatedly chromatographed on MCI Gel CHP 20P (100 ml) using 80% MeOH to give a fraction containing V (Fr. 1, 2.2 g) and VI (300 mg) and VII (600 mg). Fr. 1 was again chromatographed on silica gel (100 g) using $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5) to give V (1.7 g). III: Colorless needles from MeOH. mp 216—217.5 °C. $[\alpha]_D^{22}+19.7°$ ($c=1.0$, MeOH). FD-MS *m/z*: 991 $[M+Na]^+$, 969 $[M+1]^+$. *Anal.* Calcd for $C_{49}H_{76}O_{19}\cdot 4H_2O$: C, 56.54; H, 8.08. Found: C, 56.84; H, 7.73. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (C=O). ^{1}H-NMR: 3.78 (3H, s, –$COOCH_3$), 9.85 (H, s, –CHO). ^{13}C-NMR: Table I. IV: Colorless needles from MeOH. mp 214—215.5 °C. $[\alpha]_D^{22}+2.2°$ ($c=1.0$, MeOH). FD-MS *m/z*: 1007 $[M+Na]^+$. *Anal.* Calcd for $C_{49}H_{76}O_{20}\cdot 3H_2O$: C, 56.67; H, 7.90. Found: C, 57.12; H, 8.00. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (C=O). ^{1}H-NMR: 3.78 (3H, s, –$COOCH_3$), 5.60 (H, br s, C_{12}-H), 5.99 (H, br s, anomeric H of rhamnosyl group), 9.82 (H, s, –CHO). ^{13}C-NMR: Table I. V: A hygroscopic white powder. $[\alpha]_D^{20}-10.2°$ ($c=1.2$, MeOH). FD-MS *m/z*: 773 $[M+Na]^+$. ^{13}C-NMR: 17.0 q, 18.7 q, 54.9 q (–OMe), 61.9 t, 66.5, 67.0, 68.1, 69.1, 69.8, 70.7, 70.9, 71.1, 73.3, 74.3, 75.3, 75.4, 75.6, 77.4, 77.6, 78.4, 78.7, 82.7 d, 88.7 d, 100.4 d, 103.8 d, 104.1 d, 105.1 d,106.0 d. VI: An amorphous powder. ^{1}H-NMR (pyridine-d_5–D_2O (1 : 1)): 3.77 (s, –$COOCH_3$), 9.84 (s, –CHO). ^{13}C-NMR (pyridine-d_5–D_2O (1 : 1)): 30.7 s (C_{20}), 36.2 s (C_{10}), 40.2 s (C_8), 42.2 s (C_{14}), 46.9 s (C_{17}), 52.1 q (–OMe), 55.0 d[23] (C_4), 123.7 d (C_{12}), 144.0 s (C_{13}), 170.3 s (–COOMe), 176.3 s (C_{28}), 210.2 d (C_{23}). VII: An amorphous powder. ^{1}H-NMR (pyridine-d_5–D_2O (1 : 1): 3.77 (s, –COOMe), 9.85 (s, –CHO). ^{13}C-NMR (pyridine-d_5–D_2O (1 : 1)): 30.7 s (C_{20}), 35.8 s (C_{10}), 40.4 s (C_8), 42.2 s (C_{14}), 49.2 s (C_{17}), 52.1 q (–OMe), 55.0 d[23] (C_4), 123.8 d (C_{12}), 144.5 s (C_{13}), 170.3 s (–COOMe), 175.8 s (C_{28}), 210.2 d (–CHO).

Partial Degradation of I and II——I and II (2 mg each) were each dissolved in 0.5 N HCl (MeOH) (0.5 ml) and the solution was stirred at room temperature for 1 h. The products were compared on TLC with the product from "momordica saponin." The product from I showed spots of III, V and VI, while that from II gave spots of IV, V and VII.

Methanolysis of III, Identification of the Aglycone and the Component Sugars——III (500 mg) was dissolved in 2 N HCl (MeOH) (5 ml) and the solution was refluxed for 30 min. The product was chromatographed on silica gel (10 g). Elution with $CHCl_3$–MeOH (20 : 1) furnished Fr. I (159 mg) and Fr. II (90 mg). Subsequent elution with $CHCl_3$–MeOH (10 : 1) gave Fr. III (120 mg) and Fr. IV (86 mg). Fr. I was chromatographed (Kieselgel 60, 20 g, 10% acetone in benzene) to give IX (69 mg): Colorless needles from MeOH. mp 245—248 °C. EI-MS *m/z*: 470.341 $[M]^+$ (Calcd for $C_{30}H_{46}O_4$: 470.340), 424, 250, 248 (base peak), 204 and 203. ^{1}H-NMR: 0.90, 0.96, 0.97, 1.01, 1.27, 1.33 (all s, $\rangle C-CH_3$), 3.31 (H, dd, $J=5$, 11 Hz, C_{18}-H_β), 4.08 (H, t, $J=8$ Hz, C_3-H_α), 5.49 (H, br s, C_{12}-H), 9.60 (H, s, –CHO). ^{13}C-NMR: Table I. IX was acetylated with Ac_2O–pyridine mixture and then methylated with diazomethane to give X: Colorless needles from $CHCl_3$–MeOH. mp 184—187 °C. $[\alpha]_D^{20}+72.8°$ ($c=0.5$, $CHCl_3$). EI-MS *m/z*; 526.363 $[M]^+$ (Calcd for $C_{33}H_{50}O_5$: 526.366), 466 $[M-AcOH]^+$, 407, 262 (base peak), 249, 203, 189. ^{1}H-NMR ($CDCl_3$): 0.75, 0.91, 0.93, 0.99, 1.09, 1.16 (all s, $\rangle C-CH_3$), 1.96 (3H, s, –OAc), 2.7—3.0 (H, m, C_{18}-H_β), 3.62 (3H, s, –$COOCH_3$), 4.98 (H, t, $J=8$ Hz, C_3-H_α), 5.29 (H, br t, $J=4$ Hz, C_{12}-H), 9.26 (H, s, –CHO). ^{13}C-NMR: Table I.

Fr. II was chromatographed twice on silica gel (15 g) using 5% MeOH in $CHCl_3$ to give a crystalline powder (VIII): Colorless needles from MeOH. mp 238—240 °C. $[\alpha]_D^{22}+36.3°$ ($c=0.65$, MeOH). ^{1}H-NMR: 0.81, 0.93, 0.96, 1.00, 1.28 (×2) (all s, $\rangle C-CH_3$), 3.1—3.5 (H, m, C_{18}-H_β), 3.72 (3H, s, –$COOCH_3$), 4.86 (H, d, $J=7$ Hz, anomeric H), 5.43 (H, br s, C_{12}-H), 9.73 (H, s, –CHO). ^{13}C-NMR: Table I.

Fr. III was chromatographed on silica gel (10 g) with $CHCl_3$–MeOH–H_2O (32 : 4 : 0.1). The thin-layer chromatographically homogeneous compound (105 mg) was acetylated with Ac_2O–pyridine mixture at room temperature. The product was chromatographed (Kieselgel 60, hexane–AcOEt (3 : 1)) to give methyl tri-*O*-Ac-α-L-

rhamnopyranoside (98 mg) ($[\alpha]_D^{27}$ −54.1° (c=2.1, $CHCl_3$)) and its β-anomer (17 mg) ($[\alpha]_D^{27}$ +26.1° (c=0.78, $CHCl_3$)). Identification was made by comparing their ^{1}H-NMR spectra and specific rotations with those of authentic samples.

Fr. IV was acetylated and chromatographed (Kieselgel 60, hexane–AcOEt (2 : 1)) to give methyl tetra-OAc-α-D-galactopyranoside (48 mg) ($[\alpha]_D^{27}$ +105.9° (c=1.6, $CHCl_3$)) and its β-anomer (35 mg) ($[\alpha]_D^{27}$ +10.8° (c=1.3, $CHCl_3$)). Identification was made by comparison of their ^{1}H-NMR spectra with those of authentic samples.

$NaBH_4$ Reduction of III——III (326 mg) was dissolved in MeOH (3 ml). $NaBH_4$ (30 mg) was added to the solution, and the whole was stirred at room temperature for 2 h. The product was suspended in water (5 ml) and extracted with BuOH (3 ml × 3). The BuOH extract was repeatedly chromatographed on silica gel (100 times the weight of materials) using $CHCl_3$–MeOH–H_2O (32 : 8 : 1) to give a less polar compound (109 mg), a more polar one (XI, 43 mg) and a mixture of the two (44.5 mg). The less polar compound was proved to have no formyl group, but retained a carbomethoxyl group (^{1}H-NMR, 3.73 (3H, s, –$COOCH_3$); ^{13}C-NMR, 52.1 q (–$COOCH_3$), 170.2 s (–$COOCH_3$)), and it was combined with the mixture and treated with $NaBH_4$ to give another crop (100 mg) of XI: Colorless needles from dil. MeOH. mp 239—241 °C. $[\alpha]_D^{22}$ +19.3° (c=1.2, MeOH). ^{1}H-NMR: The signals of a methoxyl group and a formyl group were not observed. 1.61 (d, J=6 Hz), 4.8—5.1 (2H, anomeric H of glucose and C_3-H_α), 5.24 (d, J=8 Hz, anomeric H of galactose), 5.44 (br s, C_{12}-H) and 5.87 (br s, anomeric H of rhamnose).

Hydrolysis of XI; Identification of the Aglycone and the Component Sugars——XI (5 mg) was dissolved in 2 N H_2SO_4 in 50% EtOH (1 ml) and the whole was heated in a boiling water bath for 2 h. Water was added (5 ml), and the EtOH was evaporated off. The aqueous solution was extracted with $CHCl_3$ (3 ml × 3). The aqueous layer was neutralized with $BaCO_3$ and filtered, then the filtrate was concentrated to dryness. The residue was dissolved in MeOH and passed through a Sephadex LH-20 column. The MeOH eluate was concentrated to dryness to give a sugar fraction. The $CHCl_3$ extract was checked by TLC (hexane–AcOEt (1 : 1)), giving the same *Rf* value and coloration (H_2SO_4, heating) as authentic hederagenin. The sugar fraction was checked by silica gel TLC ($CHCl_3$–MeOH–H_2O (25 : 17 : 3)) and Avicel SF TLC (BuOH–pyridine–H_2O (6 : 2 : 3), top layer + pyridine (1)) to reveal the presence of L-rhamnose, D-glucose and D-galactose.

Methylation of XI——XI (20 mg) and NaH (200 mg) were added to anhydrous tetrahydrofuran (3 ml), and the mixture was stirred for 10 min. CH_3I (2 ml) was added and the whole was stirred at room temperature for 26 h. Then MeOH (5 ml) was added, and the reaction mixture was neutralized with dil. HCl. The solvent was evaporated off and the residue was extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on silica gel (Woelm, 10 g) using 10% acetone in benzene to give XII (15 mg) as a resin. The IR spectrum (CCl_4) showed no hydroxyl group absorption. ^{1}H-NMR ($CDCl_3$): 4.15—4.35 (2H, m, anomeric H of glucose and C_3-H_α), 4.48 (d, J=7 Hz, anomeric H of galactose), 5.19 (H, br s, anomeric H of rhamnose), 5.26 (br s, C_{12}-H).

Methanolysis of XII; Identification of the Component Methylated Sugars by TLC and GC——XII (5 mg) was dissolved in 2 N HCl (MeOH) (1 ml) and heated in a sealed tube for 3 h, then worked up in a usual manner to give the methanolysate. The product was subjected to TLC and GC. The results are shown in Table II.

Partial Methanolysis of III; Structure Determination of the Bioside XIII——III (200 mg) was dissolved in 0.2 N H_2SO_4 (MeOH) (2 ml) and the solution was refluxed for 1 h. After being diluted with MeOH (5 ml) the solution was neutralized with $BaCO_3$, and the precipitates were removed by centrifugation. The supernatant was concentrated and the residue was chromatographed (Kieselgel 60, 20 g, $CHCl_3$–MeOH–H_2O (32 : 8 : 1) to obtain XIII (50 mg) and III (80 mg). XIII (50 mg) and $NaBH_4$ (50 mg) were dissolved in MeOH (1 ml) and the mixture was stirred for 30 min. The solution was treated in the same manner as described for the reduction of III. The product was purified by column chromatography (Kieselgel 60, 5 g, $CHCl_3$–MeOH–H_2O (32 : 8 : 1)) to give XIV (15 mg) as an amorphous powder from dil. MeOH. mp 232—235 °C. $[\alpha]_D^{22}$ +33.1° (c=0.13, MeOH). The ^{1}H-NMR spectrum showed no signal of a carbomethoxyl group or a formyl group. XIV (15 mg) was methylated and purified essentially in the same way as described for methylation of XI, and XV (7 mg) was obtained. It showed no IR absorption of a hydroxyl group (CCl_4). The ^{1}H-NMR ($CDCl_3$) spectrum showed signals at 4.25 (d, J=8 Hz, anomeric H of glucose), 4.62 (d, J=7 Hz, anomeric H of galactose) and 5.27 (br s, C_{12}-H). XV was methanolyzed in the same way as described for methanolysis of XII, and the methylated sugars were checked by TLC and GC. The results are shown in Table II.

Structure Determination of IV——The experimental procedures were essentially the same as those for the structure determination of III, although some minor modifications were made. The product data are given below. XVI: Colorless needles from dil. MeOH. mp 256—263 °C. EI-MS m/z: 486.335 $[M]^+$ (Calcd for $C_{30}H_{46}O_5$: 486.335), 468 $[M-H_2O]^+$, 264, 246 (264 − H_2O), 201 (246 − COOH). ^{1}H-NMR: 0.96, 1.01, 1.07, 1.19, 1.35, 1.59 (all s, >C–CH_3), 3.63 (H, dd, J=4, 14 Hz, C_{18}-H_β), 4.10 (H, t, J=8 Hz, C_3-H_α), 5.18 (H, br s, C_{16}-H_β), 5.63 (H, br s, C_{12}-H), 9.62 (H, s, –CHO). ^{13}C-NMR: Table I. XVII: Colorless prisms from MeOH. mp 205—207 °C. $[\alpha]_D^{20}$ +1.4° (c=0.5, $CHCl_3$). EI-MS m/z: 584.370 $[M]^+$ (Calcd for $C_{35}H_{52}O_7$: 584.371), 524 $[M-AcOH]^+$, 464 $[M-2AcOH]^+$, 260 (base peak), 201. ^{1}H-NMR ($CDCl_3$): 0.73, 0.93, 1.00 (×2), 1.09, 1.27 (all s, >C–CH_3), 1.96 (3H, s, –$OCOCH_3$), 2.09 (3H, s, –$OCOCH_3$), 3.11 (H, dd, J=4, 13 Hz, C_{18}-H_β), 3.63 (3H, s, –$COOCH_3$), 4.99 (H, t, J=8 Hz, C_3-H_α), 5.43 (H, br s, C_{12}-H), 5.64 (H, br s, C_{16}-H_β), 9.27 (H, s, –CHO). ^{13}C-NMR: Table I. XVIII: Amorphous solid. IR (CCl_4): no hydroxyl group absorption. FD-MS m/z: 1124 $[M]^+$. EI-MS m/z: 189 (i), 219 (ii), 260(iv), 292 (iii), 375, 405, 465, 497. ^{1}H-NMR ($CDCl_3$): 3.79 (3H, s, –$COOCH_3$), 4.37 (H, d, J=7 Hz, anomeric H of galactose), 4.53 (H, d, J=5 Hz, anomeric H of glucuronate), 5.11 (H, d, J=1.5 Hz, anomeric H of rhamnose), 5.33 (br s, C_{12}-H), 9.50 (H, s, –CHO). The signal of

TABLE II. GC and TLC of Component Sugars of XII, XV, XX and XXIII

	GC (t_R)[a]				TLC (Rf)[b]			
	140 °C		160 °C		AcOEt–MeOH (50 : 1)		Benzene–acetone (2 : 1)	
Methanolysate of XII	1.9[c]	2.1	1.3	3.6[d]	0.64	0.41[d]		
	8.0[d]	8.9	4.0	20.9	0.34	0.16		
Methanolysate of XV	8.0[d]	8.9	3.6[d]	4.0			0.47[d]	0.33
	15.7	19.2[e]	6.7	8.1[e]			0.28[e]	0.23
Methanolysate of XX			1.3	4.1	0.64	0.40[d]		
			3.6[d]	21.0	0.34	0.16		
Methanolysate of XXIII			3.6[d]	4.1			0.47[d]	0.34
			6.8	8.1[e]			0.28[e]	0.23
Methyl glycoside of								
2,3,4-Me–α-L-rham.pyr.	2.1		1.3		0.64			
2,3,4,6-Me–α-D-gal.pyr.	8.9		4.0		0.34		0.33	
2,3,6-Me–α-D-glu.pyr.	26.0		10.3				0.23	
2,4,6-Me–α-D-glu.pyr.	27.0		10.9				0.18	
3,4,6-Me–α-D-glu.pyr.	15.7		6.7				0.23	
2,6-Me–α-D-glu.pyr.			32.9					
3,6-Me–α-D-glu.pyr.			21.3		0.19			
4,6-Me–α-D-glu.pyr.			20.9		0.16			

a) Column: 2% ECNSS-M on Chromosorb WAW DMCS (60—80 mesh); 2 m × 3 mm i.d. glass column; carrier gas, He 30 ml/min.
b) Kieselgel 60 F_{254} plate.
The unidentified peaks might be due to methyl glycosides of *c*) 2,3,4-Me–β-L-rhm.pyr.; *d*) 2,3,4,6-Me–β-D-gal.pyr.; and *e*) 3,4,6-Me–β-D-glu.pyr.

$C_{28}OOCH_3$ could not be distinguished from other methoxyl signals. XIX: Amorphous solid. ^{1}H-NMR: 1.63 (3H, d, J=5 Hz, >CH–CH_3), 4.7—5.5 (4H, signals of the anomeric protons of glucose and galactose, and of C_3-H_α and C_{16}-H_β appeared in this region; the signals were not assigned), 5.64 (H, br s, C_{12}-H), 5.89 (H, br s, anomeric H of rhamnose). No signals of carbomethoxyl or formyl protons were observed. ^{13}C-NMR: Table I. XX: Amorphous solid. No IR absorption due to a hydroxyl group (CCl_4). FD-MS m/z: 1126 $[M]^+$. ^{1}H-NMR ($CDCl_3$): 4.27 (H, d, J=7 Hz, anomeric H of glucose), 4.49 (H, d, J=8 Hz, anomeric H of galactose), 5.20 (H, d, J=1.5 Hz, anomeric H of rhamnose), 5.30 (H, perturbed t, C_{12}-H). XXI: Colorless needles from dil. MeOH. mp 205—208 °C. $[\alpha]_D^{15}$ +15.0° (c=1.75, MeOH). ^{1}H-NMR: 3.71 (–$COOCH_3$), 4.90 (H, d, J=7 Hz, anomeric H of glucuronate), 5.1—5.3 (2H, m, anomeric H of galactose and C_{16}-H_β), 5.60 (H, br s, C_{12}-H), 9.90 (H, s, –CHO). ^{13}C-NMR: 31.0 s (C_{20}), 36.2 s (C_{10}), 40.1 s (C_8), 42.1 s (C_{14}), 48.8 s (C_{17}), 52.0 q (–OCH_3), 55.0 d[23] (C_4), 62.1 t (C_6 of glucose), 70.1, 72.5, 74.4, 74.6, 74.8, 76.7, 77.1, 77.4, 82.2, 83.4, 103.1 d (anomeric C), 106.2 d (anomeric C), 121.9 d (C_{12}), 145.2 s (C_{13}), 170.2 s (–$COOCH_3$), 180.0 s (C_{28}), 209.2 d (–CHO). XXII: Not crystallized. ^{1}H-NMR: no signals of carbomethoxyl or formyl protons were observed. XXIII: Not crystallized. No IR absorption due to a hydroxyl group was observed (CCl_4). ^{1}H-NMR ($CDCl_3$): 3.94 (H, br s, C_{16}-H_β or C_3-H_α), 4.25 (H, d, J=7 Hz, anomeric H of glucose), 4.62 (H, d, J=7 Hz, anomeric H of galactose), 5.32 (H, br s, C_{12}-H).

Methanolysis of V and Identification of the Component Sugars——V (10 mg) was dissolved in 2 N HCl (MeOH) (1 ml) and the solution was refluxed for 1 h. The methanolysate was acetylated in Ac_2O–pyridine (1 : 1) (2 ml) at room temperature, and the product was subjected to GC analysis (2% ECNSS-M on Chromosorb WAW DMCS (60—80 mesh); 2 m × 3 mm i.d. glass column; column temperature, 195 °C; carrier gas, He 30 ml/min). t_R values (min) and identification were as follows: 3.3 (Me tri-OAc–α-L-rha.pyr.), 3.7 (Me tri-OAc–α-D-fuc.pyr.), 4.5 (Me tri-OAc–α-D-xyl.pyr.), 5.5 (Me tri-OAc–β-D-xyl.pyr:), 18.9 (Me tetra-OAc–α-D-glu.pyr.), 22.7 (Me tetra-OAc–β-D-glu.pyr.). The identities of all methyl glycoside acetates were confirmed by co-chromatography with authentic samples.

Methylation of V——NaH (500 mg) was suspended in anhydrous dimethyl sulfoxide (DMSO) (7 ml) and heated at 80 °C for 10 min. A solution of V (200 mg) in DMSO (3 ml) was added and the mixture was stirred at room temperature for 10 min, then CH_3I (5 ml) was added. The whole was stirred for 3 h at room temperature. The reaction mixture was poured into water, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water. The water layer and washing were combined and again extracted with AcOEt. The $CHCl_3$ and AcOEt extracts were combined and purified by column chromatography (Kieselgel 60, benzene–acetone (4 : 1)) to give a thin-layer chromatographically homogeneous colorless solid (XXIV): IR (CCl_4): no hydroxyl group absorption. $[\alpha]_D^{19}$ −29.0° (c=1.2, MeOH). ^{1}H-

NMR ($CDCl_3$): 1.24 (6H, d, $J=6$ Hz, C_5-CH_3 of L-rhamnopyranosyl and D-fucopyranosyl groups), 4.65—4.95 (5H, anomeric protons, Fig. 2c).

Methanolysis of XXIV and Identification of the Component Methylated Monosaccharides by GC-CI-MS——A solution of XXIV (20 mg) in 2 N HCl (MeOH) (2 ml) was refluxed for 2 h. The reaction mixture was worked up in a usual manner to give a mixture of methylated monosaccharides. The methanolysate was acetylated with Ac_2O–pyridine (1 : 1) mixture (2 ml), and the product was checked by GC-CI-MS. The results are shown in Fig. 1a.

Partial Methanolysis of XXIV, and Isolation of XXV and XXVI——XXIV (100 mg) was dissolved in 1 N HCl (MeOH) (4 ml) and the solution was stirred for 70 h at room temperature. The reaction mixture was worked up in a usual manner. The methanolysate was subjected to column chromatography (Silica Woelm TSC, benzene–acetone (3 : 1)) to give thin-layer chromatographically homogeneous XXV (28 mg) and XXVI (20 mg) as solids. XXV: $[\alpha]_D^{19}-21.9°$ ($c=1.4$, MeOH). ^{1}H-NMR ($CDCl_3$): 1.24 (6H, d, $J=6$ Hz). XXVI: $[\alpha]_D^{19}+20.1°$ ($c=1.0$, MeOH). ^{1}H-NMR ($CDCl_3$): 1.2—1.3 (6H, m). The ^{1}H-NMR signals of the anomeric protons of XXV and XXVI are shown in Fig. 2b and 2a, respectively.

Methylation of XXV and XXVI——XXV (25 mg) and XXVI (13 mg) were each dissolved in tetrahydrofuran (2 ml). NaH (200 mg) was added to the solution and the mixture was stirred for 10 min, then CH_3I (2 ml) was added and the whole was stirred at room temperature for 3 h. MeOH (5 ml) was added and the solvents were evaporated off *in vacuo*. The $CHCl_3$-soluble part of the residue gave a single spot on TLC (benzene–acetone (2 : 1)). The product was purified by column chromatography (Silica Woelm TSC, benzene–acetone (4 : 1)) to give XXVII (29 mg) from XXV, and XXVIII (13 mg) from XXVI, both as colorless solids. XXVII and XXVIII showed no hydroxyl group absorption in their IR spectra (CCl_4).

Methanolysis of XXVII and XXVIII, and Identification of Component Methylated Monosaccharides——XXVII (9 mg) and XXVIII (3 mg) were each dissolved in 2 N HCl (MeOH) (1 ml) and refluxed for 2 h. The reaction mixture was worked up in a usual manner and the methanolysate was acetylated with Ac_2O–pyridine (1 : 1) mixture. The products obtained from XXVII and XXVIII were subjected to GC-CI-MS. The results are shown in Fig. 1b and 1c.

Partial Methanolysis of V——V (100 mg) was dissolved in 2 N HCl (MeOH) (3 ml) and the solution was stirred for 22 h at room temperature. The methanolysate was chromatographed (Kieselgel 60, $CHCl_3$–MeOH–H_2O (32 : 8 : 1, 7 : 3 : 0.5) to give XXIX (18 mg) in addition to methyl α-D-xylopyranoside (34 mg), a mixture (20 mg) of methyl α-D-glucopyranoside and a methyl bioside. In addition, V (22 mg) was recovered. XXIX was obtained as a colorless solid. $[\alpha]_D^{20}+12.9°$ ($c=0.9$, MeOH). FD-MS m/z: 509 $[M+Na]^+$. ^{1}H-NMR: 1.4—1.7 (6H, C_5-CH_3 groups of L-rhamnopyranosyl and D-fucopyranosyl groups), 3.43 (3H, s, $-OCH_3$), 5.18 (H, d, $J=7$ Hz, anomeric H of β-D-glucopyranosyl group), 5.27 (H, d, $J=3$ Hz, anomeric H of α-D-fucopyranosyl group) and 5.63 (H, d, $J=2$ Hz, anomeric H of α-L-rhamnopyranosyl group). ^{13}C-NMR: 17.1 d, 18.6 d (C_6 of L-rhamnopyranosyl and D-fucopyranosyl groups), 55.0 q ($-OCH_3$), 62.2 t (C_6 of D-glucopyranosyl group), 66.6, 69.7, 70.0, 71.1, 71.4, 72.9, 73.4, 75.9, 78.1, 78.2, 78.5, 83.7. anomeric C: 100.6, 104.6 and 106.3.

Enzymatic Hydrolysis of XXIX——XXIX (15 mg) was dissolved in H_2O (3 ml), and cellulase (20 mg) was added. The mixture was incubated at 37 °C for 45 h, then the H_2O was evaporated off *in vacuo* and the residue was extracted with MeOH. The MeOH extract showed 3 spots on TLC (AcOEt–MeOH (4 : 1), and $CHCl_3$–MeOH–H_2O (25 : 17 : 3)). They gave the same *Rf* values and colorations (5% H_2SO_4–MeOH) as those of methyl α-D-fucopyranoside (orange), L-rhamnose (yellow) and D-glucose (black). The hydrolysate was acetylated with Ac_2O–pyridine (1 : 1) (4 ml) at room temperature, and the acetylation product was chromatographed twice (Kieselgel 60, hexane–AcOEt (4 : 1 and 5 : 1)) to give acetates of methyl α-D-fucopyranoside (4 mg), α-L-rhamnopyranose (4 mg), β-L-rhamnopyranose (3 mg) and α-D-glucopyranose (8 mg). They were identified by comparison of their ^{1}H-NMR spectra with those of authentic samples.

Treatment of I and II with Cation-Exchange Resin and Preparation of Methyl Esters XXX and XXXI——I (200 mg) was dissolved in H_2O (5 ml) and passed through a column of Amberlite IRC-84 (20 g). The effluent was concentrated *in vacuo* to give a white powder (I′). I′ was dissolved in 50% MeOH and an ether solution of diazomethane was added portionwise. When the solution became turbid during addition of the ether solution, sufficient volume of MeOH and water was added to keep the reaction mixture clear. The solvents were evaporated off and the methylation procedure was repeated 3 times. The reaction product was subjected to column chromatography (Kieselgel 60, $CHCl_3$–MeOH–H_2O (25 : 17 : 3)) to give XXX (60 mg): White powder. FAB-MS (triethanolamine matrix, Xe gas) m/z: 1709 $[M+Na]^+$. ^{1}H-NMR (pyridine-d_5–D_2O (1 : 1): 3.82 ($-COOCH_3$), 9.82 (–CHO). ^{13}C-NMR (pyridine-d_5–D_2O (1 : 1)): anomeric C; 95.0 (anomeric C of β-D-fucopyranosyl group), 101.9, 102.8, 103.4, 104.1, 104.7, 105.4, 105.6. 124.0 d (C_{12}), 144.0 s (C_{13}), 170.4 s (ester carbonyl C of methyl glucuronate), 176.5 s (C_{28}) and 210.4 d (C_{23}).

II (150 mg) was treated and methylated in the same manner to give XXXI as a white powder (57 mg). FAB-MS (triethanolamine matrix, Xe gas) m/z: 1725 $[M+Na]^+$. ^{1}H-NMR (pyridine-d_5–D_2O (1 : 1)): 9.27 (s, –CHO), the signal of carbomethoxyl protons was masked by H_2O signal. ^{13}C-NMR (pyridine-d_5–D_2O (1 : 1)): anomeric C; 94.7 (the anomeric C of a β-D-fucopyranosyl group), 104.1, 104.7, other anomeric carbon signals were not distinguishable: 124.1 d (C_{12}), 143.9 s (C_{13}), 170.9 s (ester carbonyl C of methyl glucuronate), 176.5 s (C_{28}), the signal of the formyl C was not distinguishable because of the low signal intensity.

Alkaline Hydrolysis of I——I (200 mg) was dissolved in aqueous 2 N KOH (5 ml) and the solution was heated in a boiling water bath for 3 h. After cooling, the reaction solution was neutralized with the cation-exchange resin Dowex 50W-X8 and evaporated *in vacuo*. The residue was purified by column chromatography (Kieselgel 60, $CHCl_3$–MeOH–H_2O (25:17:3)). The purified hydrolysate (80 mg) was treated with diazomethane in the same manner as described for the methylation of I′ to give XXXII as a white powder (23 mg). ^{1}H-NMR: 3.70, 3.78 (–$COOCH_3$), 9.86 (s, –CHO). ^{13}C-NMR: 103.1, 103.7 and 104.4 (anomeric C), 144.1 s (C_{13}), 170.2 s (ester carbonyl C of methyl glucuronate), 177.9 s (C_{28}), 210.3 d (C_{23}). The signal of C_{12} was masked by pyridine signals. The methyl ester of III obtained by treatment of III with diazomethane gave an identical ^{13}C-NMR spectrum.

Acetic Anhydride–Triethylamine Degradation of I; Preparation of XXXIII and XXXIV——I (300 mg) was added to a mixture of Ac_2O (4 ml) and triethylamine (2 ml), and the whole was heated in a sealed tube at 90 °C for 2 h. The solvents were evaporated off *in vacuo* and the residue was chromatographed (Kieselgel 60, benzene–acetone (9:1)) to yield two fractions (Fr. I and II). Fr. I (60 mg) was rechromatographed (Kieselgel 60, hexane–AcOEt (3:1)) to give 1,2,3,4-tetra-OAc–α-L-rhamnopyranose (11 mg) and its β-anomer (5 mg) and 1,2,3,4,6-penta-OAc–α-D-galactopyranose (38 mg). Fr. II (190 mg) was twice chromatographed (Kieselgel 60; the first run, 10% acetone in benzene and the 2nd run, hexane–AcOEt (1:1)) to give 172 mg of XXXIII: A white powder from MeOH. mp 161—164 °C. $[\alpha]_D^{21}$ −18.8° (c=1.0, MeOH). ^{1}H-NMR: 6.06 (H, d, J=8 Hz, anomeric H of β-D-fucopyranosyl group), 9.56 (H, s, –CHO). ^{13}C-NMR: 94.0, 97.6, 99.3, 101.2 and 101.7 (anomeric C), 123.7 d (C_{12}), 143.8 s (C_{13}), 169—171 (acetyl carbonyl C), 176.2 s (C_{28}), 204. 7 d (C_{23}). XXXIII (50 mg) was suspended in 0.1 N MeONa (MeOH) (5 ml) and the suspension was stirred at room temperature for 1 h. The solution was neutralized with Amberlite IRC-84 and evaporated. The residue was chromatographed (Kieselgel 60, $CHCl_3$–MeOH–H_2O (7:3:0.5)) to give XXXIV (28 mg): Colorless needles from dil. EtOH. mp 274—277 °C (dec.). $[\alpha]_D^{17}$ −2.5° (c=0.9, MeOH). FD-MS m/z: 1211 $[M+Na]^+$. ^{13}C-NMR: 95.0, 101.7, 105.0, 105.1, 106.2 (anomeric C), 123.7 d (C_{12}), 144.1 s (C_{13}), 176.3 s (C_{28}), 207.2 d (C_{23}).

Partial Methanolysis of XXXIV——XXXIV (38 mg) was dissolved in 0.5 N HCl (MeOH) (2 ml) and the solution was stirred at room temperature for 7 h. The reaction mixture was neutralized in a usual manner and the reaction product was subjected to column chromatography (Kieselgel 60, $CHCl_3$–MeOH–H_2O (7:3:0.5)) to give an aglycone fraction, XXXIV (4 mg) and a sugar (20 mg). The aglycone fraction was again chromatographed (benzene–acetone (9:1)) to give the aglycone as a white powder (8 mg). The aglycone was acetylated with Ac_2O–pyridine and the acetate was treated with diazomethane. The product was purified by column chromatography to give a thin-layer chromatographically homogeneous methyl ester acetate (5 mg). It crystallized as colorless needles from MeOH. mp 185—187 °C. Its IR (KBr) spectrum was identical with that of acetyl gypsogenin methyl ester. The sugar gave the same coloration and Rf value on TLC, and the same ^{13}C-NMR spectrum, as V.

Alkaline Hydrolysis of II and Preparation of XXXV——II (100 mg) was hydrolyzed with 2 N KOH and treated in the same manner as described for I to give a thin-layer chromatographically homogeneous hydrolysis product (58 mg), which was methylated with diazomethane and purified by column chromatography to give XXXV (23 mg) as a white powder: ^{1}H-NMR: 3.69, 3.78 (–$COOCH_3$), 9.82 (H, s, –CHO). ^{13}C-NMR: 103.6, 104.3, 104.5 (anomeric C), 123.7 d (C_{12}), 144.4 s (C_{13}), 170.2 s (ester carbonyl C of methyl glucuronate), 177.6 s (C_{28}), 210.1 d (C_{23}). The methyl ester of IV gave an identical ^{13}C-NMR spectrum.

Acetic Anhydride–Triethylamine Degradation of II and Preparation of XXXVI and XXXVII——II (300 mg) was treated in the same way described for the degradation of I to give XXXVI (182 mg): A white powder from dil. MeOH. mp 164—167 °C. $[\alpha]_D^{21}$ −32.7° (c=1.0, MeOH). ^{1}H-NMR: 9.56 (H, s, –CHO). ^{13}C-NMR: 93.9, 97.8, 99.9, 101.2, 101.7 (anomeric C), 123.8 d (C_{12}), 144.3 s (C_{13}), 176.1 s (C_{28}), 204.7 d (C_{23}). XXXVI (115 mg) was deacetylated to give XXXVII (65 mg):[24] Colorless needles from dil. MeOH. mp 260—263 °C (dec.). $[\alpha]_D^{19}$ −16.3° (c=0.5, MeOH). FD-MS m/z: 1227 $[M+Na]^+$. ^{1}H-NMR: 9.63 (H, s, –CHO). ^{13}C-NMR: 95.2, 101.5, 104.8, 104.9, 106.2 (anomeric C), 123.8 d (C_{12}), 144.5 s (C_{13}), 175.8 s (C_{28}), 207.3 d (C_{23}).

Partial Methanolysis of XXXVII——XXXVII (28 mg) was treated with 0.5 N HCl (MeOH) as described for XXXIV to give the aglycone and a methyl oligosaccharide. The aglycone was converted to the methyl ester acetate. It crystallized from MeOH as colorless prisms: mp 202—205 °C. The IR (KBr) spectrum was identical with that of quillaic acid methyl ester 3,16-diacetate. The methyl oligosaccharide gave a ^{13}C-NMR spectrum identical with that of V.

Acknowledgement The authors are grateful to Mr. M. Nishi and Mr. Y. Furukawa, former staff of this Faculty, for measurements of NMR and MS spectra. This work was supported in part by a grant from the Central Research Institute of this University.

References and Notes

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19) The instruments and materials used in this work were as follows: Yanaco micromelting point apparatus (melting points), Hitachi EPI-G3 grating infrared spectrophotometer and Hitachi 295 infrared spectrophotometer (IR spectra), JASCO DIP-4 digital polarimeter (specific rotations), JEOL JNM FX-100 (25 MHz) spectrometer (^{13}C-NMR spectra), Hitachi R-22 (90 MHz) spectrometer (^{1}H-NMR spectra), JEOL D-300 and DX-300 mass spectrometers (MS spectra), Shimadzu GC-7A gas chromatograph (GC), Shimadzu gas chromatograph-mass spectrometers Auto GCMS-6020 with a GC-MSPAC 500 FDG data analyzer (GC-CI-MS spectra). Kieselgel 60, 70—230 mesh (E. Merck), Silica Woelm TSC (Woelm Pharma GmbH and Co.), LiChroprep RP-18, 25—40 μm (E. Merck), MCI Gel CHP 20P, 150—300 μ (Mitsubishi Chemical Industries Ltd.), precoated Kieselgel 60 F_{254} plate (E. Merck), RP-18 F_{254} plate (E. Merck). The cellulase was Type II, from *Aspergillus niger* (Sigma Chemical Company). ^{1}H-NMR and ^{13}C-NMR spectra were measured in pyridine-d_5 unless otherwise specified, and chemical shifts were expressed on the δ-scale using TMS as an internal standard.
20) Specific rotations of the acetylated methyl glycosides isolated were in good agreement with those of authentic samples.
21) The yields of methyl tri-OAc-β-L-rhamnopyranoside and other furanoside derivatives were very low and their peaks might be concealed in the other large peaks.
22) These two peaks were obtained when glucuronic acid was methanolyzed and acetylated under the same conditions. Identification was not attempted.
23) This doublet collapsed to a singlet on irradiation at the frequency of the formyl proton, indicating the presence of geminal coupling between the formyl proton and C_4.
24) Under these deacetylation conditions, 38 mg of the less polar compound was obtained. It was presumed to be the 16-*O*-acetate of XXXVII on the basis of ^{1}H-NMR spectral analysis.