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Studies on the Constituents of Zizyphi Fructus. V.[1] Structures of Glycosides of Benzyl Alcohol, Vomifoliol and Naringenin

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New glycosides, zizybeoside I(I) and II(II), zizyvoside I(IV) and II(V), and a mixture of 6,8-di-C-glucosyl-2(*S*)-naringenin and 6,8-di-C-glucosyl-2(*R*)-naringenin (compound A), together with vomifoliol and roseoside, were isolated from the ethanol extract of Zizyphi Fructus. On the basis of spectral and chemical evidence the structures of the new glycosides were determined as benzyl β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (I), benzyl β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (II), vomifoliol 9-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (IV), vomifoliol 9-O-β-glucopyranosyl-(1→2)-[β-glucopyranosyl-(1→3)]-β-glucopyranoside (V), 6,8-di-C-glucosyl-2(*S*)-naringenin (VII) and 6,8-di-C-glucosyl-2(*R*)-naringenin (VIII).

Keywords——Zizyphi Fructus; Rhamnaceae; zizybeoside I; zizybeoside II; zizyvoside I; zizyvoside II; roseoside; vomifoliol; 6,8-di-C-glucosyl-2(*S*)-naringenin; 6,8-di-C-glucosyl-2(*R*)-naringenin

In earlier studies on the pharmacologically active principles in Zizyphi Fructus (*Zizyphus jujuba* Mill. var. *inermis* Bunge), we reported the isolation of pentacyclic triterpenoids and dammarane-type saponins.[1] Further examination of the glycosidic fraction, having hypotensive and sedative activities, has resulted in the isolation and characterization of four new O-glycosides of benzyl alcohol (I, II) and vomifoliol (IV, V), along with vomifoliol (VI), roseoside (III) and two new C-glycosides, obtained as compound A(VII, VIII). This paper deals with the structure elucidation of these glycosides.

The EtOH extractive of commercial Zizyphi Fructus was subjected to Amberlite XAD-2 column chromatography and eluted successively with H_2O, EtOH–H_2O (1:1) and $CHCl_3$–MeOH (2:1). The EtOH–H_2O eluate was treated as described in the experimental section to afford compounds I, II, III, IV, V, VI and A.

Compound I (zizybeoside I), mp 192—193°C, $[\alpha]_D$ −32.6°, $C_{19}H_{28}O_{11}$ (I), has two glycosidic linkages as revealed by the anomeric carbon signals at δ 105.4 and 101.7 in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. In addition, the signals at δ 138.2, 128.2, 127.6 and 127.3 suggest the presence of a mono-substituted benzene ring. A triplet signal at δ 70.4 is attributable to a methylene carbon having an oxygen function. I was hydrolyzed with crude hesperidinase to give D-glucose and benzyl alcohol, the former being identical with an authentic sample as judged by thin-layer co-chromatography and by the analysis of optical rotation, while the latter was identical with an authentic sample on gas chromatography (GLC). The mode of two glucosidic linkages in I was deduced to be β-form in the $^4C_1$ conformation from the fact that large coupling constants (J=7.0) of the anomeric proton signals are observed in the proton magnetic resonance (^{1}H-NMR) spectrum. A per-O-methyl derivative (Ia) prepared from I by Hakomori's method[2] was methanolyzed to yield methyl 2,3,4,6-tetra-O-methyl-α(β)-D-glucopyranosides and methyl 3,4,6-tri-O-methyl-α-D-glucopyranoside, which were identical with authentic samples on GLC. Based upon these chemical results, the linkage between the two glucose units was determined to be at C_2 and C_1. Furthermore, this conclusion was supported by the ^{13}C-NMR spectrum of I which exhibits sugar carbon signals analogous to those of methyl β-sophoroside (Table). Consequently,the structure of zizybeoside I is represented by I.

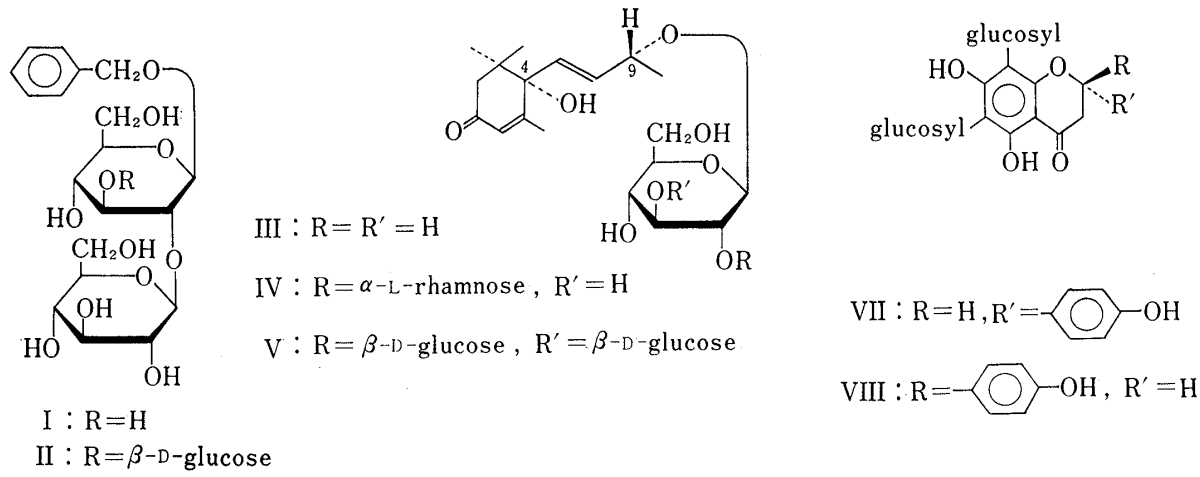
Compound II (zizybeoside II), mp 237—238°C, $[\alpha]_D$ −39.0°, $C_{25}H_{38}O_{16}\cdot H_2O$(II), gives a ^{13}C-NMR spectrum similar to that of I, and the anomeric proton signals appear at δ 4.97 (1H, d, J=7.0), 5.34 (1H, d, J=7.0) and 5.47 (1H, d, J=7.0) in the ^{1}H-NMR spectrum. On acid hydrolysis, II afforded D-glucose. Therefore, it is demonstrated that each D-glucose moiety is combined through a β-linkage in the $^4C_1$ conformation. A per-O-methyl derivative (IIa) prepared from II by Hakomori's method was methanolyzed to furnish methyl 2,3,4,6-tetra-O-methyl-$\alpha(\beta)$-D-glucopyranosides and methyl 4,6-di-O-methyl-α-D-glucopyranoside (GLC). This finding agrees with the ^{13}C-NMR spectral data indicating that the signals assignable to the C-3′, C-2′ and C-4′ were shifted by +8.9 ppm, −2.3 ppm and −1.4 ppm, respectively, in comparison with those of I (Table). Thus, the position of the additional glucose unit is demonstrated to be at C-3′ of I.[3] Based on the above evidence, zizybeoside II is formulated as structure(II).

Compound III (roseoside), $[\alpha]_D$ +108.9°, $C_{19}H_{30}O_8\cdot H_2O$ (III), has the molecular ion peak at m/z 386 in the field desorption mass spectrum (FD–MS) and signals in the ^{13}C-NMR (δ 198.1)

TABLE. ^{13}C-NMR Spectral Data (in C_5D_5N)

Compound	I	II	VI	III	IV	V	Methyl β-sophoroside
C-1	138.2	138.3	197.6	198.1	197.6	197.5	
2	127.6	127.5	126.4	126.4	126.4	126.4	
3	128.2	128.2	164.4	164.4	164.9	163.8	
4	127.3	127.3	78.8	78.7	78.6	78.6	
5	128.2	128.2	41.6	41.5	41.5	41.4	
6	127.6	127.5	50.2	50.0	50.0	50.0	
	70.4	70.8					
7			128.8	130.9	131.1	131.2	
8			136.8	134.2	134.2	134.5	
9			67.2	76.1	76.6	77.4	
10			24.4	21.2	21.3	21.8	
11			24.4	24.6	24.6	24.6	
Methyl			23.5	23.4	23.4	23.4	
groups			19.5	19.4	19.3	19.0	
Glucose							
1′	101.7	102.2		102.2	100.7	101.3	103.7
2′	83.2	80.9		74.8	79.3	81.7	83.5
3′	78.0	86.9		77.9	77.3	87.1	78.6
4′	70.8	69.4		71.3	c)	69.8	71.2
5′	a)	b)		77.9	77.8	d)	78.3
6′	62.1	62.2		62.5	62.5	62.2	62.5
Glucose or rhamnose							
1	105.4	104.4			101.4	104.6	106.0
2	76.1	75.7			c)	75.9	76.4
3	a)	b)			c)	d)	78.9
4	70.8	71.2			73.7	71.3	71.5
5	a)	b)			69.1	d)	78.3
6	62.1	62.2			18.5	62.2	62.5
Glucose							
1		104.4				104.6	
2		74.8				74.9	
3		b)				d)	
4		71.2				71.3	
5		b)				d)	
6		62.2				62.2	

a) 77.8 and 77.4 ppm not assigned.
b) 78.2, 78.0 and 77.5 ppm not assigned.
c) 73.7, 72.1 and 71.6 ppm not assigned.
d) 78.2 and 77.4 ppm not assigned.



Chart

and infrared absorption (IR) (1650 cm^{-1}) spectra suggest the presence of an α,β-unsaturated carbonyl group. The ^{1}H-NMR spectrum of III exhibits signals attributed to an anomeric proton [δ 4.90 (1H, d, J=8.0)], geminal dimethyl groups [δ 1.15, 1.25 (3H, each, s)] and *trans* olefinic protons [δ 6.09 (1H, d, J=16.0), 6.32 (1H, q, J=16.0, 6.0)]. III was hydrolyzed with crude hesperidinase to afford D-glucose and vomifoliol,[4] $C_{13}H_{20}O_3$, mp 115—116°C, $[\alpha]_D$ +182.6° (VI). The coupling constant of anomeric proton signals in the ^{1}H-NMR spectrum of III and the molecular rotation difference between III and VI prove that D-glucose has a β-linkage in the $^4C_1$ conformation. The position of the glucose unit in III is regarded as being at C-9 in VI from the ^{13}C-NMR spectrum of III which shows the signals assignable to C-9 and C-10 shifted by +8.9 ppm and −3.2 ppm, respectively, as compared with those of VI (Table).[5] Based on the above evidence, the structure of compound III is represented as III (roseoside), which has been isolated from *Vinca rosea* (Apocyanaceae).[6]

Compound IV (zizyvoside I), $[\alpha]_D$ +44.8°, $C_{25}H_{40}O_{12}\cdot 1/2\ H_2O$ (IV), shows a ^{13}C-NMR spectrum analogous to that of III, besides signals due to the carbons of an additional rhamnose moiety (Table). IV gave vomifoliol, D-glucose and L-rhamnose on enzymatic hydrolysis. The ^{13}C-NMR [an anomeric carbon: δ 100.7 (J=155.9)][7] and ^{1}H-NMR [anomeric proton: δ 4.84 (1H, d, J=8.0)] spectra of IV indicate that D-glucose possesses a β-configuration in $^4C_1$ conformation. The configuration of L-rhamnose was proved to have an α-linkage in the $^1C_4$ conformation by comparison of the C–H coupling constant (J=172.1) of the anomeric carbon with those of methyl α- and β-L-rhamnopyranosides.[8] A per-O-methyl derivative (IVa) prepared from IV by Hakomori's method displays fragment ion peaks due to the permethylated disaccharide residue (m/z 393 and 361) and the terminal permethylated rhamnose residue (m/z 189 and 157) in the mass spectrum (MS). On methanolysis, IVa gives methyl 3,4,6-tri-O-methyl-α-D-glucopyranoside and 2,3,4-tri-O-methyl-α-L-rhamnopyranoside (GLC). Therefore, it is established that an additional rhamnose unit is linked with C-2′ of III. This conclusion is consistent with the result that the signals due to C-2 and C-1 of the glucose moiety were shifted by +4.5 ppm and −1.5 ppm, respectively, on comparison of the ^{13}C-NMR spectral data of IV with those of III (Table). Based on the above results, zizyvoside I can be illustrated as the structure (IV).

Compound V (zizyvoside II), $[\alpha]_D$ +49.6°, $C_{31}H_{50}O_{18}\cdot 2H_2O$ (V), appears to contain glycosidic linkages as indicated by the anomeric carbon signals at δ 104.6 and 101.3, and shows the signals of an aglycone moiety analogous to those of III in the ^{13}C-NMR spectrum (Table). V was subjected to acid hydrolysis yielding vomifoliol (VI) and glucose. The ^{1}H-NMR spectrum of V shows signals at δ 4.84 (1H, d, J=7.0), 5.32 (1H, d, J=7.0) and 5.40 (1H, d, J=7.0) corresponding to three anomeric protons, indicating that the D-glucose moieties

are combined with β-linkages in the $^4C_1$ conformation. A per-O-methyl derivative (Va) prepared from V by Hakomori's method was methanolyzed to produce methyl 2,3,4,6-tetra-O-methyl-$\alpha(\beta)$-D-glucopyranosides and methyl 4,6-di-O-methyl-α-D-glucopyranoside (GLC). Furthermore, this result is supported by the ^{13}C-NMR spectrum, which shows glucose unit signals analogous to those of II (Table). Therefore, it is concluded that two glucose units are linked with C-2′ and C-3′ in III. Consequently, the structure of zizyvoside II can be expressed as V.

Compound A (VII, VIII) shows positive color reactions of flavonoid derivatives ($FeCl_3$ and Mg-HCl) and a negative color reaction to Zn-HCl. Compound A has strong absorption bands assignable to hydroxyl groups (3360 cm^{-1}) and an α,β-unsaturated carbonyl group (1620 cm^{-1}). The bathochromic shift in the ultraviolet (UV) absorption spectrum of compound A caused by addition of NaOMe, $AlCl_3$ or NaOAc solution suggested the presence of free hydroxyl groups at the 5, 7 and 4′ positions.[9] From the results obtained it appears that compound A is a flavanone derivative. The ^{1}H-NMR spectrum of compound A indicates the presence of four aromatic protons at the 2′, 3′, 5′ and 6′ positions in the B ring of a flavanone moiety. The appearance of signals due to sugar carbons in the C-glycoside in the ^{13}C-NMR spectrum, and the absence of signals attributable to aromatic protons in the A ring of a flavanone moiety in the ^{1}H-NMR spectrum and of signals assignable to an anomeric carbon in the ^{13}C-NMR spectrum suggest that compound A is the flavanone C-glycoside having two sugar units in the A ring. A comparative study on the ^{13}C-NMR spectral data of compound A and naringenin[10] in DMSO-d_6 revealed that the signals assignable to C-6 and C-8 in compound A are shifted by +9.0 and +10.2 ppm, respectively. These results are supported by the finding that compound A produced no sugar moiety on acid hydrolysis with 10% H_2SO_4 for 2 h. The appearance of split signals due to the hydroxyl group at C-5 in the ^{1}H-NMR spectrum and of split signals corresponding to an aromatic carbon and a carbonyl carbon in the ^{13}C-NMR spectrum suggest that compound A is a mixture of C-glycosyl naringenins having glycosyl moieties at both C_6 and C_8. Compound A was acetylated in the usual way to give two acetates of VIIa, $C_{49}H_{54}O_{26}$, $[\alpha]_D$ −40.8°, MS (*m/z*) :1058 $[M]^+$ and VIIIa, $C_{49}H_{54}O_{26}$, $[\alpha]_D$ −43.1°, MS (*m/z*): 1058 $[M]^+$, each of which shows acetyl groups (1780, 1755 cm^{-1}) and an α,β-unsaturated carbonyl group (1695 cm^{-1}) in the IR spectrum. On refluxing VIIa or VIIIa with 0.1 N HCl–MeOH for 6 h, the generation of a mixture of C-hexosyl naringenins which was similar to compound A was demonstrated by analysis of the ^{1}H-NMR and ^{13}C-NMR spectra. Furthermore, on usual acetylation, the mixtures of C-hexosyl naringenins reverted to VIIa and VIIIa. A glucose moiety was detected as a product of ferric chloride oxidation[11] of compound A, together with arabinose due to oxidative cleavage of the C_1–C_2 bond of the glucosyl residue. Since only glucose was detected as the sugar residue of compound A, it is possible that interconversion mediated by a chalcone occurred in the hydrolysis of VIIa or VIIIa to give the C-2 isomer in the flavanone. The circular dichroism (CD) spectra of VIIa and VIIIa are apparently antipodal near 335 and 305 nm, and the CD spectrum of VIIIa near 335 nm is analogous to that of (−)-naringin having 2(*R*)-configuration. These spectral data demonstrate that VIIa and VIIIa have the 2(*S*)- and 2(*R*)-configuration, respectively. Consequently, the structures 6,8-di-C-glucosyl-2(*S*)-naringenin(VII) and 6,8-di-C-glucosyl-2(*R*)-naringenin(VIII) in compound A can be deduced.

The existence of a di-C-glucosyl residue in VII and VIII is the first example of its occurrence in flavanones. Pharmacological studies on the isolated compounds are in progress.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. IR, ^{1}H-NMR and ^{13}C-NMR spectra were obtained with KOKEN DS-301, JEOL PS-100H and JEOL FX-100 instruments, respectively. MS and FD-MS were recorded on JEOL JMS-01SG and JEOL JMS-D300 mass spectrometers, respectively.

Optical rotatory dispersion (ORD) and CD spectra were recorded on a JASCO ORD/CD J-20 unit. GLC was run on a Shimadzu GC-48M machine with a flame ionization detector using a glass column (2m×3 mm) packed with 5% 1,4-butanediol succinate on Shimalite W (60—80 mesh): *a*) column temperature, 177°C; N_2, 38 ml/min, *b*) column temperature, 180°C; N_2, 85 ml/min. TLC of sugars was conducted on Avicel SF (Funakoshi) with the upper layer of *n*-BuOH–pyridine–H_2O(6: 2: 3)+pyridine (1) as the solvent and aniline hydrogen phthalate as the spraying reagent. TLC of other compounds was performed on Kieselgel $60F_{254}$ (Merck) and detection was done by spraying dilute H_2SO_4 reagent followed by heating. Column chromatography was carried out with Kieselgel 60 (70—230 mesh, Merck), Kieselgel 60 silanisiert (70—230 mesh, Merck), Diaion HP-20AG (100—200 mesh, Mitsubishi Chemical) and Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemicals). The ratios of solvent and reagent are given in v/v.

Isolation——The dried commercial fruits (50 kg) of Zizyphi Fructus were extracted three times with boiling EtOH for 2 h each. A suspension of the EtOH extract (25.2 kg) in H_2O was applied to an Amberlite XAD-2 column and eluted with H_2O, EtOH–H_2O (1: 1) and $CHCl_3$–MeOH (2: 1) successively. The EtOH–H_2O fraction (405.1 g) was partitioned between $CHCl_3$ and H_2O, and the H_2O layer was extracted with *n*-BuOH. The *n*-BuOH extract (181 g) was applied to a Diaion HP-20AG column and eluted with H_2O, 10% MeOH–H_2O, 20% MeOH–H_2O and MeOH successively. The 10% MeOH–H_2O fraction (55 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (10: 2: 0.2)] to give fractions A and B. Fraction A was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (8: 2: 0.2)] to afford fractions A-1 and A-2. Fraction A-1 was subjected to chromatography over Sephadex LH-20 (acetone) and silica gel [EtOAc–MeOH–H_2O (20: 1: 0.1)] to furnish compound III (65 mg). Fraction A-2 was crystallized from MeOH to give zizybeoside I (350 mg). The mother liquor was chromatographed over Sephadex LH-20 (acetone) and silanized silica gel (10% MeOH–H_2O) to yield zizyvoside I (250 mg). Fraction B was chromatographed over Sephadex LH-20 (H_2O) to afford fractions A-2-1 and A-2-2. Fraction A-2-1 was crystallized from MeOH to give zizybeoside II (400 mg), and the mother liquor was chromatographed over silanized silica gel (5% MeOH–H_2O), Sephadex LH-20 (acetone) and silica gel [$CHCl_3$–MeOH–H_2O (8: 2: 0.2)] to furnish zizyvoside II (17 mg). Fraction A-2-2 was chromatographed over silanized silica gel (5% MeOH–H_2O) and Sephadex LH-20 (EtOH) to give compound A (180 mg). The 20% MeOH–H_2O fraction (50 g) was subjected to repeated chromatography over silica gel [$CHCl_3$–MeOH (8: 1)], [*n*-hexane–acetone (4: 1)] and [$CHCl_3$–MeOH (50: 1)] to provide vomifoliol (12 mg).

Zizybeoside I (I)——Colorless needles, mp 192—193°C (MeOH–H_2O), $[\alpha]_D^{23}$ −32.6° (*c*=1.03, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3320. FD-MS (*m/z*): 455 $[M+Na]^+$. *Anal.* Calcd for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.53. Found: C, 52.60; H, 6.59. ^{1}H-NMR (C_5D_5N) δ: 4.80 (1H, d, *J*=12, α-CH), 5.03 (1H, d, *J*=7, anomeric H), 5.12 (1H, d, *J*=12, α-CH), 5.36 (1H, d, *J*=7, anomeric H), 7.2—7.7 (5H, aromatic H).

Enzymatic Hydrolysis of I——A solution of I (90 mg) in H_2O (2 ml) was incubated with crude hesperidinase (20 mg) at 37° for 30 min and the mixture was extracted with ether. The organic layer was evaporated to dryness *in vacuo* to yield benzyl alcohol (23 mg) [GLC(*a*); t_R(min): 2.37]. ^{1}H-NMR ($CDCl_3$) δ: 2.20 (1H, br s, OH), 4.62 (2H, s, α-CH_2), 7.31 (5H, s, aromatic H). The aqueous layer was evaporated to dryness *in vacuo* and the residue was chromatographed over Sephadex LH-20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (8: 2: 0.1)] to give D-glucose, as a colorless syrup (20 mg), *Rf* 0.37, $[\alpha]_D^{26}$ +48.5° (24 h later) (*c*=1.00, H_2O).

Permethylation of I——According to Hakomori's method, 50% NaH (120 mg) washed with *n*-hexane was added to DMSO (1 ml) and the mixture was heated for 45 min at 60°C. A solution of I (20 mg) in DMSO (0.1 ml) was added to the above solution and the mixture was stirred at room temperature for 1 h. Then, methyl iodide (1 ml) was added to the reaction mixture and stirring was continued for 1 h. The reaction mixture was poured into water and extracted with ether. The organic layer was washed with water and evaporated to dryness. The residue was chromatographed over silica gel [*n*-hexane–acetone (8: 1)] to give the hepta-O-methyl derivative (Ia) (12 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: no hydroxyl group. MS (*m/z*): 529 $[M-1]^+$, 423 [permethylated disaccharide residue], 391 [423-MeOH], 219 (terminal permethylated glucose residue], 187 [219-MeOH].

Methanolysis of Ia——A solution of Ia (5 mg) in 10% HCl–MeOH (2 ml) was heated for 2 h under reflux. The reaction mixture, after cooling, was neutralized with Ag_2CO_3 and the insoluble material was filtered off. The filtrate was evaporated to dryness and the residue was subjected to GLC analysis under condition(*a*) to give benzyl alcohol and methyl sugar [t_R(min): benzyl alcohol, 2.37; methyl pyranosides of 2,3,4,6-tetra-O-methyl-β-D-glucose, 3.41; 2,3,4,6-tetra-O-methyl-α-D-glucose, 4.57; 3,4,6-tri-O-methyl-α-D-glucose, 9.11. Ref. methyl pyranosides of 2,3,4-tri-O-methyl-α-D-glucose, 10.67; 2,3,6-tri-O-methyl-α-D-glucose, 13.28; 2,4,6-tri-O-methyl-α-D-glucose, 13.75].

Zizybeoside II (II)——Colorless needles, mp 237—238°C (MeOH–H_2O), $[\alpha]_D^{26}$ −39.0° (*c*=1.00, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3320. FD-MS (*m/z*): 617 $[M+Na]^+$. *Anal.* Calcd for $C_{25}H_{38}O_{16}\cdot H_2O$: C, 49.01; H, 6.58. Found: C, 49.24; H, 6.56. ^{1}H-NMR (C_5D_5N) δ: 4.89 (1H, d, *J*=12, α-CH), 4.97 (1H, d, *J*=7, anomeric H), 5.12 (1H, d, *J*=12, α-CH), 5.34 (1H, d, *J*=7, anomeric H), 5.47 (1H, d, *J*=7, anomeric H), 7.2—7.7 (5H, aromatic H).

Acid Hydrolysis of II——A solution of II (30 mg) in 10% H_2SO_4 (5 ml) was heated for 2 h under reflux. The solution was diluted with water and extracted with ether to yield benzyl alcohol. The aqueous layer, after neutralization with Amberlite IRA 400, was evaporated to dryness *in vacuo* and the residue was sub-

jected to chromatography over Sephadex LH-20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (8:2:0.1)] to give D-glucose (9 mg), which was identified by comparison with an authentic sample (optical rotation measurement).

Permethylation of II——Methylation of II (25 mg) was carried out in the same way as for I to give the nona-O-methyl derivative (IIa) (17 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: no hydroxy group. MS (*m/z*): 219 [terminal permethylated glucose residue], 187 [219-MeOH].

Methanolysis of IIa——IIa (5 mg) was worked up in the same way as for Ia to give benzyl alcohol and methyl sugars which were identified by GLC under condition(*b*) [t_R(min): benzyl alcohol, 1.27; methyl pyranosides of 2,3,4,6-tetra-O-methyl-β-D-glucose, 1.78; 2,3,4,6-tetra-O-methyl-α-D-glucose, 2.35; 4,6-di-O-methyl-α-D-glucose, 12.98. Ref. methyl pyranosides of 3,4-di-O-methyl-α-D-glucose, 11.53; 3,6-di-O-methyl-α-D-glucose, 12.82; 2,4-di-O-methyl-α-D-glucose, 16.81; 2,3-di-O-methyl-α-D-glucose, 19.18; 2,6-di-O-methyl-α-D-glucose, 20.54].

Compound III (Roseoside)——Colorless syrup, $[\alpha]_D^{26}$ +108.9° (*c*=1.00, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650. FD-MS (*m/z*): 387 $[M+1]^+$, 386 $[M]^+$. *Anal.* Calcd for $C_{19}H_{30}O_8 \cdot H_2O$: C, 56.42; H, 7.98. Found: C, 56.70; H, 7.96. ^{1}H-NMR (C_5D_5N) δ: 1.15, 1.25 (each 3H, s, CH_3), 1.37 (3H, d, *J*=6, C_{10}–H), 2.01 (3H, d, *J*=15, C_{11}–H), 2.37, 2.68 (each 1H, d, *J*=16, C_6–H), 4.70 (1H, quintet, *J*=6, C_9–H), 4.90 (1H, d, *J*=8, anomeric H), 6.07 (1H, br s, C_2–H), 6.09 (1H, d, *J*=16, C_7–H), 6.32 (1H, q, *J*=16, 6, C_8–H). ORD (*c*=0.09, MeOH) [α] (nm): +5222° (262), 0° (247), −14889° (210). CD (*c*=0.002, MeOH) Δε (nm): −0.71° (316), +7.80° (240).

Enzymatic Hydrolysis of III——A solution of III (40 mg) in H_2O (2 ml) was incubated with crude hesperidinase (15 mg) at 37°C for 2 h and the hydrolysate was extracted with ether. The organic layer was evaporated to dryness and the residue was chromatographed over silica gel [$CHCl_3$–MeOH (50: 1)] to give vomifoliol (VI) (10 mg) as colorless needles, mp 115—116°C (benzene), $[\alpha]_D^{26}$ +182.6° (*c*=1.00, EtOH), IR ν_{max}^{KBr} cm^{-1}: 3380, 1660. *Anal.* Calcd for $C_{13}H_{20}O_3$: C, 69.61; H, 8.99. Found: C, 69.44; H, 8.99. The aqueous layer was evaporated to dryness and the residue was chromatographed over Sephadex LH-20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (8: 2: 0.1)] to give D-glucose (11 mg), which was identified by comparison with an authentic sample (optical rotation measurement).

Acetylation of III——III (15 mg) was acetylated in the usual way and the product was chromatographed over silica gel [*n*-hexane–acetone (4: 1)] to give a tetra-O-acetyl derivative (IIIa) (12 mg), colorless needles, mp 157—158°C (EtOH), $[\alpha]_D^{27}$ +123.3° (*c*=0.72, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 1750, 1640. *Anal.* Calcd for $C_{27}H_{38}O_{12}$: C, 58.47; H, 6.91. Found: C, 58.58; H, 6.97. ^{1}H-NMR ($CDCl_3$) δ: 1.01, 1.08 (each 3H, s, CH_3), 1.23 (3H, d, *J*=6, C_{10}–H), 1.89 (3H, d, *J*=1.5, C_{11}–H), 2.01, 2.03, 2.04, 2.09 (each 3H, s, CH_3CO), 2.20, 2.46 (each 1H, d, *J*=16, C_6–H), 3.64 (1H, m, C_5–H), 4.12—4.32 (3H, m, C_9 and $C_{6'}$–H), 4.56 (1H, d, *J*=7, anomeric H), 4.86—5.20 (3H, m, $C_{2'}$, $C_{3'}$ and $C_{4'}$–H), 5.78—5.90 (3H, m, olefinic H). MS (*m/z*): 554 $[M]^+$, 498 $[M-56]^+$, 331 [peracetylated glucose residue], 169.

Zizyvoside I (IV)——Colorless syrup, $[\alpha]_D^{26}$ +44.8° (*c*=1.00, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650. *Anal.* Calcd for $C_{25}H_{40}O_{12} \cdot 1/2H_2O$: C, 55.44; H, 7.63. Found: C, 55.08; H, 7.59. ^{1}H-NMR (C_5D_5N) δ: 1.20, 1.26 (each 3H, s, CH_3), 1.50 (3H, d, *J*=6, C_{10}–H), 1.70 (3H, d, *J*=6, deoxy hexose-CH_3), 2.04 (3H, br s, C_{11}–H), 2.40, 2.76 (each 1H, d, *J*=16, C_6–H), 4.84 (1H, d, *J*=8, anomeric H), 6.50 (1H, d, *J*=16, C_7–H), 6.08 (1H, br s, C_2–H), 6.35 (1H, q, *J*=16, 7, C_8–H), 6.36 (1H, br s, anomeric H). ORD (*c*=0.158, MeOH) [α] (nm): +2063° (261), 0° (245), −4937° (211). CD (*c*=0.003, MeOH) Δε (nm): −0.11° (317), +2.64° (240).

Enzymatic Hydrolysis of IV——A solution of IV (60 mg) in H_2O (3 ml) was incubated with crude hesperidinase (20 mg) at 37°C for 2 h. The hydrolysate was treated in the same way as for III to give vomifoliol (VI) (12 mg), D-glucose (7 mg) (identified by comparison of the optical rotation with that of an authentic sample) and L-rhamnose (8 mg), as a colorless syrup (8 mg), *Rf* 0.63, $[\alpha]_D^{25}$ +6.1° (24 h later) (*c*=0.80, H_2O).

Permethylation of IV——Methylation of IV (50 mg) was carried out in the same way as for I to give the per-O-methyl derivative (IVa) (7 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: no hydroxy group. MS (*m/z*): 393 [permethylated disaccharide residue]$^+$, 361 [393−MeOH]$^+$, 189 [terminal permethylated rhamnose residue]$^+$, 157 [189 −MeOH]$^+$.

Methanolysis of IVa——IVa (3 mg) was worked up in the same way as for Ia to give methyl sugars which were identified by GLC under condition(*a*) [t_R(min): methyl pyranosides of 2,3,4-tri-O-methyl-α-L-rhamnose, 1.71; 3,4,6-tri-O-methyl-α-D-glucose, 9.11].

Zizyvoside II (V)——Colorless syrup, $[\alpha]_D^{26}$ +49.6° (*c*=1.70, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650. FD-MS (*m/z*): 733 $[M+Na]^+$. *Anal.* Calcd for $C_{31}H_{50}O_{18} \cdot 2H_2O$: C, 49.86; H, 7.29. Found: C, 50.03; H, 7.27. ^{1}H-NMR (C_5D_5N) δ: 1.11, 1.21 (each 3H, s, CH_3), 1.48 (3H, d, *J*=6, C_{10}–H), 2.00 (3H, br s, C_{11}–H), 2.32, 2.60 (each 1H, d, *J*=16, C_6–H), 4.84 (1H, d, *J*=7, anomeric H), 5.32 (1H, d, *J*=7, anomeric H), 5.40 (1H, d, *J*=7, anomeric H), 6.01 (1H, d, *J*=16, C_7–H), 6.05 (1H, br s, C_2–H), 6.33 (1H, q, *J*=16, 7, C_8–H). ORD (*c*= 0.15, MeOH) [α] (nm): +3117° (262), 0° (245), −6800° (209). CD (*c*=0.002, MeOH) Δε (nm): −0.19° (317), +4.07° (240).

Acid Hydrolysis of V——A solution of V (3 mg) in 10% H_2SO_4 (0.5 ml) was heated for 2 h under reflux. The reaction mixture was diluted with water and extracted with ether. The organic layer was washed with water and evaporated to dryness. The residue was shown by TLC to contain vomifoilol (IV). The aqueous

layer, after neutralization with Amberlite IRA 400, was evaporated to dryness *in vacuo* and the residue was shown by Avicel TLC to contain glucose.

Permethylation of V——Methylation of V (11 mg) was carried out in the same way as for I to give the per-O-methyl derivative (Va) (1.8 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: no hydroxy group. MS (*m/z*): 219 [terminal permethylated glucose], 187 [219—MeOH].

Methanolysis of Va——Va (1 mg) was worked up in the same way as for Ia to give methyl sugars which were identified by GLC under condition(*b*) [t_R(min): methyl pyranosides of 2,3,4,6-tetra-O-methyl-β-D-glucose, 1.78; 2,3,4,6-tetra-O-methyl-α-D-glucose, 2.35; 4,6-di-O-methyl-α-D-glucose, 12.98].

Compound A (VII, VIII)——White powder, IR ν_{max}^{KBr} cm^{-1}: 3360, 1620. UV λ_{max}^{MeOH} nm: 292, 332 (sh), $\lambda_{max}^{MeOH+NaOMe}$ nm: 245, 327, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 312, 370, $\lambda_{max}^{MeOH+NaOAc}$ nm: 285 (sh), 331. ^{1}H-NMR (DMSO-d_6) δ: 5.42 (1H, m), 6.78 (2H, d, J=8), 7.33, 7.35 (each 1H, d, J=8), 9.45, 9.54 (total 1H, br s), 12.76, 12.79 (total 1H, br s, C_5–OH). ^{13}C-NMR (DMSO-d_6) δ naringenin moiety: 196.5 and 196.8(4), 163.6(7), 160.5(5), 159.7 (9), 156.6 and 156.9 (4′), 128.4 and 128.9 (1′), 127.5 (2′ or 6′), 126.9 (2′ or 6′), 114.7 (3′, and 5′), 104.8 (6 and 8), 101.2 and 101.4 (10), 77.4 (2); sugar moiety: 81.0, 78.0, 73.2, 70.7, 69.4, 60.5 (sugar C-atoms not assigned), (C_5D_5N) δ naringenin moiety: 196.6 and 196.9 (4), 165.1 (7), 162.4 and 162.5 (5), 160.8 (9), 158.5 and 158.7 (4′), 129.2 and 129.6 (1′), 128.2 (2′ or 6′), 127.7 (2′ or 6′), 116.0 (3′ and 5′), 106.3 (6), 105.1 (8), 102.5 and 102.7 (10), 78.6 (20), 43.2 and 43.5 (3); sugar moiety: 82.6, 80.0, 79.7, 75.2, 72.9, 71.2, 62.1 (sugar C-atoms not assigned).

Ferric Chloride Oxidation of Compound A——A solution of compound A (60 mg) and ferric chloride (600 mg) in water (4 ml) was heated under reflux for 6 h. The dark-colored reaction mixture, after cooling, was filtered. The dark yellow filtrate was treated with Amberlite MB-3 to remove ions and the neutral solution was concentrated. The residue was chromatographed on Sephadex LH-20 (EtOH), and the eluate was evaporated to dryness. Avicel TLC showed the residue to contain glucose and arabinose.

Acetylation of Compound A——Compound A (100 mg) was acetylated in the usual way and the products were chromatographed over silica gel [$CHCl_3$–EtOAc (2: 1)] to give two undeca-O-acetyl derivatives [VIIa (45 mg) and VIII (25 mg)]. On TLC [$CHCl_3$–EtOAc (1: 1); double development] the spots due to VIIa and VIIIa were discriminated around *Rf* 0.3.

Compound VIIa——White powder, $[\alpha]_D^{21}$ −40.8° (*c*=2.05, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1780, 1755, 1695. MS (*m/z*): 1058 $[M]^+$. *Anal.* Calcd for $C_{49}H_{54}O_{26}$: C, 55.67; H, 5.14. Found: C, 55.09; H, 5.29. ^{1}H-NMR ($CDCl_3$) δ: 1.78, 1.91, 1.96, 2.02, 2.04 (each 3H, s, CH_3CO), 2.05 (6H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 2.32 (3H, s, C_4′–$OCOCH_3$), 2.43 (3H, s, C_5–$OCOCH_3$), 2.48 (3H, s, C_7–$OCOCH_3$), 7.20 (2H, d, J=8, C_3′ and C_5′–H), 7.59 (2H, d, J=8, C_2′ and C_6′–H). ^{13}C-NMR ($CDCl_3$) δ naringenin moiety: 188.3 (4), 166.9—170.3 (5, 7, 9, 4′ and $CH_3\underline{C}O$), 135.5 (1′), 126.1 (2′ and 6′), 121.8 (3′ and 5′), 116.4 (6), 115.2 (8), 112.0 (10), 78.6 (2), 44.9 (3), 20.1—21.0 ($\underline{C}H_3CO$); sugar moiety: 76.5, 74.2, 73.9, 73.2, 72.3, 69.1, 68.1, 62.1, 61.8 (sugar C-atoms not assigned). CD (*c*=0.0009, MeOH) $\Delta\varepsilon$ (nm): +5.38° (336), −8.14° (305), +3.76° (253).

Compound VIIIa——White powder, $[\alpha]_D^{21}$ −43.1° (*c*=1.48, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1780, 1755, 1695. MS (*m/z*): 1058 $[M]^+$. *Anal.* Calcd for $C_{49}H_{54}O_{26}$: C, 55.57; H, 5.14. Found: C, 55.59; H, 5.55. ^{1}H-NMR ($CDCl_3$) δ: 1.78, 1.80, 1.92 (each 3H, s, CH_3CO), 2.02 (9H, s, CH_3CO), 2.04, 2.06 (each 3H, s, CH_3CO), 2.32 (3H, s, C_4′–$OCOCH_3$), 2.43 (3H, s, C_5–$OCOCH_3$), 2.48 (3H, s, C_7–$OCOCH_3$), 7.22 (2H, d, J=8, C_3′ and C_5′–H), 7.60 (2H, d, J=8, C_2′ and C_6′–H). ^{13}C-NMR ($CDCl_3$) δ naringenin moiety: 188.7 (4), 166.8—170.3 (5, 7, 9, 4′ and $CH_3\underline{C}O$), 135.1 (1′), 126.7 (2′ and 6′), 121.9 (3′ and 5′), 116.8 (6), 115.3 (8), 110.7 (10), 78.8 (2), 44.5 (3), 20.2—21.1 ($\underline{C}H_3CO$); sugar moiety: 76.4, 74.2, 72.9, 72.5, 69.0, 68.1, 61.8 (sugar C-atoms not assigned). CD (*c*=0.003, MeOH) $\Delta\varepsilon$ (nm): −3.95° (335), +3.16° (303), +10.95° (257).

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