Comparison of Cytoprotective Effects of Saponins Isolated from Leaves of *Aralia elata* SEEM. (Araliaceae) with Synthesized Bisdesmosides of Oleanoic Acid and Hederagenin on Carbon Tetrachloride-Induced Hepatic Injury

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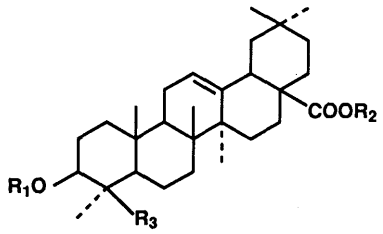
Glycosylations of 3-*O*-{2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl(1→2)-3,4-di-*O*-acetyl-α-L-arabinopyranosyl}-23-*O*-acetylhederagenin (15) with mono- (16), di- (17) and trisaccharide bromide (18) gave the bisdesmoside peracetates 19, 20 and 22, respectively, which were treated with 5% KOH in MeOH to give the bisdesmosides 25—27. Hydrolysis of the glycosides 6 and 9 having β-D-glucopyranose as a terminal sugar component with β-glucosidase in acetate buffer (pH 4.7) gave compounds 28 and 29, respectively. Cytoprotective effects of the synthesized triterpenoidal saponins against CCl_4-induced hepatic injury were compared with those of saponins isolated from the leaves of *Aralia elata* SEEM. (Araliaceae) using isolated hepatocytes from rat liver. Although the monodesmosides 1—4 having neutral sugar components only at the *O*-3 position on the aglycones showed no cytoprotective effect, bisdesmosides having sugar components at both the *O*-3 and *O*-28 positions on the aglycones had potent effects, even when the species of the sugar components were different. The bisdesmosides 10, 11, and 27 having five monosaccharides in the molecules exhibited the most potent cytoprotective effects.

Keywords cytoprotective effect; carbon tetrachloride-induced hepatic injury; bisdesmoside; glycosylation; oleanoic acid; hederagenin

In a previous paper,[1] we reported the isolation of eleven saponins (**1**—**11**) from the leaves of *Aralia elata* SEEM. (Araliaceae) and structural analysis of the saponins. In this paper, we describe the cytoprotective effects of the isolated saponins on carbon tetrachloride (CCl_4)-induced hepatic injury. Synthesis of bisdesmosides of oleanoic acid (**12**) and hederagenin (**13**) and a comparison of their cytoprotective effects are also reported.

Chemistry In order to investigate the relationship between the cytoprotective effect and the differences of number and species of sugar components, several bisdesmosides were obtained by synthetic and enzymic degradation procedures. In the synthetic procedure, the monodesmoside **2**, which was isolated in relatively large quantity from the leaves of *Aralia elata*, but showed no cytoprotective effect, was used as the starting material for the syntheses of the bisdesmosides **25**—**27**, in which β-D-glucopyranose, β-D-galactopyranosyl(1→4)-β-D-glucopyranose and β-D-galactopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranose, respectively, were linked at the *O*-28 position of **2**. 3-*O*-{2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl(1→2)-3,4-di-*O*-acetyl-α-L-arabinopyranosyl}-23-*O*-acetylhederagenin (**15**) was obtained by acetylation of **2**. The fast atom bombardment mass spectrum (FAB-MS) of **15** showed the quasimolecular ion peak at *m/z* 1025 $[M+Na]^+$, and the ^{1}H-NMR spectrum of **15** exhibited two anomeric proton signals due to α-L-arabino- and α-L-rhamnopyranose rings at δ 4.44 (d, J=6.3 Hz) and 5.03 (d, J=1.0 Hz), respectively, together with the signals of six acetyl groups at δ 1.97—2.14. Glycosylation of **15** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **16**[4] in the presence of Ag_2CO_3 in dry CH_2Cl_2 gave compound **19** in 54.3% yield. The FAB-MS of **19** showed the quasimolecular ion peak at *m/z* 1355



	R_1	R_2	R_3
1	Rha(1→2)Ara	H	CH_3
2	Rha(1→2)Ara	H	CH_2OH
3	Glc(1→3)Rha(1→2)Ara	H	CH_3
4	Glc(1→3)Rha(1→2)Ara	H	CH_2OH
5	Rha(1→2)Ara	Xyl(1→6)Glc	CH_3
6	Rha(1→2)Ara	Glc(1→6)Glc	CH_3
7	Rha(1→2)Ara	Xyl(1→6)Glc	CH_2OH
8	Rha(1→2)Ara	Glc(1→6)Glc	CH_2OH
9	Glc(1→3)Rha(1→2)Ara	Glc(1→6)Glc	CH_3
10	Rha(1→2)Ara	Rha(1→4)Glc(1→6)Glc	CH_2OH
11	Glc(1→3)Rha(1→2)Ara	Glc(1→6)Glc	CH_2OH
12	H	H	CH_3
13	H	H	CH_2OH

Glc = β-D-glucopyranose Ara = α-L-arabinopyranose
Rha = α-L-rhamnopyranose Xyl = β-D-xylopyranose

Chart 1

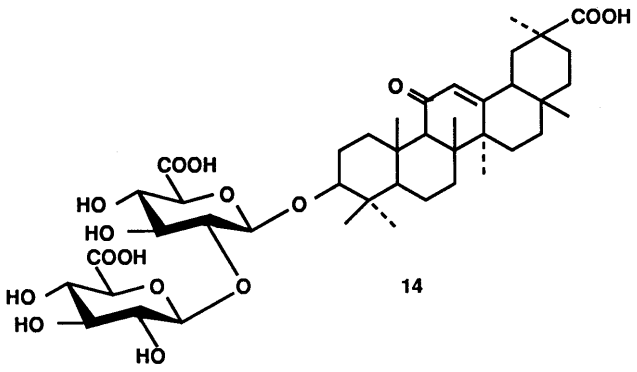


Chart 2

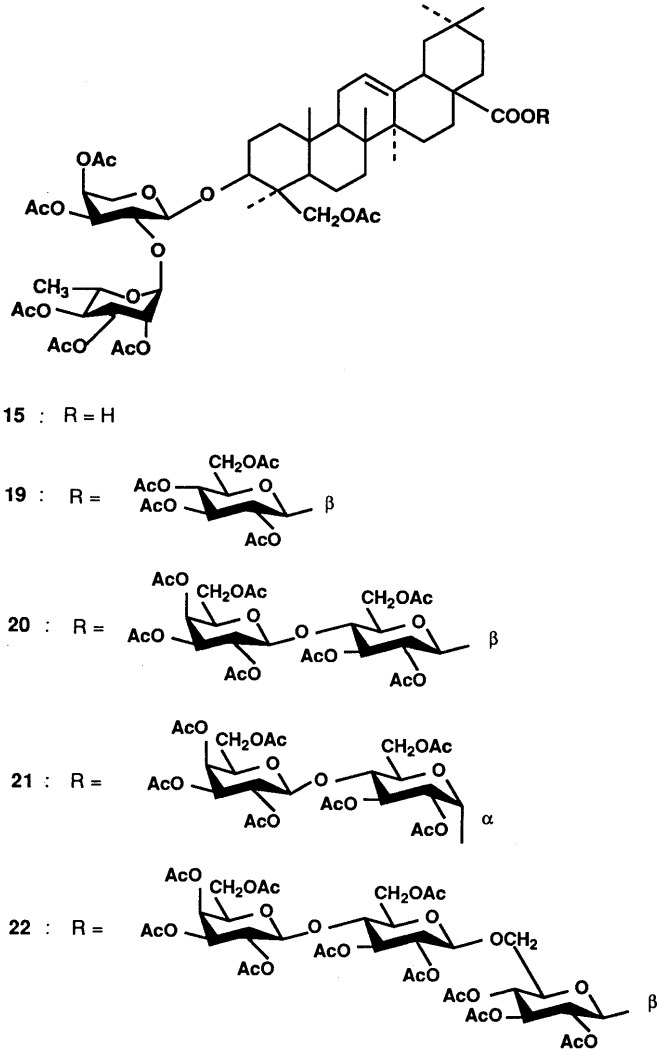


Chart 3

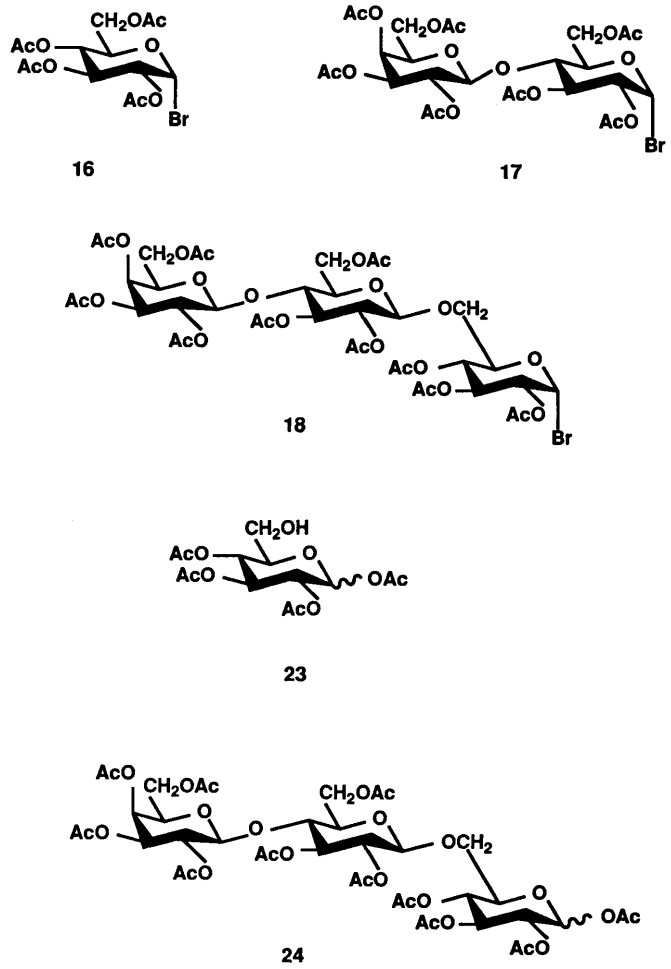
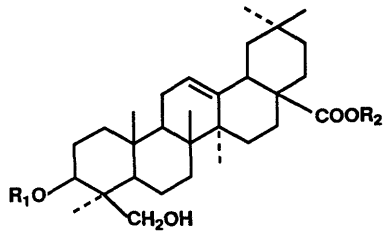


Chart 4

$[M+Na]^+$. In the ^{1}H-NMR spectrum of **19**, the signal of an anomeric proton due to the β-D-glucopyranose ring was observed at δ 5.56 (d, $J=7.9$ Hz) together with those due to α-L-arabino- and α-L-rhamnopyranose rings at δ 4.45 (d, $J=6.3$ Hz) and 5.05 (d, $J=1.0$ Hz), respectively. In the ^{13}C-NMR spectrum of **19**, an anomeric carbon signal was observed at δ 91.6 due to β-D-glucopyranose in addition to those due to α-L-arabinopyranose (δ 103.6) and α-L-rhamnopyranose (δ 98.0). Glycosylation of **15** with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide **17**[5] under similar reaction conditions to those of **15** with **16** gave compounds **20** and **21** in the yields of 51.3 and 4.5%, respectively. Both **20** and **21** showed the quasimolecular ion peaks at *m/z* 1643 $[M+Na]^+$. In the ^{1}H-NMR spectrum of **20**, four anomeric protons due to α-L-arabino-, β-D-galacto-, α-L-rhamno- and β-D-glucopyranose rings were observed at δ 4.42 (d, $J=6.6$ Hz), 4.46 (d, $J=8.1$ Hz), 5.04 (d, $J=1.0$ Hz) and 5.53 (d, $J=8.1$ Hz), respectively. On the other hand, the ^{1}H-NMR spectrum of **21** exhibited the signal of an anomeric proton due to α-D-glucopyranose at δ 6.26 (d, $J=3.7$ Hz) in addition to those of three anomeric protons due to α-L-arabino-, β-D-galacto- and α-L-rhamnopyranoses at δ 4.20 (d, $J=6.2$ Hz), 4.48 (d, $J=7.7$ Hz) and 5.05 (d, $J=1.0$ Hz), respectively. In the ^{13}C-NMR spectra, **20** showed two additional anomeric carbon signals due to β-D-glucopyranose and β-D-galactopyranose at δ 91.4 and 100.9, respectively, and **21** showed those due to α-D-glucopyranose and β-D-galactopyranose at δ 89.1 and 101.8, respectively. Prior to the preparation of **22**, the acetylated trisaccharide bromide **18** was synthesized: 1,2,3,4-tetra-*O*-acetyl-D-glucopyranose (**23**)[6,7] was reacted with **17** in the presence of Ag_2CO_3 in dry CH_2Cl_2 to give the trisaccharide peracetate (**24**), which was treated with 20% HBr in acetic acid to give **18**. The FAB-MS of **18** showed the quasimolecular ion peak at *m/z* 1009 $[M+Na]^+$. The ^{1}H-NMR spectrum of **18** exhibited the



	R_1	R_2
25	Rha(1→2)Ara	Glc
26	Rha(1→2)Ara	Gal(1→4)Glc
27	Rha(1→2)Ara	Gal(1→4)Glc(1→6)Glc

Chart 5

TABLE I. ^{13}C-NMR Spectral Data for Compounds **4** and **25**—**27** in d_5-Pyridine[a]

	24	**25**	**26**	**27**
Aglycone[b]				
C-3	81.0	81.0	81.0	81.0
C-12	122.5	122.9	123.0	122.9
C-13	144.0	144.0	144.0	144.1
C-23	61.6	62.2	61.7	61.9
C-28	180.1	176.4	176.4	176.5
Ara[c]				
C-1	104.1	104.1	104.2	104.2
C-2	75.2	75.8	75.9	75.8
C-3	74.4	74.3	74.5	74.5[f]
C-4	69.5	69.6	69.7	69.7
C-5	65.4	65.3	65.5	65.5
Rha				
C-1	101.4	101.5	101.6	101.6
C-2	72.4[d]	72.4[d]	72.3[d]	72.2[d]
C-3	72.1[d]	72.2[d]	72.4[d]	72.3[d]
C-4	73.9	74.0	74.1	74.1
C-5	69.1	69.1	69.2	69.2[e]
C-6	18.4	18.4	18.5	18.5
Glc				
C-1		95.6	95.2	95.7
C-2		74.0	72.5	72.4
C-3		78.7	77.2[d]	78.6
C-4		71.1	81.5	70.8
C-5		79.1	77.2[d]	77.2
C-6		62.2	62.0	69.3[e]
Glc′				
C-1				104.9
C-2				72.5
C-3				76.4[f]
C-4				81.8
C-5				75.1
C-6				62.0
Gal				
C-1			105.8	105.7
C-2			73.7	73.9
C-3			75.1	74.6
C-4			70.0	70.0
C-5			77.0	76.5
C-6			61.7	62.0

a). The signal assignments were based on the ^{1}H–^{13}C-COSY method. *b*) Only characteristic signals of aglycones are listed. *c*) Abbreviations Ara, Rha, Glc and Gal represent α-L-arabino-, α-L-rhamno-, β-D-gluco- and β-D-galactopyranose. *d*—*f*) These values may be interchangeable in each column.

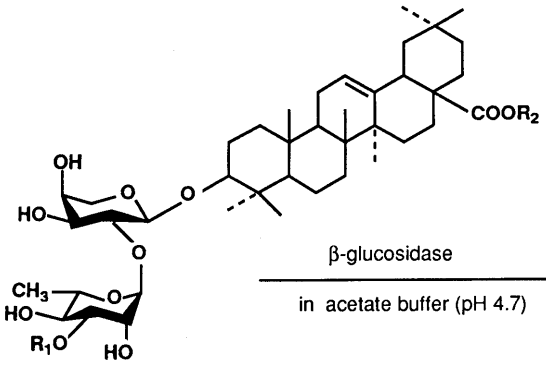
TABLE II. ^{13}C-NMR Spectral Data for Compounds **6**, **9**, **28** and **29** in d_5-Pyridine[a]

		6	**9**	**28**	**29**
Aglycone[b]					
	C-3	88.7	88.7	88.8	88.7
	C-12	122.8	122.8	122.9	122.9
	C-13	144.1	144.2	144.1	144.1
	C-28	178.5	176.5	176.4	176.4
3-*O*-Sugar					
Ara	C-1	104.6	105.1	104.8	105.4
	C-2	75.9	75.8[d]	76.0	75.6
	C-3	73.4	74.3	74.1[d]	74.6[d]
	C-4	69.8	69.8	68.4	69.8
	C-5	64.3	65.6	64.4	65.8
Rha	C-1	101.6	101.6	101.7	101.6
	C-2	72.4	71.6	72.3[e]	71.8
	C-3	72.2	83.1	72.6[e]	83.4
	C-4	73.9[d]	72.9	74.0[d]	73.1
	C-5	68.4	69.3	69.9	69.4
	C-6	18.5	18.5	18.5	18.5
Glc	C-1		106.6		106.9
	C-2		75.7[d]		76.0
	C-3		78.4		78.7
	C-4		71.5		71.5[e]
	C-5		78.5		79.4
	C-6		62.6		62.5
28-*O*-Sugar					
Inner Glc					
	C-1	95.6	95.7	95.7	95.8
	C-2	73.8[d]	73.8	73.5	74.2[d]
	C-3	78.5[e]	78.7	78.9	78.6
	C-4	70.7	70.9	72.2	71.2[e]
	C-5	77.8	77.9	79.2	79.0
	C-6	69.2	69.2	62.3	62.3
Outer Glc					
	C-1	105.0	105.3		
	C-2	75.0	75.2		
	C-3	78.2	78.4		
	C-4	71.4	71.5		
	C-5	78.2[e]	78.4		
	C-6	62.5	62.6		

a) The signal assignments were based on the reported spectral data.[8-10,13,14] *b*) Only characteristic signals of aglycones are listed. *c*) Abbreviations Ara, Rha, Glc and Gal represent α-L-arabino-, α-L-rhamno-β-D-gluco- and β-D-galactopyranose. *d*, *e*) These values may be interchangeable in each column.

signals of three anomeric protons at δ 6.61 (d, J=4.0 Hz), 4.50 (d, J=7.7 Hz) and 4.48 (d, J=8.1 Hz). Glycosylation of **15** with **18** under similar reaction conditions to those used in the case of **15** with **16** gave compound **22** in 29.4% yield. The ^{1}H-NMR spectrum of **22** exhibited the signals of three anomeric protons due to three β-arranged pyranoses, one β-D-galacto- and two β-D-glucopyranoses, at δ 4.46 (d, J=7.9 Hz), 4.47 (d, J=7.9 Hz) and 5.52 (d, J=8.1 Hz), respectively, in addition to those of two anomeric protons due to α-L-arabino- and α-L-rhamnopyranose at δ 4.42 (d, J=6.3 Hz) and 5.05 (d, J=1.0 Hz), respectively. Hydrolyses of **19**, **20** and **22** in 5% KOH in MeOH gave compounds **25**—**27**. The structures of **25**—**27** were confirmed by ^{13}C-NMR spectral data (Table I) and FAB-MS, which showed the quasimolecular ion peaks at m/z 935, 1097 and 1259 for **25**, **26** and **27**, respectively.

The degradation of glycosides **3**, **6** and **9**, having terminal β-D-glucopyranose, with β-glucosidase was examined. The reaction of **3** having β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranose, Glc(1→3)Rha(1→2)Ara, linked to the *O*-3 position on the aglycone with



	R_1	R_2		R_1	R_2
3	Glc	H	–		
6	H	Glc(1→6)Glc	**28**	H	Glc
9	Glc	Glc(1→6)Glc	**29**	Glc	Glc

Chart 6

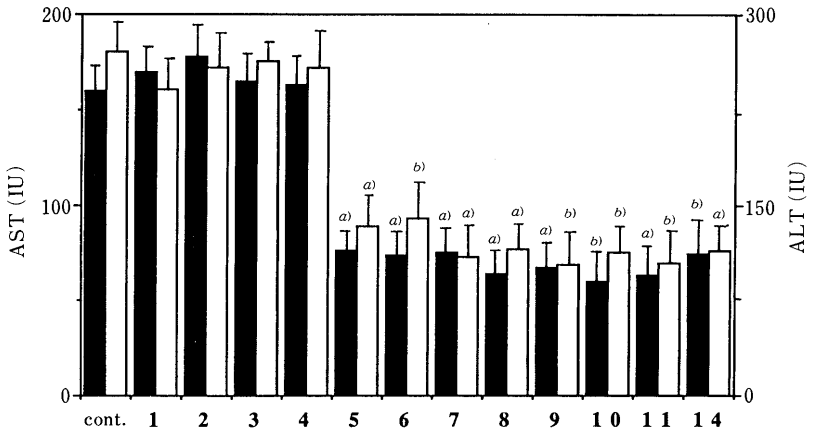


Fig. 1. Cytoprotective Effects of Isolated Glycosides at the Lower Concentration on CCl_4-Induced Hepatic Injury

A final concentration of 3.2×10^{-7} M isolated glycosides (**1**—**11**) was added to an incubation mixture. Both AST and ALT were assayed as described in the text. Closed bars indicate activities of AST and blank ones those of ALT. Significantly different from the control: *a*) $p<0.01$, *b*) $p<0.05$.

β-glucosidase in acetate buffer (pH 4.7) gave no product, but the reaction of **6** having β-D-glucopyranosyl(1→6)-β-D-glucopyranose, Glc(1→6)Glc, linked to the *O*-28 position on the aglycone with β-glucosidase in the same buffer gave **28** in 88.6% yield. The ^{13}C-NMR spectrum of **28** (Table II) lacked the carbon signals due to the terminal β-glucopyranose. Thus, it was found that the terminal β-D-glucopyranose linked to the *O*-6 position of the β-D-glucopyranose was readily hydrolyzed by β-glucosidase, whereas those linked to the *O*-28 position on the aglycone, which formed an ester glycosidic linkage, and to the *O*-3 position of the α-L-rhamnopyranose were not. It is therefore predicted from these results that **9** having the trisaccharide, Glc(1→3)Rha(1→2)Ara, and disaccharide, Glc(1→6)Glc, at the *O*-3 and 28 positions, respectively, on the aglycone would give a hydrolyzed product (**29**) in the reaction with β-glucosidase. Actually the reaction of **9** with the enzyme under the same reaction conditions as used for **6** gave the predicted product **29** in 75.0% yield. The FAB-MS of **29** showed the quasimolecular ion peak at *m/z* 1081 $[M+Na]^+$, which suggests that a β-D-glucopyranose had been lost. In the ^{13}C-NMR spectrum of **29**, the signals of the carbons due to two β-D-glucopyranoses in addition to those due to α-L-arabino- and α-L-rhamnopyranose were observed. From the result of the hydrolysis of **6** and the observation of glycosylation shift[8-10] at the carbon at the *O*-3 position on α-L-rhamnopyranose and the disappearance of the shift on the carbon at the C-6 position of the ester glycosidic β-D-glucopyranose in the ^{13}C-NMR spectrum of **29**, it was suggested that one of the other two remaining β-D-glucopyranoses links to the *O*-28 position on the aglycone and the other one to the *O*-3 position of α-L-rhamnopyranose.

Cytoprotective Activity Glycyrrhizin (**14**) isolated from *Glycyrrhiza glabra* L. and allied plants (Leguminosae) is a monodesmoside consisting of glycyrrhetic acid as an aglycone and an acidic sugar component, β-D-glucuronopyranosyl-β(1→2)-β-D-glucuronopyranose, and has a potent cytoprotective effect.[2] The saponins **1**—**11** are mono- and bisdesmosides that consist of oleanoic acid **12** or hederagenin **13** as the aglycone and only neutral sugar components. The cytoprotective effects of these saponins **1**—**11** on CCl_4-induced hepatic injury *in vitro* were compared with that of glycyrrhizin **14**, as estimated by assay

of aspartate transaminase (AST) and alanine transaminase (ALT) released from CCl_4-treated hepatocytes (Fig. 1). The reaction suspensions for the assay were composed of hepatocytes (2×10^6 cells) and glycosides (3.2×10^{-7} M) in Hanks solution (total 1.1 ml), and the suspension for the control was composed of only hepatocytes (2×10^6 cells) in Hanks solution (total 1.1 ml). The suspensions were exposed to CCl_4 vapor at 37 °C for 1 h. After incubation of the cell suspensions, the supernatants were collected by centrifugation at $1000 \times g$ for 30 s. The activities of AST and ALT were assayed by the reported procedures.[3] Bisdesmosides **5**—**11** decreased the release of both AST and ALT from the hepatocytes injured with CCl_4, as did **14**, indicating that these glycosides had potent cytoprotective effects, while the monodesmosides **1**—**4** showed almost the same values as that of the control, indicating no effect (Fig. 1). From these results, it is suggested that the presence of sugar components at both the *O*-3 and *O*-28 positions on the aglycones is essential for the appearance of a potent cytoprotective effect, and furthermore, a difference of the substituents at the C-24 position (CH_3 and CH_2OH for oleanoic acid and hederagenin, respectively) does not alter the effects.

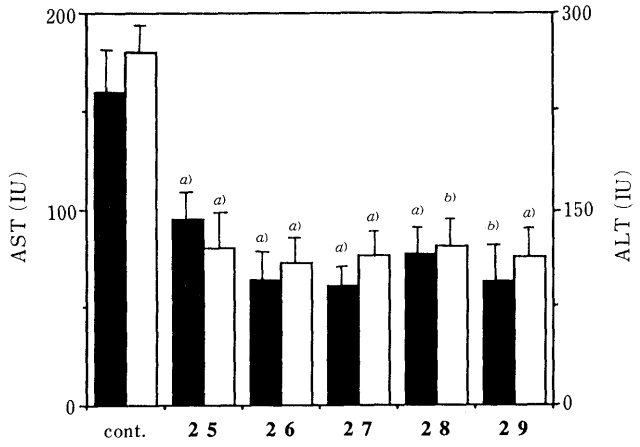


Fig. 2. Cytoprotective Effects of Synthesized Glycosides at the Lower Concentration on CCl_4-Induced Hepatic Injury

A final concentration of 3.2×10^{-7} M synthesized glycosides (**25**—**29**) was added to an incubation mixture. Both AST and ALT were assayed as described in the test. Closed bars indicate activities of AST and blank ones those of ALT. Significantly different from the control: *a*) $p<0.01$, *b*) $p<0.05$.

The cytoprotective effects of the synthesized glycosides **25**—**29** were compared with those of glycyrrhizin **14** and some of the isolated glycosides at the concentrations of 3.2×10^{-7} M and 1.3×10^{-6} M. In the case of the lower concentration (3.2×10^{-7} M), glycosides **25**—**29** decreased the release of both AST and ALT from the hepatocytes injured with CCl_4 almost as potently as glycyrrhizin **14** (Fig. 2), which indicated that the bisdesmosides **25**—**29** have a potent cytoprotective effect, like that of **14**.

The cytoprotective effects of **25**—**29**, the isolated glycosides **8**, **10** and **11**, and glycyrrhizin **14** were compared at the higher concentration (1.3×10^{-6} M) (Fig. 3). Glycosides **25** and **28** having three monosaccharide units showed increased release of both AST and ALT as compared with the lower concentration. A similar increase in the release of the enzymes was also shown in the case of **14**. On the other hand, **8**, **26** and **29** having four monosaccharide units had almost no effect on the release of the enzymes at both the lower and higher concentrations. Furthermore, **9**, **10**, **11** and **27** having five monosaccharide units progressively decreased the release of the enzymes. The main mechanism of cytoprotective effect must involve interaction between the cell membrane and saponin, as discussed previously.[11] Therefore the high concentration of saponin may cause disturbance of the cell membrane surface, since cytoprotective effects at high concentration were weaker than those at the lower concentration.

Thus, although monodesmosides **1**—**4** having only neutral sugar components at the *O*-3 position on the aglycones did not show any cytoprotective effect against CCl_4-induced hepatic injury, bisdesmosides **6**—**11** and **25**—**29**, which also consist of only neutral sugar components, showed potent effects. These results indicated that for the appearance of potent cytoprotective effects of triterpenoidal saponins composed of oleanoic acid or hederagenin as the aglycone and only neutral sugar components, it is essential that the sugar components are linked at both the *O*-3 and *O*-28 positions. The effects of the bisdesmosides **9**, **10**, **11** and **27** having five monosaccharide units, were significantly enhanced. It seems that the greater the number of sugar components the bisdesmosides have, the more potent their cytoprotective effects against CCl_4-induced hepatic injury.

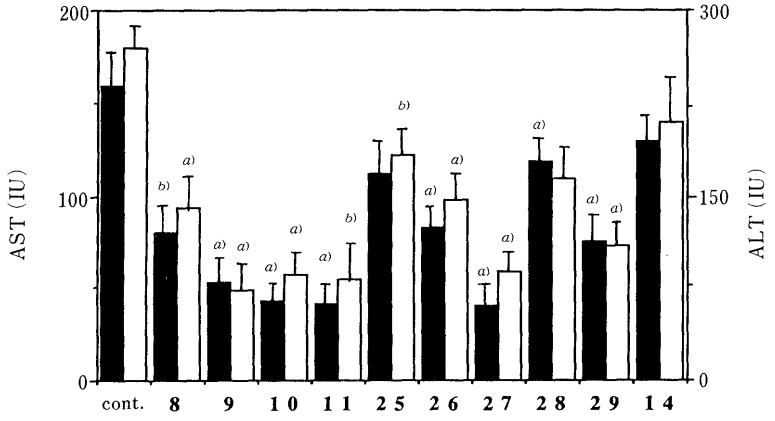


Fig. 3. Comparison of Cytoprotective Effects of Isolated (**8**—**11**) and Synthesized Glycosides (**25**—**29**) and Glycyrrhizin **14** at the Higher Concentration on CCl_4-Induced Hepatic Injury.

A final concentration of 1.3×10^{-6} M glycosides (**8**—**11**, **14** and **25**—**29**) was added to an incubation mixture. Both AST and ALT were assayed as described in the text. Closed bars indicate activities of AST and blank ones those of ALT. Significantly different from the control: *a*) $p<0.01$, *b*) $p<0.05$.

Experimental

Materials Dry dichloromethane (CH_2Cl_2) was obtained by refluxing with NaH followed by distillation. β-Glucosidase (from sweet almonds) was purchased from P-L Biochemicals, Inc. (Milwaukee). Other chemicals and solvents were of reagent grade, and were obtained from commercial sources.

Measurements The thin-layer chromatography (TLC) utilized Kieselgel HF_{254} (Merck), and spots were detected by spraying the plates with dilute H_2SO_4 followed by heating at 100 °C for 10 min. Column chromatography was carried out on Wakogel C-200. An SSC-6300 apparatus (Senshu Scientific Co., Ltd.) equipped with an SSC-3000A was employed for analytical HPLC using an ODS-1251-D column (4.6 mm × 250 mm), with an SSC autoinjector 6310 and an SSC fraction collector 6320 for preparative HPLC using an ODS-4251-D column (10 mm × 250 mm). ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM-GX NMR spectrometer at 270 and 67.8 MHz, respectively, and chemical shifts are given in ppm with tetramethylsilane as an internal standard. FAB-MS were recorded on a JEOL JMS-DX 300 mass spectrometer. The activities of AST and ALT were assayed by using an autoanalyzer COBAS MRA (Roche) with commercial kits based on the principle of the AST and ALT assay method.[3] Statistical examination was carried out with Student's *t* test.

Preparation of Hepatocytes Isolated rat hepatocytes were prepared using 150—180 g Wistar rats by the published procedure.[12]

Preparation of 15 Compound **2** (10 g) was dissolved in pyridine (50 ml) and acetic anhydride (50 ml) and the solution was allowed to stand overnight at room temperature. The reaction mixture was coevaporated with toluene (200 ml × 5) to give a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 5%) to give compound **15** (11.49 g, 85.8%). FAB-MS *m/z*: 1025 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.97—2.14 (Ac × 6); (aglycone; only assignable signals of the aglycone are listed) 0.74, 0.79, 0.91, 0.93, 0.95, 1.11 (each CH_3), 3.55 (dd, J=11.4, 4.7 Hz, H-3), 5.32 (dd, J=3.3, 3.3 Hz, H-12), 2.81 (dd, J=13.6, 4.0 Hz, H-18), 3.90 (d, J=12.0 Hz, H-23), 4.13 (d, J=12.0 Hz, H-23′); (Ara) 4.44 (d, J=6.3 Hz, H-1), 3.86 (dd, J=8.8, 6.3 Hz, H-2), 4.96 (dd, J=8.8, 3.3 Hz, H-3), 5.25 (ddd, J=3.7, 3.3, 1.6 Hz, H-4), 3.95 (dd, J=12.8, 3.7 Hz, H-5), 3.58 (dd, J=12.8, 1.6 Hz, H-5′); (Rha) 5.03 (d, J=1.0 Hz, H-1), 5.21—5.28 (H-2, 3), 5.04 (dd, J=10.0, 10.0 Hz, H-4), 4.12 (m, H-5), 1.21 (d, J=6.2 Hz, H-6), 9.53—11.0 (COOH). ^{13}C-NMR ($CDCl_3$) δ: 81.3 (C-3), 122.5 (C-12), 143.6 (C-13), 41.0 (C-18), 65.1 (C-23), 188.8 (C-28) (only characteristic signals of the aglycone are listed), 20.6—21.0 (6 × CH_3 of acetyl groups), 169.7—170.4 (6 × C=O of acetyl groups), 103.5 (Ara-1), 74.5 (Ara-2), 71.8 (Ara-3), 69.6 (Ara-4), 62.6 (Ara-5), 98.3 (Rha-1), 71.0 (Rha-2), 67.8 (Rha-3), 69.6 (Rha-4), 67.2 (Rha-5), 17.4 (Rha-6). *Anal.* Calcd for $C_{53}H_{78}O_{18}$: C, 63.45; H, 7.84. Found: C, 63.26; H, 7.77.

Glycosylation of 15 with 16 Compound **16** (1.2 g) was added to a suspension of compound **15** (1.0 g), Drierite (1.0 g) and Ag_2CO_3 (850 mg) in dry CH_2Cl_2 (20 ml), and the mixture was stirred for 20 h at room temperature, then filtered. The filtrate was poured into ice-water (150 ml) and extracted with CH_2Cl_2 (150 ml × 3). The combined organic extracts were successively washed with $NaHCO_3$-saturated water and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a residue, which was subjected to column chromatography (gradient elution with benzene–AcOEt up to 12%) to give compound **19** (722 mg, 54.3%). FAB-MS *m/z*: 1355 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.98—2.15 (Ac × 10); (aglycone; only assignable signals of the aglycone are listed) 0.72, 0.80, 0.90, 0.91, 0.95, 1.08 (each CH_3), 3.57 (dd, J=11.4, 4.7 Hz, H-3), 5.32 (dd, J=3.3, 3.3 Hz, H-12), 2.81 (dd, J=13.6, 4.0 Hz, H-18), 3.90 (d, J=11.9 Hz, H-23), 4.15 (d, J=11.9 Hz, H-23′); (Ara) 4.45 (d, J=6.3 Hz, H-1), 3.87 (dd, J=8.9, 6.3 Hz, H-2), 4.97 (dd, J=8.9, 3.3 Hz, H-3), 5.26 (ddd, J=3.7, 3.3, 1.6 Hz, H-4), 3.95 (dd, J=12.8, 3.7 Hz, H-5), 3.59 (dd, J=12.8, 1.6 Hz, H-5′); (Rha) 5.05 (d, J=1.0 Hz, H-1), 5.20—5.28 (H-2, 3), 5.05 (dd, J=10.0, 9.9 Hz, H-4), 4.12 (m, H-5), 1.21 (d, J=6.0 Hz, H-6); (Glc) 5.56 (d, J=7.9 Hz, H-1), 5.12 (dd, J=9.5, 7.9 Hz, H-2), 5.15—5.28 (H-3), 3.70—3.90 (H-4), 3.79 (H-5), 4.27 (dd, J=12.0, 4.0 Hz, H-6), 4.09 (H-6′). ^{13}C-NMR ($CDCl_3$) δ: 82.0 (C-3), 123.0 (C-12), 143.0 (C-13), 41.0 (C-18), 65.0 (C-23), 176.0 (C-28) (only characteristic signals of the aglycone are listed), 20.5—21.0 (10 × CH_3 of acetyl groups), 169.0—170.5 (10 × C=O of acetyl groups), 103.6 (Ara-1), 74.1 (Ara-2), 72.0 (Ara-3), 69.6 (Ara-4), 62.8 (Ara-5), 98.0 (Rha-1), 71.0 (Rha-2), 67.9 (Rha-3), 69.9 (Rha-4), 67.1 (Rha-5), 17.3 (Rha-6), 91.6 (Glc-1), 68.6 (Glc-2), 68.0 (Glc-3), 73.0 (Glc-4), 72.5 (Glc-5), 61.5 (Glc-6). *Anal.* Calcd for $C_{67}H_{96}O_{27}$: C, 60.35; H, 7.26. Found: C, 60.01; H, 7.31.

Glycosylation of 15 with 17 The bromide **17** (4.0 g), silver trifluoromethanesulfonate (380 mg) and 1,1,3,3-tetramethylurea (220 μl) were added to a suspension of compound **15** (1.0 g), Drierite (1.0 g) and Ag_2CO_3 (850 mg) in dry CH_2Cl_2 (30 ml), and the mixture was stirred for 36 h at room temperature. The reaction mixture was treated according to the preparative method for **19** to give a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 7.5%) followed by preparative HPLC (solvent system, 20% H_2O in acetone; flow rate, 1.0 ml/min; column temperature, 35 °C) to give compounds **20** (830 mg, 51.3%) and **21** (73 mg, 4.5%). FAB-MS of **20** *m/z*: 1643 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.97—2.16 (Ac × 13); (aglycone; only assignable signals are listed) 0.71, 0.80, 0.90, 0.90, 0.95, 1.08 (each CH_3), 3.55 (dd, J=11.3, 4.8 Hz, H-3), 5.30 (dd, J=3.3, 3.3 Hz, H-12), 2.80 (dd, J=13.5, 4.0 Hz, H-18), 3.91 (d, J=11.9 Hz, H-23), 4.18 (d, J=11.9 Hz, H-23′); (Ara) 4.42 (d, J=6.6 Hz, H-1), 3.85 (dd, J=8.9, 6.6 Hz, H-2), 4.99 (dd, J=8.8, 3.3 Hz, H-3), 5.25 (ddd, J=3.5, 3.3, 1.6 Hz, H-4), 3.94 (dd, J=12.7, 3.5 Hz, H-5), 3.58 (dd, J=12.7, 1.6 Hz, H-5′); (Rha) 5.04 (d, J=1.0 Hz, H-1), 5.20—5.30 (H-2, 3), 5.06 (dd, J=10.1, 9.7 Hz, H-4), 4.12 (H-5), 1.21 (d, J=6.4 Hz, H-6); (Glc) 5.53 (d, J=8.1 Hz, H-1), 5.03—5.13 (H-2), 5.20—5.27 (H-3), 3.70—3.90 (H-4), 3.72 (m, H-5), 4.37 (dd, J=11.8, 1.0 Hz, H-6), 4.00—4.10 (H-6′); (Gal) 4.46 (d, J=8.1 Hz, H-1), 5.06 (dd, J=10.3, 8.1 Hz, H-2), 4.93 (dd, J=10.3, 3.4 Hz, H-3), 5.45 (d, J=3.4 Hz, H-4), 3.50—4.00 (H-5, 6, 6′). ^{13}C-NMR ($CDCl_3$) δ: 82.3 (C-3), 123.4 (C-12), 143.0 (C-13), 41.0 (C-18), 65.1 (C-23), 175.4 (C-28) (only characteristic signals of the aglycone are listed), 20.5—21.0 (13 × CH_3 of acetyl groups), 169.0—170.3 (13 × C=O of acetyl groups), 103.6 (Ara-1), 74.1 (Ara-2), 72.0 (Ara-3), 69.6 (Ara-4), 62.8 (Ara-5), 98.0 (Rha-1), 71.0 (Rha-2), 67.9 (Rha-3), 70.3 (Rha-4), 67.1 (Rha-5), 17.3 (Rha-6), 91.4 (Glc-1), 68.6 (Glc-2), 69.1 (Glc-3), 70.8 (Glc-4), 73.3 (Glc-5), 61.8 (Glc-6), 100.9 (Gal-1), 71.1 (Gal-2), 72.7 (Gal-3), 66.7 (Gal-4), 75.8 (Gal-5), 60.6 (Gal-6). *Anal.* Calcd for $C_{79}H_{112}O_{35}$: C, 58.51; H, 6.96. Found: C, 58.51; H, 7.01. FAB-MS of **21** *m/z*: 1643 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.96—2.17 (Ac × 13); (aglycone; only assignable signals are listed) 0.74, 0.80, 0.91, 0.92, 0.94, 1.13 (each CH_3), 3.55 (dd, J=11.4, 4.7 Hz, H-3), 5.35 (dd, J=3.3, 3.3 Hz, H-12), 2.85 (dd, J=13.6, 4.1 Hz, H-18), 3.80—4.20 (H-23, 23′); (Ara) 4.20 (d, J=6.2 Hz, H-1), 3.80—3.90 (H-2), 4.95—5.10 (H-3), 5.10—5.25 (H-4), 3.80—4.00 (H-5), 3.56 (dd, J=12.8, 1.7 Hz, H-5′); (Rha) 5.05 (d, J=1.0 Hz, H-1), 5.20—5.30 (H-2, 3), 5.00—5.10 (H-4), 4.10—4.20 (H-5), 1.22 (d, J=5.9 Hz, H-6); (Glc) 6.26 (d, J=3.7 Hz, H-1), 5.00 (dd, J=9.5, 3.7 Hz, H-2), 5.21 (dd, J=9.9, 9.5 Hz, H-3), 3.80 (dd, J=9.9, 9.9 Hz, H-4), 3.95 (m, H-5), 4.45 (dd, J=12.0, 1.1 Hz, H-6), 4.05—4.20 (H-6′); (Gal) 4.48 (d, J=7.7 Hz, H-1), 5.12 (dd, J=10.3, 7.7 Hz, H-2), 4.95 (dd, J=10.3, 3.3 Hz, H-3), 5.27 (d, J=3.3 Hz, H-4), 3.50—4.00 (H-5, 6, 6′). ^{13}C-NMR ($CDCl_3$) δ: 82.2 (C-3), 123.4 (C-12), 143.3 (C-13), 41.6 (C-18), 65.5 (C-23), 175.9 (C-28) (only characteristic signals of the aglycone are listed), 20.0—22.2 (13 × CH_3 of acetyl groups), 169.5—170.9 (13 × C=O of acetyl groups), 104.0 (Ara-1), 74.6 (Ara-2), 71.6 (Ara-3), 69.5 (Ara-4), 63.3 (Ara-5), 98.5 (Rha-1), 71.1 (Rha-2), 67.5 (Rha-3), 70.1 (Rha-4), 67.0 (Rha-5), 17.7 (Rha-6), 89.1 (Glc-1), 69.9 (Glc-2), 69.0 (Glc-3), 71.5 (Glc-4), 74.6 (Glc-5), 62.0 (Glc-6), 101.8 (Gal-1), 71.4 (Gal-2), 72.4 (Gal-3), 65.5 (Gal-4), 76.5 (Gal-5), 61.2 (Gal-6). *Anal.* Calcd for $C_{79}H_{112}O_{35}$: C, 58.51; H, 6.96. Found: C, 58.19; H, 7.11.

Preparation of 24 The bromide **17** (13 g) was added to a suspension of 1,2,3,4-tetra-*O*-acetyl-D-glucopyranose **23**[7] (5.4 g), Drierite (1.0 g) and Ag_2CO_3 (4.3 g) in dry CH_2Cl_2 (100 ml). The mixture was stirred overnight at room temperature and filtered. The filtrate was poured into ice-water (300 ml) and extracted with CH_2Cl_2 (200 ml × 3). The combined organic extracts were successively washed with $NaHCO_3$-saturated water and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a residue, which was subjected to column chromatography (gradient elution with CH_2Cl_2–MeOH up to 1%) to obtain compound **24** (7.3 g, 48.7%). FAB-MS *m/z*: 989 $[M+Na]^+$. *Anal.* Calcd for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.41; H, 5.88.

Preparation of 18 Compound **24** (5.6 g) was dissolved in 30% HBr in acetic acid (150 ml) at 0 °C. The solution was stirred for 1 h at the same temperature, then poured into ice-water (500 ml) and extracted with CH_2Cl_2 (200 ml × 3). The combined organic extracts were successively washed with ice-water, $NaHCO_3$-saturated water and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a residue, which was subjected to column chromatography (gradient elution with benzene–acetone up to 1%) to afford compound **18** (3.4 g, 59.6%). FAB-MS *m/z*: 1009 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.96—2.16 (Ac × 10), 6.61 (d, J=4.0 Hz, Glc-1), 4.79 (dd, J=9.9, 4.0 Hz, Glc-2), 5.19 (dd, J=9.9, 9.2 Hz, Glc-3), 5.07 (dd, J=10.3, 9.2 Hz, Glc-4), 4.23 (ddd, J=10.3, 4.8, 2.6 Hz, Glc-5), 4.14—3.70 (Glc-6, 6′), 4.48 (d, J=8.1 Hz, Glc′-1), 4.80 (dd, J=9.2, 8.1 Hz, Glc′-2), 5.53 (dd, J=9.5, 9.2 Hz, Glc′-3),

3.57 (dd, J=9.5, 9.5 Hz, Glc'-4), 4.14—3.70 (Glc'-5), 4.43 (dd, J=12.8, 1.8 Hz, Glc'-6), 4.14—3.70 (Glc'-6'), 4.50 (d, J=7.7 Hz, Gal-1), 5.11 (dd, J=11.6, 7.7 Hz, Gal-2), 5.95 (dd, J=11.6, 3.7 Hz, Gal-3), 5.34 (dd, J=3.3, 1.1 Hz, Gal-4), 3.61 (m, Gal-5), 4.14—3.70 (Gal-6, 6'). *Anal.* Calcd for $C_{38}H_{51}BrO_{25}$: C, 46.21; H, 5.20. Found: C, 45.95; H, 5.43.

Glycosylation of 15 with 18 Drierite (1.0 g), Ag_2CO_3 (1.37 g) and the bromide **18** (800 mg) were added to a solution of compound **15** (1.0 g) in dry CH_2Cl_2 (10 ml), and the mixture was stirred for 28 h at room temperature, then worked up as described for **19** to give a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 24.5%) followed by preparative HPLC (solvent, 10% H_2O in acetone; flow rate, 1.0 ml/min; column temperature, 35 °C) to afford compound **22** (560 mg, 29.4%). ^{1}H-NMR ($CDCl_3$) δ: 1.96—2.15 (Ac × 16); (aglycone; only assignable signals are listed) 0.73, 0.81, 0.91, 0.91, 0.96, 1.09 (each CH_3), 3.45—3.62 (H-3), 5.30 (dd, J=3.3, 3.3 Hz, H-12), 2.80 (dd, J=13.6, 4.0 Hz, H-18), 3.80—4.20 (H-23, 23'); (Ara) 4.42 (d, J=6.3 Hz, H-1), (Rha) 5.05 (d, J=1.0 Hz, H-1), (inner Glc) 5.52 (d, J=8.1 Hz, H-1), (Gal) 4.46 (d, J=7.9 Hz), (outer Glc) 4.77 (d, J=7.9 Hz, H-1) (only anomeric protons on pyranoses are listed). FAB-MS could not be obtained because the molecular weight was too large. *Anal.* Calcd for $C_{91}H_{128}O_{43}$: C, 57.22; H, 6.76. Found: C, 56.96; H, 6.95.

Deacetylation of 19 A solution of compound **19** (830 mg) in 5% KOH in MeOH (2 ml) was stirred for 1 h at room temperature. The solution was neutralized with acetic acid and subjected to ion exchange resin column chromatography (Amberlite MB-3). The eluate was evaporated to give a residue, which was subjected to column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer) to give compound **25** (440 mg, 77.4%). FAB-MS m/z: 935 $[M+Na]^+$. $[\alpha]_D^{20}$ +10.3 (c = 1.5, pyridine). ^{13}C-NMR spectrum: see Table I. *Anal.* Calcd for $C_{47}H_{76}O_{19}\cdot H_2O$: C, 60.63; H, 8.44. Found: C, 60.55; H, 8.47.

Deacetylation of 20 A solution of **20** (450 mg) in 5% KOH in MeOH (2 ml) was stirred for 1 h at room temperature. The reaction mixture was treated as described for **25** to give a residue, which was subjected to column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer) to afford compound **26** (240 mg, 80.4%). FAB-MS m/z: 1097 $[M+Na]^+$. $[\alpha]_D^{20}$ +11.2 (c = 0.83, pyridine). ^{13}C-NMR spectrum: see Table I. *Anal.* Calcd for $C_{53}H_{86}O_{22}\cdot H_2O$: C, 58.23; H, 8.11. Found: C, 57.96; H, 8.23.

Deacetylation of 22 A solution of **22** (280 mg) in 5% KOH in MeOH (2 ml) was stirred for 1 h at room temperature, then worked up as described for **25** to give a residue. The residue was subjected to column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer) followed by preparative HPLC (solvent, 20% H_2O in MeOH; flow rate, 1.0 ml/min; column temperature, 35 °C) to give compound **27** (75 mg, 41.3%). FAB-MS m/z: 1259 $[M+Na]^+$. $[\alpha]_D^{20}$ +14.5 (c = 1.2, pyridine). ^{13}C-NMR spectrum: see Table I. *Anal.* Calcd for $C_{59}H_{96}O_{27}\cdot H_2O$: C, 56.45; H, 7.87. Found: C, 56.27; H, 7.90.

Reaction of 3 with β-Glucosidase A solution of compound **3** (200 mg), Triton X-100 (0.5 ml) and β-glucosidase (10 mg) in 0.1 M acetate buffer (pH 4.7, 4 ml) was incubated at 37 °C for 14 h. No product could be isolated.

Reaction of 6 with β-Glucosidase A solution of compound **6** (200 mg), Triton X-100 (0.5 ml) and β-glucosidase (10 mg) in 0.1 M acetate buffer (pH 4.7, 4 ml) was incubated at 37 °C for 14 h. Then EtOH (2 ml) was added and the whole was heated at 80 °C for 10 min, and filtered. The filtrate was evaporated to give a residue, which was subjected to column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer) to give compound **28** (150 mg, 88.6%). FAB-MS m/z: 919 $[M+Na]^+$. $[\alpha]_D^{20}$ −8.9 (c = 2.3, pyridine). ^{13}C-NMR spectrum: see Table II. *Anal.* Calcd for $C_{47}H_{76}O_{16}\cdot H_2O$: C, 61.69; H, 8.59. Found: C, 61.33; H, 8.72.

Reaction of 9 with β-Glucosidase A solution of **9** (200 mg), Triton X-100 (0.5 ml) and β-glucosidase (10 mg) in 0.1 M acetate buffer (pH 4.7, 4 ml) was incubated at 37 °C for 14 h. The reaction mixture was treated as described for **28** to give compound **29** (130 mg, 75.0%). FAB-MS m/z: 1082 $[M+Na]^+$. $[\alpha]_D^{20}$ −9.7 (c = 1.8, pyridine). ^{13}C-NMR spectrum: see Table II. *Anal.* Calcd for $C_{53}H_{87}O_{21}\cdot H_2O$: C, 59.04; H, 8.32. Found: C, 58.75; H, 8.51.

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