Preparation of Glycyrrhetic Acid β-Glycosides Having β(1→2)-Linked Disaccharides by the Use of 2-*O*-Trichloroacetyl-β-D-pyranosyl Chlorides and Their Cytoprotective Effects on Hepatic Injury *in Vivo*

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Stepwise glycosidation was adopted for the construction of glycyrrhetic acid β-glycosides (27—30) having β(1→2)-linked disaccharides such as 2-*O*-β-D-glucuronopyranosyl-β-D-glucopyranose, 2-*O*-β-D-glucuronopyranosyl-β-D-galactopyranose, 2-*O*-β-D-glucopyranosyl-β-D-glucuronopyranose and 2-*O*-β-D-galactopyranosyl-β-D-glucuronopyranose. In the first glycosidation, 2-*O*-trichloroacetyl-β-D-pyranosyl chlorides (9—11) were utilized as starting sugar derivatives to react with methyl glycyrrhetinate (5): Glycosidation of 5 with 9 and 10 gave β- and α-monoglycosides (12) and (13), and (15) and (16), respectively. Treatment of the β-glycosides 12 and 15 with ammonia-saturated ether gave products (14) and (17), respectively. The glycosidation of 5 with 11 followed by treatment with ammonia-saturated ether gave compounds (18) and (19), respectively. The second step glycosidations of 14 and 17 with methyl 2,3,4-tri-*O*-acetyl-α-D-glucuronatopyranosyl bromide (20) gave diglycoside derivatives (23) and (24), respectively, and that of 18 with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (21) and -α-D-galactopyranosyl bromide (22) gave diglycoside derivatives (25) and (26), respectively. The removal of the protecting groups of 23—26 gave diglycosides 27—30, respectively, having a β-D-glucuronopyranose (β-D-glcUA) as one of two sugar components in the molecules. The cytoprotective effects of the synthesized glycosides 27—30 on carbon tetrachloride (CCl_4)-induced hepatotoxicity *in vivo* were compared with diglycosides 31—33 having only neutral sugar components, and naturally occurring glycyrrhizin (34) having two acidic sugar components (β-D-glcUA). While glycosides 31—33 had no cytoprotective effect, glycosides 27—30 showed potent effects. Especially, 27 and 28, having a β-D-glcUA as the terminal sugar component, were more effective materials against hepatic injury than glycyrrhizin 34.

Keywords 2-*O*-trichloroacetyl-β-D-pyranosyl chloride; glycosidation; cytoprotective effect; glycyrrhetic acid β-diglycoside; carbon tetrachloride-induced hepatic injury

In naturally occurring triterpenoidal saponins, most D-pyranose which link directly at the C-3 position of the aglycons arrange in a β-configuration. It has been reported that the glycosidation using a β(1→2)-linked disaccharide derivative such as 2-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (**1**) gives only α-glycosides,[1] because of the absence of an acetyl group at the C-2 position of **1**, which takes part in neighboring-group participation to the anomeric carbon resulting in the formation of a dioxolane cation intermediate.[2] The authors[3] reported the preparation of glycyrrhetic acid β-glycosides having β(1→2)-linked disaccharides. In the syntheses, 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-glucopyranose (**2**),[4] -D-galactopyranose (**3**)[5] and benzyl 2-*O*-acetyl-3,4-di-*O*-benzyl-D-glucuronatopyranose (**4**)[3] were utilized in the first step glycosidation with methyl glycyrrhetinate **5** to obtain corresponding monoglycosides **6**—**8** which were further reacted, after deacetylation, with acetylated sugar bromides **20**,[6] **21**[7] and **22**[7] to construct the desired diglycosides. However, the synthesis of these benzylated sugar derivatives **2**—**4** from the corresponding pyranose peracetates required several steps. Consequently, the synthesis of desired diglycosides required many steps and resulted in a decrease of the total yields. Brigl[8] and Lemieux and Huber[9] obtained 2-*O*-trichloroacetyl-β-D-glucopyranosyl chloride (**9**) by the reaction of β-D-glucopyranose peracetate with PCl_5, and the authors[10] prepared 2-*O*-trichloroacetyl-β-D-galactopyranosyl chloride (**10**) and methyl 2-*O*-trichloroacetyl-β-D-glucuronatopyranosyl chloride (**11**) by the same reactions of β-D-galacto- and methyl β-D-glucuronatopyranose peracetates, respectively. These 2-*O*-trichloroacetyl-pyranosyl chlorides **9**—**11** seemed to be more convenient sugar derivatives for con-

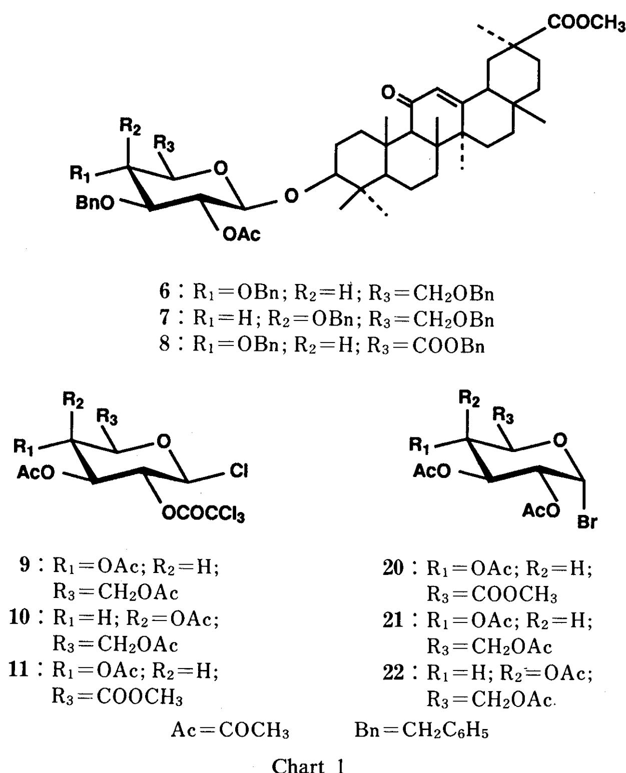

Chart 1

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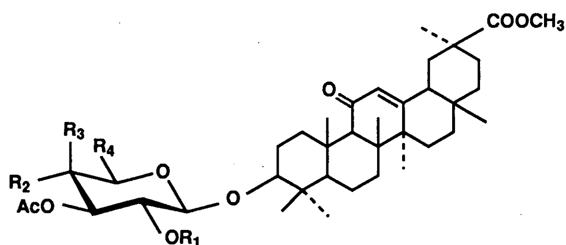
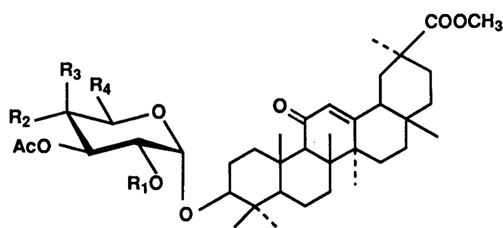


12 : R_1=$COCCl_3$; R_2=OAc; R_3=H; R_4=CH_2OAc
14 : R_1=R_3=H; R_2=OAc; R_4=CH_2OAc
15 : R_1=$COCCl_3$; R_2=H; R_3=OAc; R_4=CH_2OAc
17 : R_1=R_2=H; R_3=OAc; R_4=CH_2OAc
18 : R_1=R_3=H; R_2=OAc; R_4=$COOCH_3$



13 : R_1=$COCCl_3$; R_2=OAc; R_3=H; R_4=CH_2OAc
16 : R_1=$COCCl_3$; R_2=H; R_3=OAc; R_4=CH_2OAc
19 : R_1=R_3=H; R_2=OAc; R_4=$COOCH_3$

Chart 2

structing β-glycosides having β(1→2)-linked disaccharides, since the trichloroacetyl groups at the C-2 position of the sugar derivatives **9**—**11** were easily removable and the resulting OH groups could be linked with other pyranose derivatives to construct the diglycosides. In this paper, we will report the synthesis of glycyrrhetic acid β-glycosides having β(1→2)-linked disaccharides using the 2-*O*-trichloroacetyl-D-pyranosyl chlorides **9**—**11** as starting sugar derivatives, and the cytoprotective effects *in vivo* of the glycyrrhetic acid glycosides against hepatic injury are also reported.

Glycosidation of **5** with **9** in the presence of silver trifluoromethanesulfonate (Ag-OTf) and 1,1,3,3-tetramethyl urea (TMU) in dry CH_2Cl_2 gave β-glycoside **12** (41.3% yield) and α-glycoside **13** (29.8% yield). Both compounds **12** and **13** showed the same pseudomolecular ion peak at *m/z* 939 $[M+Na]^+$ in fast atom bombardment mass spectra (FAB-MS). In the proton magnetic resonance (^{1}H-NMR) spectra (Table I), **12** exhibited an anomeric proton at δ 4.69 with the coupling constant of 8.0 Hz. On the other hand, **13** exhibited one at δ 5.37 with the coupling constant of 4.0 Hz. Treatment of the β-glycoside **12** with ammonia-saturated ether gave compound (**14**) in 95.5% yield. The FAB-MS of **14** showed a pseudomolecular ion peak at *m/z* 795 $[M+Na]^+$. In the ^{1}H-NMR spectrum of **14**, an anomeric proton was observed at δ 4.40 (d, J=7.7 Hz) and an H-2 proton on the pyranose ring shifted at a higher field (δ 3.63) than that of **12** (δ 5.11). Glycosidation of **5** with **10** in the same reaction as that of **5** with **9** gave β-glycoside **15** (43.2% yield) and α-glycoside **16** (26.5% yield). The FAB-MS of **15** and **16** showed the same pseudomolecular ion peak at *m/z* 939 $[M+Na]^+$ as those of **12** and **13**. The ^{1}H-NMR spectra of **15** and **16** showed anomeric protons at δ 4.68 (d, J=7.9 Hz) and 5.45 (d, J=3.3 Hz), respectively. Treatment of **15** with ammonia-saturated ether gave compound **17** (97.1% yield). The FAB-MS of **17** showed the same pseudomolecular ion peak at *m/z* 795 $[M+Na]^+$ as that of **14**. In the ^{1}H-NMR spectrum of **17**, an anomeric proton was observed at δ 4.40 (d, J=7.7 Hz) and an H-2 proton of the pyranose ring was shifted at a higher field (δ 3.85) than that of **15** (δ 5.30). Glycosidation of **5** with **11** in the same reaction condition as **5** with **9** followed by the treatment with ammonia-saturated ether gave β-glycoside **18** (41.7% yield) and α-glycoside **19** (26.4% yield). Both glycosides **18** and **19** showed pseudomolecular ion peaks at *m/z* 781 $[M+Na]^+$. In the ^{1}H-NMR spectra, a doublet signal due to an anomeric proton of **18** appears at δ 4.47 (J=7.7 Hz) and that of **19** appears at δ 5.18 (J=4.0 Hz).

Glycosidations of **14** with **20**, **17** with **20**, **18** with **21**, and **18** with **22** in the presence of Ag-OTf and TMU gave β-diglycoside derivatives having β(1→2)-linked disaccharides **23**, **24**, **25** and **26** in 58.3, 60.4, 62.6, and 61.9% yields, respectively. All these diglycosides showed the same pseudomolecular ion peak at *m/z* 1111 $[M+Na]^+$. In the ^{1}H-NMR spectra (Table II), **23**, **24**, **25** and **26** exhibited a pair of doublet signals due to anomeric protons at δ 4.44 (J=8.2 Hz) and 4.73 (J=9.1 Hz), 4.43 (J=7.7 Hz) and 4.75 (J=8.1 Hz), 4.50 (J=7.7 Hz) and 4.70 (J=8.9 Hz), and 4.50 (J=7.7 Hz) and 4.66 (J=7.7 Hz), respectively. Deacetylation followed by demethylation of **23**—**26** gave, after purification by column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer) followed by preparative HPLC (solvent system, MeOH : H_2O : AcOH = 64.5 : 35 : 0.5; flow rate, 1.0 ml/min; column temp. 35 °C), diglycosides **27**, **28**, **29** and **30** in 51.8, 50.4, 50.9 and 51.2% yields, respectively. These glycosides **27**—**30** were identified with authentic samples[3] by HPLC and ^{13}C-NMR spectra.

The cytoprotective effects of the glycyrrhetic acid glycosides on CCl_4-induced hepatotoxicity were estimated by assay of aspartate transaminase (AST) and alanine transaminase (ALT) which were released from the injured hepatocytes.[11] ddY male mice weighing about 30 g (6 weeks old) received experimental hepatic injury by i.p. injection with 0.4 ml/kg of 25% CCl_4 in olive oil.[12] Three hours following the i.p. with the CCl_4 solution, the mice were peritoneally injected with 10 mg/kg of glycosides **27**—**33** and glycyrrhizin (**34**) in saline (0.1 ml), and an equal volume of saline as a control. At 9 h after the injection of the glycosides, blood samples were collected through the orbital fossa of the mice and centrifuged at 10000 rpm for 10 min to obtain serum solutions. The activity of AST and ALT in the serum solutions was assayed by the reported procedure[13] as shown in Fig. 1. Glycosides **31**—**33**,[3] constituted of only neutral pyranoses as sugar components in the molecules, released both AST and ALT from the hepatocytes injured with CCl_4, in amounts equal to the control, which suggests that glycosides **31**—**33** have no cytoprotective effect. On the other hand, glycosides **27**—**30**, having one β-D-glcUA in the glycoside molecules, decreased the release of both AST and ALT similarly to that of glycyrrhezin **34**, indicating potent cytoprotective effects. Especially, glycosides **27** and **28** having β-D-glcUA as the terminal sugar component at the C-3 position of the aglycons indicated stronger cytoprotective effects on

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TABLE I. ^{1}H-NMR Spectral Data of Compounds 12—19[a)]

	12	13	14	15
Aglycon[c)]				
H-3	3.17 (dd, 8.9, 6.7)[b)]	3.27 (dd, 10.5, 5.7)	3.18 (dd, 8.4, 8.1)	3.18 (dd, 10.6, 6.2)
H-9	2.30 (s)	2.35 (s)	2.35 (s)	2.31 (s)
H-12	5.66 (s)	5.74 (s)	5.67 (s)	5.66 (s)
H-18	2.82 (br d, 13.3)	2.81 (br d, 13.9)	2.78 (br d, 13.3)	2.81 (br d, 13.5)
OCH_3	3.69	3.70	3.67	3.69
CH_3	0.78, 0.80, 0.93, 1.11, 1.13, 1.14, 1.34	0.81, 0.88, 1.00, 1.05, 1.13, 1.15, 1.36	0.79, 0.83, 1.00, 1.11, 1.13, 1.13, 1.34	0.80, 0.80, 0.94, 1.12, 1.14, 1.14, 1.35
Sugar				
H-1	4.69 (d, 8.0)	5.37 (d, 4.0)	4.40 (d, 7.7)	4.68 (d, 7.9)
H-2	5.11 (dd, 9.6, 8.0)	4.95 (dd, 9.9, 4.0)	3.63 (dd, 9.5, 7.7)	5.30 (dd, 10.6, 7.9)
H-3	5.37 (dd, 9.6, 9.6)	5.58 (dd, 9.9, 9.9)	4.96 (dd, 9.5, 9.5)	5.17 (dd, 10.6, 3.3)
H-4	5.04 (dd, 9.6, 9.6)	5.10 (dd, 9.9, 9.9)	5.11 (dd, 9.5, 9.5)	5.40 (d, 3.3)
H-5	3.75 (ddd, 9.6, 5.6, 2.5)	4.12 (m)	3.67 (m)	3.95 (dd, 7.3, 6.2)
H-6	4.12 (dd, 12.1, 2.5)	4.23 (dd, 11.3, 3.9)	4.05 (dd, 12.1, 2.6)	4.12 (dd, 11.3, 6.2)
H-6′	4.28 (dd, 12.1, 5.6)	4.10 (dd, 11.3, 1.5)	4.24 (dd, 12.1, 5.9)	4.22 (dd, 11.3, 7.3)
$COCH_3$	1.99, 2.02, 2.08	2.01 2.06, 2.11	2.01, 2.04, 2.06	1.97, 2.05, 2.18
OCH_3	—	—	—	—

	16	17	18	19
Aglycon[c)]				
H-3	3.26 (dd, 9.9, 5.9)	3.21 (dd, 8.1, 8.1)	3.21 (dd, 8.2, 8.2)	3.24 (m)
H-9	2.33 (s)	2.35 (s)	2.33 (s)	2.32 (s)
H-12	5.66 (s)	5.67 (s)	5.66 (s)	5.66 (s)
H-18	2.82 (br d, 13.6)	2.81 (br d, 13.6)	2.79 (br d, 12.5)	2.86 (br d, 13.9)
OCH_3	3.69	3.68	3.65 or 3.70	3.69 or 3.74
CH_3	0.80, 0.85, 1.04, 1.12, 1.15, 1.15, 1.35	0.81, 0.87, 1.03, 1.13, 1.15, 1.15, 1.36	0.81, 0.85, 1.02, 1.12, 1.14, 1.16, 1.36	0.81, 0.90, 1.07, 1.13, 1.15, 1.15, 1.35
Sugar				
H-1	5.45 (d, 3.3)	4.40 (d, 7.7)	4.47 (d, 7.7)[d)]	5.18 (d, 4.0)
H-2	5.39 (dd, 10.0, 3.3)	3.85 (dd, 10.3, 7.7)	3.65[d)]	3.69 (overlapped with OCH_3)
H-3	5.21 (dd, 10.0, 3.3)	4.94 (dd, 10.3, 3.7)	5.11—5.21[d)] (H-3, H-4)	5.23 (dd, 9.9, 9.9)
H-4	5.52 (d, 3.3)	5.35 (d, 3.7)		5.10 (dd, 9.9, 9.9)
H-5	4.41 (dd, 7.0, 6.2)	3.89 (dd, 7.3, 7.3)	4.01[d)]	4.40 (d, 9.9)
H-6	4.13 (dd, 11.0, 7.0)	4.08 (dd, 11.2, 7.3)	—	—
H-6′	4.13 (dd, 11.0, 6.2)	4.19 (dd, 11.2, 7.3)	—	—
$COCH_3$	1.98, 2.05, 2.17	2.03, 2.04, 2.17	2.02, 2.08	2.04, 2.10
OCH_3	—	—	3.65 or 3.70	3.69 or 3.74

a) The signal assignments were based on a decoupling method. *b*) Coupling constants (*J* in Hz) are given in parentheses. *c*) Only assignable protons on the aglycons are listed. *d*) Virtual long-range spin–spin couplings among these protons are observed.[15,16)]

CCl_4-induced hepatic injury. In these *in vivo* experiments, it is suggested that the β-D-glcUA in the glycyrrhetic acid glycosides is essential for a cytoprotective effect and the terminal β-D-glcUA seems to be more important.

Experimental

Materials Methyl glycyrrhetinate was prepared by the methylation of glycyrrhetic acid according to the published method.[14)] Dry dichloromethane (CH_2Cl_2) was obtained by refluxation with NaH, followed by distillation. 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 with dilute H_2SO_4, followed by heating at 80 °C for 10 min. Column chromatography was carried out on Wakogel C-200. An SSC-6300 (Senshu Scientific Co., Ltd.) equipped with an SSC-3000 A was employed for analytical HPLC using ODS-1251-D (4.6 mm × 250 mm), and it was further equipped with an SSC autoinjector 6310 and an SSC fraction collector 6320 for preparative HPLC using ODS-4251-D (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 were given in ppm with tetramethylsilane as an internal standard. FAB-MS spectra were recorded on a JEOL JMS-DX 300 mass spectrometer. The activities of AST and ALT were assayed by the autoanalyzer COBAS MRA (Roche) using commercial kits based on the principal of the AST and ALT assay method.

Glycosidation of 5 with 9 To a solution of **5** (5.0 g) and **9** (7.5 g) in dry CH_2Cl_2 (100 ml), Ag-OTf (4.3 g) and TMU (2.0 ml) were added, and the mixture was stirred under shielding from light for 20 h at room temperature. The reaction mixture was filtered, and the filtrate was poured into ice-water (300 ml) and extracted with CH_2Cl_2 (100 ml × 3). The combined organic extracts were successively washed with $NaHCO_3$-saturated aqueous solution 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 1.5%) to give β-monoglycoside **12** (3.91 g, 41.3%) and α-monoglycoside **13** (2.82 g, 29.8%). FAB-MS of **12** *m/z*: 939 [M+Na]$^+$. *Anal.* Calcd for $C_{45}H_{65}Cl_3O_{13}$: C, 58.85; H, 6.92. Found: C, 58.82; H, 6.97. FAB-MS of **13** *m/z*: 939 [M+Na]$^+$. *Anal.* Calcd for $C_{45}H_{63}Cl_3O_{13}$: C, 58.85; H, 6.92. Found: 58.77; H, 7.01.

Treatment of 12 with Ammonia Compound **12** (3.5 g) was added to ammonia-saturated ether (100 ml) at 0 °C. The mixture was vigorously shaken for 10 min at 0 °C, suctioned until the solution had no remaining odor of ammonia, and evaporated to give a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 4.5%) to give compound **14** (2.83 g, 95.5%). FAB-MS of **14** *m/z*: 795 [M+Na]$^+$. *Anal.* Calcd for $C_{43}H_{64}O_{12}$: C, 66.82; H, 8.35. Found C, 66.79; H, 8.39.

Glycosidation of 5 with 10 To a solution of **5** (5.0 g) and **10** (9.5 g) in dry CH_2Cl_2 (100 ml), Ag-OTf (4.3 g) and TMU (2.0 ml) were added. The mixture was stirred under shielding from light for 18 h at room temperature. The reaction mixture was treated according to the

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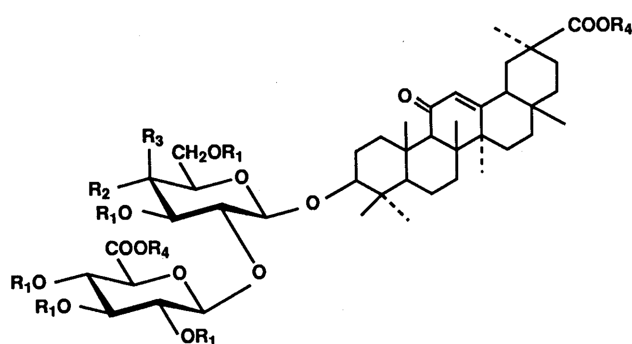
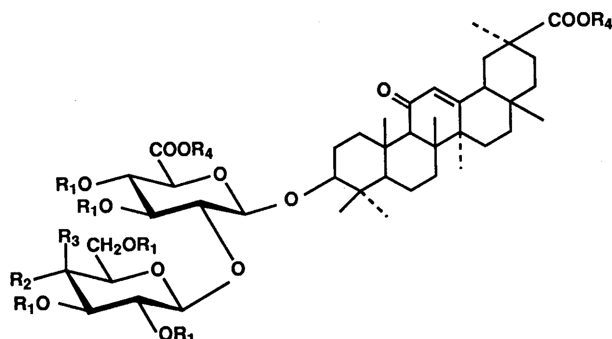


23 : R_1=Ac; R_2=OAc; R_3=H; R_4=CH_3
24 : R_1=Ac; R_2=H; R_3=OAc; R_4=CH_3

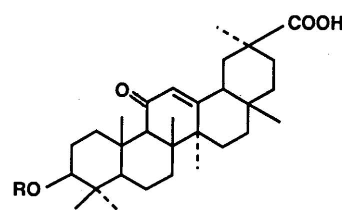


25 : R_1=Ac; R_2=OAc; R_3=H; R_4=CH_3
26 : R_1=Ac; R_2=H; R_3=OAc; R_4=CH_3

Chart 3

glycosidation of **5** with **9** to give a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 1.5%) to give β-monoglycoside **15** (4.09 g, 43.2%) and α-monoglycoside **16** (2.51 g, 26.5%). FAB-MS of **15** *m/z*: 939 $[M+Na]^+$. *Anal.* Calcd for $C_{45}H_{63}Cl_3O_{13}$: C, 58.85; H, 6.92. Found: C, 58.82; H, 6.93. FAB-MS of **16** *m/z*: 939 $[M+Na]^+$. *Anal.* Calcd for $C_{45}H_{63}Cl_3O_{13}$: C, 58.85; H, 6.92. Found: C, 58.57; H, 7.11.

Treatment of 15 with Ammonia Compound **15** (4.0 g) was added to ammonia-saturated ether (250 ml) at 0 °C. The mixture was vigorously shaken for 10 min at 0 °C, and suctioned until the solution had no remaining odor of ammonia. The mixture was evaporated to give a residue which was subjected to column chromatography (gradient elution with



	R		R
27	-glc$\overset{2\beta}{----}$glcUA	31	-glc$\overset{2\beta}{----}$glc
28	-gal$\overset{2\beta}{----}$glcUA	32	-gal$\overset{2\beta}{----}$glc
29	-glcUA$\overset{2\beta}{----}$glc	33	-gal$\overset{2\beta}{----}$gal
30	-glcUA$\overset{2\beta}{----}$gal	34	-glcUA$\overset{2\beta}{----}$glcUA

glc : β-D-glucopyranose gal : β-D-galactopyranose
glcUA : β-D-glucuronopyranose

Chart 4

TABLE II. ^{1}H-NMR Spectral Data of Compounds **23**—**26**[a)]

	23	**24**	**25**	**26**
Aglycon[b)]				
H-3	3.11 (dd, 9.1, 9.1)[c)]	3.12 (dd, 6.8, 6.6)	3.14 (dd, 7.8, 7.6)	3.13 (dd, 7.7, 7.7)
H-9	2.34 (s)	2.32 (s)	2.31 (s)	2.32 (s)
H-12	5.66 (s)	5.66 (s)	5.66 (s)	5.66 (s)
H-18	2.78 (br d, 13.6)	2.68 (br d, 13.5)	2.77 (br d, 11.6)	2.79 (br d, 13.2)
OCH_3	3.69 or 3.71	3.69 or 3.71	3.69 or 3.71	3.69 or 3.75
CH_3	0.81, 0.84, 1.04, 1.12, 1.14, 1.15, 1.36	0.81, 0.86, 1.04, 1.12, 1.12, 1.15, 1.36	0.81, 0.82, 1.04, 1.12, 1.12, 1.15, 1.36	0.81, 0.86, 1.09, 1.12, 1.13, 1.15, 1.36
Inner sugar				
H-1	4.44 (d, 8.2)	4.43 (d, 7.7)	4.50 (d, 7.7)	4.50 (d, 7.7)
H-2	3.81 (dd, 10.0, 8.2)	3.95 (dd, 9.9, 7.7)	3.84 (dd, 9.4, 7.7)	3.87 (dd, 9.5, 7.7)
H-3	5.16 (dd, 10.0, 10.0)	4.95 (dd, 9.9, 7.7)	5.21 (dd, 9.4, 9.4)	5.22 (dd, 9.5, 9.5)
H-4	4.89 (dd, 10.0, 10.0)	5.27 (d, 3.3)	5.11 (dd, 9.4, 9.4)	5.11 (dd, 9.5, 9.5)
H-5	3.69 (overlapped with OCH_3)	3.85 (dd, 6.9, 6.9)	4.00 (d, 9.4)	4.00 (d, 9.5)
H-6	4.05 (dd, 12.1, 5.9)	4.10 (dd, 11.0, 6.9)	—	—
H-6'	4.25 (dd, 12.1, 1.1)	4.16 (dd, 11.0, 6.9)	—	—
Outer sugar				
H-1	4.73 (d, 9.1)	4.75 (d, 8.1)	4.70 (d, 8.9)	4.66 (d, 7.7)
H-2	4.92 (dd, 9.1, 9.1)	4.92 (dd, 8.1, 8.1)	4.91 (dd, 8.9, 8.9)	5.10 (dd, 10.3, 7.7)
H-3	5.14 (dd, 9.1, 9.1)	5.19 (dd, 9.5, 8.1)	5.12 (dd, 8.9, 8.9)	4.93 (dd, 10.3, 3.3)
H-4	5.23 (dd, 9.1, 9.1)	5.16 (dd, 9.5, 9.5)	5.04 (dd, 8.9, 8.9)	5.33 (d, 3.3)
H-5	3.98 (d, 9.1)	4.10 (d, 9.5)	3.67 (m)	3.87 (m)
H-6	—	—	4.05 (dd, 12.4, 2.2)	4.06 (dd, 8.8, 3.7)
H-6'	—	—	4.24 (dd, 12.4, 5.0)	4.13 (dd, 8.8, 5.1)
OCH_3	3.69 or 3.71	3.69 or 3.71	3.69 or 3.71	3.69 or 3.75
$COCH_3$	2.00, 2.00, 2.02, 2.02, 2.05, 2.10	1.98, 2.00, 2.00, 2.02, 2.07, 2.15	1.99, 2.00, 2.02, 2.03, 2.08, 2.11	1.97, 2.00, 2.04, 2.06, 2.10, 2.13

a) The signal assignments were based on decoupling and ^{1}H–^{13}C-COSY methods. *b*) Only assignable protons on the aglycons are listed. *c*) Coupling constants (*J* in Hz) are given in parentheses.

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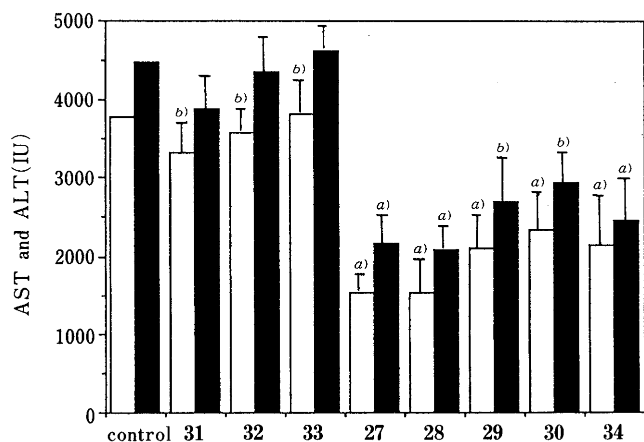

Fig. 1. Cytoprotective Effects of Glycyrrhetic Acid Glycosides on CCl_4-Induced Hepatic Injury *in Vivo*

Both AST and ALT were assayed as described in text. Blank bars indicate AST activities and closed ones ALT activities. Significantly different from the control: *a*) $p<0.01$, *b*) $p<0.05$.

benzene–acetone up to 5%) to give compound **17** (3.26 g, 97.1%). FAB-MS of **17** *m*/*z*: 795 $[M+Na]^+$. *Anal.* Calcd for $C_{43}H_{64}O_{12}$: C, 66.82; H, 8.35. Found: C, 66.56; H, 8.47.

Glycosidation of 5 with 11 Followed by Treatment with Ammonia To a solution of **5** (7.5 g) and **11** (14.3 g) in dry CH_2Cl_2 (100 ml), Ag-OTf (6.4 g) and TMU (3.0 ml) were added, and the mixture was stirred under shielding from light for 20 h at room temperature. The reaction mixture was treated according to the glycosidation of **5** with **9** to afford a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 3%) to a mixture (9.1 g) of α- and β-glycosides. The mixture was dissolved in ammonia-saturated ether (350 ml) and vigorously shaken for 10 min at 0 °C. The reaction mixture was treated according to the preparative method of **17** to afford a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 5%) to give compounds **18** (4.9 g, 41.7%) and **19** (3.1 g, 26.4%). FAB-MS of **18** *m*/*z*: 781 $[M+Na]^+$. *Anal.* Calcd for $C_{42}H_{62}O_{12}$: C, 66.47; H, 8.23. Found: C, 66.19; H, 8.33. FAB-MS of **19** *m*/*z*: 781 $[M+Na]^+$. *Anal.* Calcd for $C_{42}H_{62}O_{12}$: C, 66.47; H, 8.23. Found: C, 66.42; H, 8.25.

Glycosidation of 14 with 20 To a solution of **14** (2.1 g) and **20** (4.5 g) in dry CH_2Cl_2 (60 ml), Ag-OTf (1.4 g) and TMU (0.6 ml) were added, and the mixture was stirred under shielding from light for 5 h at room temperature. The reaction mixture was filtered, and the filtrate was poured into ice-water (200 ml) and extracted with CH_2Cl_2 (100 ml × 3). The combined organic extracts were successively washed with a $NaHCO_3$-saturated aqueous solution and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to afford a residue which was subjected to column chromatography (gradient elution with benzene–acetone up to 3%) to give compound **23** (1.75 g, 58.3%). FAB-MS of **23** *m*/*z*: 1111 $[M+Na]^+$. *Anal.* Calcd for $C_{56}H_{80}O_{21}$: C, 61.75; H, 7.40. Found: C, 61.59; H, 7.51.

Glycosidation of 17 with 20 To a solution of **17** (1.5 g) and **20** (3.1 g) in dry CH_2Cl_2 (60 ml), Ag-OTf (1.0 g) and TMU (0.46 ml) were added, and the mixture was stirred under shielding from light for 4 h at room temperature. The reaction mixture was treated according to the preparative method of **23** to afford a residue which was subjected to column chromatography (gradient elution with benzene–acetone, gradient up to 3%) to give compound **24** (1.8 g, 69.8%). FAB-MS of **24** *m*/*z*: 1111 $[M+Na]^+$. *Anal.* Calcd for $C_{56}H_{80}O_{21}$: C, 61.75; H, 7.40. Found: C, 61.69; H, 7.48.

Glycosidation of 18 with 21 To a solution of **18** (3.6 g) and **21** (7.8 g) in dry CH_2Cl_2 (100 ml), Ag-OTf (2.4 g) and TMU (1.1 ml) were added, and the mixture was stirred under shielding from light for 4 h at room temperature. The reaction mixture was treated according to the preparative method of **23** to afford a residue. The residue was subjected to column chromatography (gradient elution with benzene–acetone up to 3%) to give compound **25** (3.2 g, 63.1%). FAB-MS of **25** *m*/*z*: 1111 $[M+Na]^+$. *Anal.* Calcd for $C_{56}H_{80}O_{21}$: C, 61.75; H, 7.40. Found: C, 61.39; H, 7.53.

Glycosidation of 18 with 22 To a solution of **18** (3.0 g) and **22** (6.5 g) in dry CH_2Cl_2 (120 ml), Ag-OTf (1.9 g) and TMU (1.0 ml) with added, and the mixture was stirred under shielding from light for 5 h at room temperature. The reaction mixture was treated according to the preparative method of **23** to afford a residue. Product **26** (2.6 g, 61.5%) was isolated by column chromatography (gradient elution with benzene–acetone up to 3%). FAB-MS of **26** *m*/*z*: 1111 $[M+Na]^+$. *Anal.* Calcd for $C_{56}H_{80}O_{21}$: C, 61.75; H, 7.40. Found: C, 61.62; H, 7.45.

Removal of the Protecting Groups of 23 A solution of **23** (1.4 g) in ammonia-saturated MeOH (45 ml) was allowed to stand overnight at room temperature. The reaction mixture was evaporated to give a residue. The residue was dissolved in 5% KOH in EtOH–H_2O (1 : 1, 20 ml), and the solution was heated under reflux for 3 h. The reaction mixture was evaporated to afford a residue. The residue was subjected to column chromatography ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer) followed by application of preparative HPLC (solvent system, MeOH : H_2O : AcOH = 64.5 : 35 : 0.5; flow rate, 1.0 ml/min; column temp. 35 °C) to give compound **27** (540 mg, 51.8%). FAB-MS of **27** *m*/*z*: 831 $[M+Na]^+$. This product was identified with the authentic sample by HPLC and ^{13}C-NMR spectrum.

The Removal of the Protecting Groups of 24—26 The removal of the protecting groups of **24**—**26** was performed by the same method as that of **23** to obtain products **28** (51.8%), **29** (50.9%) and **30** (56.2%), respectively. All these products were also identified with the authentic samples by HPLC and ^{13}C-NMR spectra.

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