Preparation of Glycyrrhetic Acid Glycosides Having Various $\beta(1\rightarrow2)$ -Linked Disaccharides and Their Cytoprotective Effects on Carbon Tetrachloride-Induced Hepatic Injury

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Glycyrrhetic acid glycosides (1–7) having $\beta(1\rightarrow2)$ -linked disaccharides such as 2-*O*- β -D-glucopyranosyl- β -D-galactopyranose, 2-*O*- β -D-galactopyranosyl- β -D-galactopyranose, 2-*O*- β -D-glucopyranosyl- β -D-glucuronopyranose, 2-*O*- β -D-galactopyranosyl- β -D-glucuronopyranose, 2-*O*- β -D-glucopyranosyl- β -D-glucopyranose, 2-*O*- β -D-glucuronopyranosyl- β -D-glucopyranose, respectively, were synthesized by stepwise construction; from glycyrrhetic acid monoglycosides to the diglycosides. The cytoprotective activities of the glycosides 1–7 and 2-*O*-(β -D-glucopyranosyl)- β -D-glucopyranosyl-11-oxoolean-12-en-30-oate (8) were compared with natural occurring glycyrrhizin (9). Among these glycosides 1–8, glycosides 3 and 7 having β -D-glucuronopyranose (glcUA) as the only terminal sugar component were more effective materials against hepatic injury than glycyrrhizin 9.

Keywords glycyrrhetic acid glycoside; glycyrrhizin; cytoprotective effect; carbon tetrachloride-induced hepatotoxicity; alanine transaminase activity; aspartate transaminase activity

Naturally occurring saponins (steroidal and triterpenoidal glycosides) isolated from plant sources have various pharmacological and biological activities.^{1,2)} The biological activities was presumed to depend not only on the structure of aglycons but on the variety, number, conformation, and linking manner of sugars in the saponin molecules.²⁾

In this paper, we will report on the preparation of glycyrrhetic acid β -glycosides (1–7) having various $\beta(1\rightarrow2)$ -linked disaccharides made up of a combination of D-glucopyranose, D-galactopyranose and D-glucuronopyranose. The comparison of the cytoprotective effects on carbon tetrachloride (CCl₄)-induced hepatic injury of the synthetic saponins 1–7 and 8³⁾ with glycyrrhizin (9) are also reported.

It has been reported that the glycosidation of 1-methyl cyclohexanol with $\beta(1\rightarrow2)$ -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 (10) gave only α -glycoside.⁴⁾ The same result was also obtained by the reaction of bromide 10 with methyl glycyrrhetinate (11) to give α -glycoside,³⁾ which was thought that because of the absence of an acetyl group at C-2 of compound 10, the anomeric carbon of 10 was unsusceptible to neighboring-group participation with the acetyl group. Thus, as it is thought that the glycosidation of 11 with the

$\beta(1\rightarrow2)$ -linked disaccharides affords undesirable α -glycosides, the strategy of stepwise construction from monosaccharide glycosides to disaccharide glycosides is performed for the preparation of desirable β -glycosides. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-glucopyranose (12) and -D-galactopyranose (13) used for the first step of glycosidation with 11 have already been made available.^{5,6)} Other methyl D-glucuronatopyranose derivatives (14) and (15) were obtained as follows: Methyl 2,3,4-tri-*O*-acetyl- α -D-glucuronatopyranosyl bromide (16)⁷⁾ was reacted with tetra-*n*-butylammonium bromide and ethanol⁸⁾ to give 1,2-orthoacetate (17) (mixture of *exo*- and *endo*-isomers). Treatment of 17 with ammonia followed by benzylation^{5,9)} gave benzylated orthoacetate (18), which was treated with 95% aqueous acetic acid¹⁰⁾ to afford 14. Acetylation of 14 gave diacetate (15) which showed the presence of two acetyl

	R		R
1	-gal- ² -glc	6	-glc- ² -gal
2	-gal- ² -gal	7	-glc- ² -glcUA
3	-gal- ² -glcUA	8	-glc- ² -glc
4	-glcUA- ² -glc	9	-glcUA- ² -glcUA
5	-glcUA- ² -gal		

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

Chart 1

Chart 2

groups (δ 1.95 and 2.06) and three benzyl groups, and the absence of OCH_3 group in the proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectrum.

Glycosidation of aglycon **11** with **13** performed in dichloromethane in the presence of cobalt bromide and trimethylsilyl chloride¹¹⁾ gave β -glycoside (**19**, 31.5%) and α -glycoside (**20**, 19%). The $^1\text{H-NMR}$ spectra of **19** and **20** showed the anomeric protons at δ 4.35 (d, $J=8.0$ Hz) and 5.19 (d, $J=3.7$ Hz), respectively. Because of the slight solubility of compound **14** in dichloromethane, the glycosidation of **11** with **15** obtained from **14** was carried out in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate¹²⁾ to give β -glycoside (**21**, 16%) and α -glycoside (**22**, 29%). The $^1\text{H-NMR}$ spectra of **21** and **22** showed the anomeric protons at δ 4.53 (d, $J=7.5$ Hz) and 5.19 (d, $J=3.6$ Hz), respectively. Compounds **19** and **21** were treated with 1.5*N* NaOMe–MeOH to obtain deacetylated products (**23**) and (**24**), respectively. In the $^1\text{H-NMR}$ spectra of **23** and **24**, the signals of acetyl groups (δ 2.02 in **19** and 1.93 in **21**) disappeared.

These glycosides **23** and **24** and the reported one **25**³⁾ were further reacted with acetylated sugar bromide **26**, **27** or **16** to produce the glycosides having various $\beta(1\rightarrow2)$ -linked disaccharides. The reaction of **23** with bromide **26** in dichloromethane in the presence of silver trifluoromethanesulfonate and 1,1,3,3-tetramethyl urea¹³⁾ gave a mixture which was chromatographed to obtain a major product (85%) showing a single spot on thin layer chromatography (TLC) (benzene–acetone, 9:1; benzene–AcOEt, 4:1). The $^1\text{H-NMR}$ spectrum of the product, however, showed that it was still contaminated with a small amount of impurity. The pure compound **28** was isolated in the yield of 53% by preparative high-performance liquid chromatography (HPLC) (solvent c). The $^1\text{H-NMR}$ spectrum of **28** (Table I) showed the two anomeric protons

at δ 4.30 (d, $J=7.7$ Hz, one of the inner glycosidic linkage) and 5.10 (d, $J=8.1$ Hz, another of the terminal glycosidic linkage), which suggests that both sugar molecules in **28** arrange in β -configuration. The glycosidations of **23** with **27** and **16** under the same reaction condition gave, after purification by column chromatography followed by preparative HPLC, products (**29**) and (**30**), respectively. Similarly the glycosidation of **24** with **26** and **27** gave products (**31**) and (**32**), respectively, and that of **25** with **27** and **16** afforded products (**33**) and (**34**), respectively. In the $^1\text{H-NMR}$ spectra of **29**–**34** (Table I), all anomeric protons showed the coupling constants of 7.7–8.1 Hz, which indicated that both sugar molecules in the products arranged in β -configuration as well as those in **28**. Removal of the protecting groups of compound **28** was performed successively by treatment with NaOMe in MeOH, hydrogenation, and treatment 5% NaOH in H_2O –EtOH (1:1). The resulting reaction mixture was chromatographed on silica gel to give the product. Although the product showed a clean single spot on TLC (CHCl_3 –MeOH– H_2O , 65:35:10, lower layer), two peaks appeared at the retention times of 52.4 and 56.7 min on HPLC (ODS-1251-D, solvent e, flow rate 0.2 ml/min, 35 °C). The major product **1**, having a retention time of 52.4 min on HPLC, was isolated by preparative HPLC. Similarly, the removal of the protecting groups of **29**–**34** gave, after purification by column chromatography followed by preparative HPLC, products (**2**–**7**), respectively.

Beaton and Spring¹⁴⁾ reported that the reaction of 18 β -glycyrrhetic acid (**35**) with alkali and strong acid gave 18 α -glycyrrhetic acid (**36**). In order to elucidate the configuration of C-18 on the aglycons, glycosides **1**–**7** were methanolized and treated with diazomethane. The all resulting methylated aglycons were identified with authentic **11** by analytical HPLC. This result suggests that the aglycons of **1**–**7** are 18 β -glycyrrhetic acid. The $^{13}\text{C-NMR}$ spectra of **1**–**7** are listed together with those of **8** and **9** in Table II.

The cytoprotective effects on CCl_4 -induced hepatotoxicity with glycyrrhetic acid glycosides **1**–**9** were estimated by assay of aspartate transaminase (AST) and alanine transaminase (ALT) which were released from CCl_4 -treated

- 19** : $\text{R}_1=\text{H}$, $\text{R}_2=\text{OBn}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_4=\text{Ac}$
21 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{H}$, $\text{R}_3=\text{COOBn}$, $\text{R}_4=\text{Ac}$
23 : $\text{R}_1=\text{H}$, $\text{R}_2=\text{OBn}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_4=\text{H}$
24 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{H}$, $\text{R}_3=\text{COOBn}$, $\text{R}_4=\text{H}$
25 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{H}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_4=\text{H}$

- 20** : $\text{R}_1=\text{H}$, $\text{R}_2=\text{OBn}$, $\text{R}_3=\text{CH}_2\text{OBn}$
22 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{H}$, $\text{R}_3=\text{COOBn}$

Chart 3

- 28** : $\text{R}_1=\text{R}_5=\text{H}$, $\text{R}_2=\text{OBn}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_4=\text{OAc}$, $\text{R}_6=\text{CH}_2\text{OAc}$
29 : $\text{R}_1=\text{R}_4=\text{H}$, $\text{R}_2=\text{OBn}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_5=\text{OAc}$, $\text{R}_6=\text{CH}_2\text{OAc}$
30 : $\text{R}_1=\text{R}_5=\text{H}$, $\text{R}_2=\text{OBn}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_4=\text{OAc}$, $\text{R}_6=\text{COOCH}_3$
31 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{R}_5=\text{H}$, $\text{R}_3=\text{COOBn}$, $\text{R}_4=\text{OAc}$, $\text{R}_6=\text{CH}_2\text{OAc}$
32 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{R}_4=\text{H}$, $\text{R}_3=\text{COOBn}$, $\text{R}_5=\text{OAc}$, $\text{R}_6=\text{CH}_2\text{OAc}$
33 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{R}_4=\text{H}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_5=\text{OAc}$, $\text{R}_6=\text{CH}_2\text{OAc}$
34 : $\text{R}_1=\text{OBn}$, $\text{R}_2=\text{R}_5=\text{H}$, $\text{R}_3=\text{CH}_2\text{OBn}$, $\text{R}_4=\text{OAc}$, $\text{R}_6=\text{COOCH}_3$

Chart 4

TABLE I. ¹H-NMR Spectra of Compounds 28–34^{a)}

	28	29	30	31
CH ₃	0.81, 0.83, 1.08, 1.12, 1.13, 1.15, 1.34	0.86, 1.11, 1.12, 1.13, 1.18, 1.20, 1.32	0.80, 0.83, 1.05, 1.11, 1.13, 1.15, 1.34	0.80, 0.81, 1.07, 1.10, 1.12, 1.15, 1.36
OCH ₃	3.69	3.66	3.69, 3.70	3.69
Aromatic protons	7.24—7.43 (15H)	7.15—7.35 (15H)	7.23—7.45 (15H)	6.79 (1H), 7.22—7.41 (14H)
—CH ₂ —	4.37 (2H, s)	4.49 and 4.75 (2H, ABq, 12.1)	4.38 (2H, s)	4.42 and 4.46 (2H, ABq, 13.9)
	4.51 and 4.80 (2H, ABq, 12.1)	4.56 and 4.99 (2H, ABq, 10.3)	4.58 (2H, s)	4.56 and 4.70 (2H, ABq, 10.3)
	4.57 and 4.63 (2H, ABq, 11.0)	4.71 and 4.75 (2H, ABq, 5.9)	4.51 and 4.79 (2H, ABq, 11.7)	4.62 and 4.85 (2H, ABq, 10.3)
H-3	3.07 (dd, 10.3, 6.2)	3.06 (dd, 10.4, 6.3)	3.06 (dd, 8.3, 6.2)	3.08 (dd, 9.1, 6.3)
H-9	2.27 (s)	2.28 (s)	2.29 (s)	2.31 (s)
H-12	5.66 (s)	5.66 (s)	5.66 (s)	5.68 (s)
H-18	2.75 (br d, 13.2)	2.77 (br d, 13.2)	2.65 (br d, 13.1)	2.80 (br d, 13.1)
H-1'	4.30 (d, 7.7)	4.31 (d, 7.7)	4.30 (d, 7.7)	4.47 (d, 7.7)
H-2'	4.08 (dd, 9.9, 7.7)	3.77 (dd, 8.5, 7.7)	4.08 (dd, 10.3, 7.7)	3.77 (dd, 9.2, 7.7)
H-3'	3.45 (dd, 9.9, 2.7)	{ 3.5—3.6	3.44 (dd, 10.3, 2.9)	{ 3.57—3.66
H-4'	3.76 (d, 2.7)		3.77 (d, 2.9)	
H-5'	{ 3.42—3.52	3.40 (m)	{ 3.43—3.52	3.97 (d, 9.5)
H-6' _a		{ 3.5—3.6		—
H-6' _b			—	
H-1''	5.10 (d, 8.1)	5.00 (d, 7.7)	5.11 (d, 8.1)	4.93 (d, 7.7)
H-2''	5.00 (dd, 8.4, 8.1)	5.18 (dd, 10.6, 7.7)	5.01 (dd, 9.8, 8.1)	4.97 (dd, 8.3, 7.7)
H-3''	5.17 (t, 8.4)	4.92 (dd, 10.6, 3.3)	5.17 (t, 9.0)	5.07 (t, 8.3)
H-4''	5.18 (t, 8.4)	5.30 (d, 3.3)	5.23 (t, 9.0)	5.09 (t, 8.3)
H-5''	3.69 (m)	3.77 (m)	4.00 (d, 9.0)	3.52 (m)
H-6'' _a	4.05 (dd, 11.5, 2.9)	4.03 (dd, 11.0, 6.5)	—	4.02 (dd, 12.1, 1.3)
H-6'' _b	4.26 (dd, 11.5, 4.0)	4.08 (dd, 11.0, 6.4)	—	4.22 (dd, 12.1, 3.6)
Ac	1.97, 2.00, 2.01, 2.10	1.95, 2.00, 2.02, 2.09	1.99, 1.99, 2.01	2.00, 2.00, 2.01, 2.03

	32	33	34
CH ₃	0.81, 0.85, 1.12, 1.12, 1.12, 1.15, 1.36	0.81, 0.89, 1.14, 1.14, 1.26, 1.26, 1.35	0.81, 0.86, 1.08, 1.13, 1.15, 1.15, 1.35
OCH ₃	3.70	3.70	3.69, 3.69
Aromatic protons	6.78 (1H), 7.22—7.41 (14H)	7.29—7.40 (15H)	7.21—7.41 (15H)
—CH ₂ —	4.40 and 4.51 (2H, ABq, 14.6)	4.58 and 4.86 (2H, ABq, 10.3)	4.53 and 4.59 (2H, ABq, 11.7)
	4.55 and 4.69 (2H, ABq, 10.3)	4.57 (2H, s)	4.59 and 4.88 (2H, ABq, 10.3)
	4.62 and 4.85 (2H, ABq, 10.3)	4.76 and 4.88 (2H, ABq, 10.3)	4.74 and 4.77 (2H, ABq, 11.4)
H-3	3.10 (dd, 10.6, 5.9)	3.11 (dd, 10.5, 6.1)	3.09 (dd, 10.6, 5.9)
H-9	2.32 (s)	2.32 (s)	2.31 (s)
H-12	5.68 (s)	5.68 (s)	5.67 (s)
H-18	2.77 (br d, 13.6)	2.81 (br d, 13.0)	2.80 (br d, 13.6)
H-1'	4.47 (d, 7.7)	4.31 (d, 7.7)	4.34 (d, 7.7)
H-2'	3.76 (dd, 9.5, 7.7)	3.87 (dd, 9.1, 7.7)	3.78 (t, 7.7)
H-3'	{ 3.57—3.66	{ 3.51—3.60	{ 3.50—3.65
H-4'			
H-5'	3.95 (d, 8.4)	3.44 (m)	3.43 (m)
H-6' _a	—	{ 3.51—3.60	{ 3.50—3.65
H-6' _b	—		
H-1''	4.92 (d, 7.7)	5.03 (d, 8.1)	5.09 (d, 7.7)
H-2''	5.17 (dd, 10.3, 7.7)	5.20 (dd, 10.3, 8.1)	4.99 (dd, 8.4, 7.7)
H-3''	4.92 (dd, 10.3, 2.6)	4.95 (dd, 10.3, 3.3)	5.13 (t, 8.4)
H-4''	5.33 (d, 2.6)	5.34 (d, 3.3)	5.23 (dd, 9.6, 8.4)
H-5''	3.76 (m)	3.71 (m)	3.89 (d, 9.6)
H-6'' _a	4.05 (dd, 11.0, 6.2)	4.07 (dd, 12.4, 5.5)	—
H-6'' _b	4.11 (dd, 11.0, 7.7)	4.13 (dd, 12.4, 5.5)	—
Ac	1.98, 2.01, 2.02, 2.13	1.99, 2.03, 2.05, 2.14	2.00, 2.01, 2.04

a) Coupling constants are given in hertz (Hz).

hepatocytes. The reaction suspensions for assay were composed of hepatocytes (2×10^6 cells) and glycosides (6.3×10^{-7} M or 12.6×10^{-7} M) in Hanks solution (total 1.1 ml), and the suspension for control (C) composed of only hepatocytes (2×10^6 cells) in Hanks solution (total 1.1 ml). The suspensions were exposed to vapor of CCl₄ 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 procedure.¹⁵⁾ In the case of lower concentration (Fig. 1), glycosides **3**, **4**, **5**, **7** and **9** having one or two β-D-glucuronopyranoses (glcUA) in the glycoside molecules decreased the releasing of both AST and ALT from hepatocytes injured with CCl₄, which showed a cytoprotective effect. However, glycosides **1**, **2**, **6** and **8** having no gluUA in the molecules showed no effect. At a higher concentration of glycosides (Fig. 2), glycosides **4** and **9** which have gluUA as the inner sugar component released much

TABLE II. ^{13}C -NMR Chemical Shifts of Saponins 1–9 in $\text{C}_5\text{D}_5\text{N}^a$

	1	2	3	4	5	6	7	8	9
C-3	88.5	88.6	88.7	88.9	86.1	88.5	88.9	88.7	88.7
C-5	55.3	55.4	55.4	55.3	55.2	55.3	55.4	55.3	53.6
C-9	61.9	60.9	61.9 ^{b)}	62.0	62.0	61.0	62.0	60.8	60.4
C-11	199.4	199.4	199.3	199.3	199.4	199.2	199.5	199.2	200.2
C-12	128.5	128.5	128.5	128.5	128.6	128.4	128.6	128.5	126.4
C-13	169.5	169.5	169.3	169.5	169.4	169.2	169.6	169.9	170.5
C-30	179.0	180.8	178.9	179.0	179.0	179.4	179.1	180.7	179.6
C-1'	105.1	104.9	105.0	105.1	105.3	104.6	104.7	104.7	101.5
C-2'	81.9	84.1	83.4	82.7	82.4	83.8	84.3	83.1	77.2
C-3'	76.1	77.6	78.0	76.9	76.5 ^{b)}	77.8 ^{b)}	78.1 ^{b)}	77.8 ^{b)}	75.0
C-4'	69.7	71.3	69.6	72.9	72.2	71.1	71.3	71.2 ^{c)}	71.2 ^{b)}
C-5'	76.7	78.0	77.5	78.1	78.4	77.3 ^{b)}	78.0 ^{b)}	77.7 ^{b)}	74.3
C-6'	62.0 ^{b)}	62.6	62.0 ^{b)}	172.2	172.0	62.4	62.6	62.0	174.4
C-1''	105.1	106.9	106.7	105.9	106.2	106.6	106.3	105.7	102.5
C-2''	75.1	74.5 ^{b)}	76.1	77.2	74.4	74.2 ^{c)}	76.5	76.7	73.7
C-3''	77.7	74.7 ^{b)}	76.4	77.7 ^{b)}	74.9	74.4 ^{c)}	77.5	77.9 ^{b)}	75.8
C-4''	71.4	69.3	72.9	71.5	70.1	69.1	73.2	71.4 ^{c)}	71.1 ^{b)}
C-5''	77.7	76.6	75.2	77.5 ^{b)}	76.8 ^{b)}	76.4	77.9 ^{b)}	77.5 ^{b)}	75.6
C-6''	62.5 ^{b)}	61.1	171.8	62.6	61.2	61.9	172.6	62.5	174.4

a) Characteristic values for the carbons of aglycons were only listed. b, c) These values may be interchangeable in each column.

TABLE III. Elemental Analyses

Compound	Formula	Calcd (Found)	
		C	H
1	$\text{C}_{42}\text{H}_{66}\text{O}_{14} \cdot \text{H}_2\text{O}$	62.05 (61.78)	8.43 (8.49)
2	$\text{C}_{42}\text{H}_{66}\text{O}_{14} \cdot \text{H}_2\text{O}$	62.05 (61.83)	8.43 (8.45)
3	$\text{C}_{42}\text{H}_{64}\text{O}_{15} \cdot \text{H}_2\text{O}$	61.00 (60.58)	8.04 (7.97)
4	$\text{C}_{42}\text{H}_{64}\text{O}_{15} \cdot \text{H}_2\text{O}$	61.00 (60.77)	8.04 (8.13)
5	$\text{C}_{42}\text{H}_{64}\text{O}_{15} \cdot \text{H}_2\text{O}$	61.00 (60.95)	8.04 (8.06)
6	$\text{C}_{42}\text{H}_{66}\text{O}_{14} \cdot 1/2\text{H}_2\text{O}$	62.74 (62.75)	8.40 (8.47)
7	$\text{C}_{42}\text{H}_{64}\text{O}_{15} \cdot \text{H}_2\text{O}$	61.00 (60.84)	8.04 (8.21)

Fig. 1. Cytoprotective Effects of Low Concentration of Glycyrrhetic Acid Glycosides on CCl_4 -Induced Hepatic Injury

A final concentration at $6.3 \times 10^{-7} \text{ M}$ of glycosides was added to an incubation mixture. Both AST and ALT were assayed as described in the text. Blank bars indicate activities of ALT and closed ones those of AST. Significantly different from the control: a) $p < 0.01$, b) $p < 0.05$.

more AST and ALT than glycosides 1, 2, 6 and 8, indicating acceleration of hepatic injury. On the other hand, glycosides 3 and 7 having glcUA as the terminal sugar component indicated strong cytoprotective effects on the CCl_4 -injury

Fig. 2. Cytoprotective Effects of High Concentration of Glycyrrhetic Acid Glycosides on CCl_4 -Induced Hepatic Injury

A final concentration at $12.6 \times 10^{-7} \text{ M}$ of glycosides was added to an incubation mixture. Both AST and ALT were assayed as described in the text. Blank bars indicate activities ALT and closed ones those of AST. Significantly different from the control: a) $p < 0.01$, b) $p < 0.05$.

Fig. 3. Feasible Conformation of Compound 9

of hepatocytes. These data suggest that glcUA is essential for cytoprotective effect and the terminal glcUA is more important than the inner one.

The feasible conformation of 9 is illustrated in Fig. 3. Figure 4 shows the proposal fitting model between active sites on the cell and glycyrrhetic acid glycosides having glcUA in the molecules. The terminal glcUA molecule must be bound to the recognition site and aglycon moiety just fixed to the cell surface of hepatocytes, resulting in the inhibition of releasing of inner enzymes (Fig. 4a). If the inner glcUA molecule is bound to the recognition site, the aglycon moiety does not become fixed to the cell surface so that the cytoprotective effect becomes weaker or the membrane is

Fig. 4. A Proposal Model of Effects of Glycyrrhetic Acid Glycosides on Hepatocytes

destroyed (Fig. 4b).

Experimental

Materials Methyl glycyrrhetinate was prepared by methylation of glycyrrhetic acid according to the published procedure.¹⁶⁾ Dry dichloromethane (CH_2Cl_2) was obtained by refluxing with NaH followed by distillation. Other chemicals and solvents were of reagent grade, and were obtained from commercial sources.

Measurements The TLC utilized Kieselgel HF₂₅₄ (Merck), and spots were detected by irradiating the plates with ultraviolet (UV) light (254 nm, Manasul-light) and 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-3000A was employed for analytical HPLC using ODS-1251-D (4.6 mm $\times$ 250 mm), and was further equipped with an SSC autinjector 6310 and an SSC fraction collector 6320 for preparative HPLC using ODS-4521-D (10 mm $\times$ 250 mm). The following solvent systems for HPLC were used: (a) 20% H_2O -acetone, (b) 22.5% H_2O -acetone, (c) 25% H_2O -acetone, (d) MeOH - H_2O - AcOH (64.5:35:0.5), and (e) MeOH - H_2O - AcOH (69.5:35:0.5). Optical rotations were measured with a JASCO J-20A spectropolarimeter. ^1H - 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. Electron impact and fast atom bombardment mass spectra (EI- and FAB-MS) were recorded on a JEOL JMS-DX 300 mass spectrometer. The activities of AST and ALT were assayed by autoanalyzer COBAS MRA (Roche) using commercial kits based on the principal of the AST and ALT assay method.¹⁵⁾

Preparation of Hepatocytes Isolated rat hepatocytes were prepared using 150–180 g Wistar rats by a slightly modified method of Seglen.¹⁷⁾

Methyl 3,4-Di-*O*-acetyl- α -D-glucuronatopyranose 1,2-*O*-(Ethyl Orthoacetate) 17 To a solution of methyl 2,3,4-tri-*O*-acetyl- α -D-glucuronatopyranosyl bromide **16** (100 g) in γ -collidine (200 ml), tetra-*n*-butylammonium bromide (20 g) and dry ethanol (14 ml) were added, and the mixture was stirred for 24 h at 50 °C. After filtration, the filtrate was poured into ice-water (500 ml) and extracted with CH_2Cl_2 (300 ml $\times$ 3). The combined extracts were washed successively with 5% aqueous HCl, aqueous-saturated NaHCO_3 , and water, then dried over Na_2SO_4 and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography to obtain **17** (50 g, 55%). The ^1H -NMR spectrum of **17** indicated the formation of *exo*- and *endo*-1,2-orthoacetate (5:1), which were not isolated. ^1H -NMR spectrum of the *exo*-1,2-orthoacetate (CDCl_3) δ : 5.86 (1H, d, J =4.8 Hz, H-1), 5.24 (1H, dd, J =2.9, 2.2 Hz, H-3), 5.15 (1H, ddd, J =7.3, 2.2, 1.1 Hz, H-4), 4.32 (1H, ddd, J =4.8, 2.9, 1.1 Hz, H-2), 4.31 (1H, d, J =7.3 Hz, H-5), 3.78 (3H, s, OCH_3), 3.55 (2H, q, J =7.0 Hz, $-\text{CH}_2-\text{CH}_3$), 2.11 and 2.10 (each 3H, Ac), 1.75 (3H, s, CH_3), 1.18 (3H, t, J =7.0 Hz, $-\text{CH}_2-\text{CH}_3$). EI-MS m/z (%): 317 (9, $\text{M}^+ - \text{OCH}_2\text{CH}_3$), 303 (8, $\text{M}^+ - \text{OAc}$), 275 (11), 257 (11), 231 (5), 215 (15), 214 (15), 197 (12), 186 (6), 173 (24), 172 (15), 157 (15), 156 (11), 155 (100). Anal. Calcd for $\text{C}_{15}\text{H}_{22}\text{O}_{10}$: C, 49.72; H, 6.12. Found: C, 49.65; H, 6.16.

Benzyl 3,4-Di-*O*-benzyl- α -D-glucuronatopyranose 1,2-*O*-(Ethyl Orthoacetate) 18 To a solution of **17** (45 g) in MeOH (300 ml), ammonia-saturated MeOH (70 ml) was added, and the mixture was allowed to stand for 20 h at room temperature. After removing ammonia by aspiration, the solution was evaporated to give a residue (38 g). The residue was dissolved in dimethylformamide (100 ml), added with benzyl bromide (50 ml) and silver oxide (35 g), and stirred in the dark for 20 h at room temperature. The reaction mixture was added with MeOH (30 ml), and stirred for another 2 h. The mixture was filtered through Kieselguhr. The filtrate was concentrated to give a residue which was subjected to column chromatography (toluene- AcOEt , 4:1) to obtain **18** (15.3 g, 23%). Anal. Calcd for $\text{C}_{31}\text{H}_{34}\text{O}_8$: C, 69.65; H, 6.41. Found: C, 70.07; H, 6.53.

Benzyl 2-*O*-Acetyl-3,4-di-*O*-benzyl-D-glucuronatopyranose 14 The fore-

going 1,2-orthoester **18** (12 g) was dissolved in 95% aqueous acetic acid (200 ml), and the mixture was allowed to stand for 15 min at room temperature. The mixture was poured into ice-water (50 ml) and extracted with CH_2Cl_2 (50 ml $\times$ 3). The combined organic extracts were washed successively with aqueous-saturated NaHCO_3 and water, dried over Na_2SO_4 , and filtered. The filtrate was evaporated to give a residue which was purified by column chromatography (benzene-acetone, gradient up to 5%) to yield pure **14** (9.4 g, 83%). This product showed a singlet peak at δ : 2.02 due to an acetyl group in the ^1H -NMR spectrum. FAB-MS m/z : 506 (M^+). Anal. Calcd for $\text{C}_{29}\text{H}_{30}\text{O}_8$: C, 68.76; H, 5.97. Found: C, 68.39; H, 6.10.

Benzyl 1,2-Di-*O*-acetyl-3,4-di-*O*-benzyl- β -D-glucuronatopyranose 15 Compound **14** (6.4 g) was dissolved in acetic anhydride (5 ml) and pyridine (5 ml), and the mixture was allowed to stand overnight at room temperature. The mixture was concentrated to give a residue (6.7 g) which was subjected to column chromatography (benzene-acetone, gradient up to 10%) to obtain **15** (3.3 g, 48%). ^1H -NMR (CDCl_3) δ : 7.36–7.21 (15H, aromatic protons), 5.78 (1H, d, J =7.3 Hz, H-1), 5.06 (1H, t, J =7.3 Hz, H-2), 4.76–4.40 (6H, $-\text{CH}_2-$ $\times$ 3 on benzyl groups), 4.20 (1H, d, J =7.0 Hz, H-5), 3.95 (1H, t, J =7.3 Hz, H-3), 3.71 (1H, dd, J =7.3, 7.0 Hz, H-4), 2.06 and 1.95 (each 3H, s, Ac). FAB-MS m/z : 548 (M^+). Anal. Calcd for $\text{C}_{31}\text{H}_{32}\text{O}_9$: C, 67.87; H, 5.88. Found: C, 67.75; H, 5.89.

Glycosidation of Methyl Glycyrrhetinate 11 with 13 To a solution of **11** (3 g) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-galactopyranose **13** (5 g) in dry CH_2Cl_2 (10 ml), anhydrous cobalt bromide (2 g), trimethylsilyl chloride (1.3 ml) and molecular sieve (4 Å, 1 g) were added. After stirring for 20 h at room temperature, the mixture was centrifuged to give a solution. The solution was washed successively with aqueous NaHCO_3 and water, dried over Na_2SO_4 , and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography (benzene-acetone, gradient up to 10%) to obtain compound **19** (1.9 g, 32%) and **20** (1.2 g, 19%). ^1H -NMR of **19** (CDCl_3) δ : 0.76, 0.80, 0.90, 1.11, 1.11, 1.14, 1.33 (each 3H, s, CH_3), 2.02 (3H, s, Ac), 2.28 (1H, s, H-9), 2.75 (1H, brd, J =12.6 Hz, H-18), 3.02 (1H, br t, J =8.0 Hz, H-3), 3.48 (1H, dd, J =9.9, 2.7 Hz, H-3'), 3.52–3.63 (3H, H-5', 6a', 6b'), 3.69 (3H, s, OCH_3), 3.91 (1H, d, J =2.7 Hz, H-4'), 4.35 (1H, d, J =8.0 Hz, H-1'), 4.43–4.93 (6H, $-\text{CH}_2-$ $\times$ 3 on benzyl groups), 5.38 (1H, dd, J =9.9, 8.0 Hz, H-2'), 5.65 (1H, s, H-12), 7.23–7.36 (15H, aromatic protons). FAB-MS m/z : 981 [$\text{M} + \text{Na}$] $^+$. Anal. Calcd for $\text{C}_{60}\text{H}_{78}\text{O}_{10}$: C, 75.12; H, 8.20. Found: C, 75.05; H, 8.26. ^1H -NMR of **20** (CDCl_3) δ : 0.80, 0.81, 0.98, 1.12, 1.45, 1.57, 1.57 (each 3H, s, CH_3), 2.05 (3H, s, Ac), 2.30 (1H, s, H-9), 2.75 (1H, brd, J =12.6 Hz, H-18), 3.11 (1H, brd, J =8.0 Hz, H-3), 3.51 (1H, dd, J =8.8, 5.5 Hz, H-6a'), 3.59 (1H, dd, J =8.8, 7.3 Hz, H-6b'), 3.92 (1H, dd, J =10.3, 3.6 Hz, H-3'), 4.01 (1H, d, J =3.6 Hz, H-4'), 4.11 (1H, br t, J =8.8 Hz, H-5'), 4.38–4.96 (6H, $-\text{CH}_2-$ $\times$ 3 on benzyl groups), 5.19 (1H, d, J =3.7 Hz, H-1'), 5.25 (dd, J =10.3, 3.7 Hz, H-2'), 5.66 (1H, s, H-12), 7.26–7.36 (15H, aromatic protons). FAB-MS m/z : 981 [$\text{M} + \text{Na}$] $^+$. Anal. Calcd for $\text{C}_{60}\text{H}_{78}\text{O}_{10}$: C, 75.12; H, 8.20. Found: C, 75.33; H, 8.15.

Deacetylation of 19 To a solution of compound **19** (1.7 g) in MeOH (40 ml), 1.5 N NaOMe - MeOH (8 ml) was added. After stirring for 20 h at room temperature, the mixture was poured into ice-water (100 ml) and extracted with CH_2Cl_2 (100 ml $\times$ 3). The combined organic extracts were washed with H_2O , dried over Na_2SO_4 and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography (benzene-acetone, gradient up to 5%) to afford compound **23** (1.4 g, 86%). ^1H -NMR (CDCl_3) δ : 0.80, 0.84, 1.02, 1.11, 1.13, 1.15, 1.34 (each 3H, s, CH_3), 2.30 (1H, s, H-9), 2.76 (1H, brd, J =10.3 Hz, H-18), 3.13 (1H, dd, J =8.8, 4.1 Hz, H-3), 3.43 (1H, dd, J =9.9, 2.9 Hz, H-3'), 3.57 (3H, m, H-5', 6a', 6b'), 3.69 (3H, s, OCH_3), 3.89 (1H, d, J =2.6 Hz, H-4'), 3.98 (1H, dd, J =9.9, 7.7 Hz, H-2'), 4.28 (1H, d, J =7.7 Hz, H-1'), 4.40–4.89 (6H, $-\text{CH}_2-$ $\times$ 3 on benzyl groups), 5.66 (1H, s, H-12), 7.26–7.37 (15H, aromatic protons). FAB-MS m/z : 939 [$\text{M} + \text{Na}$] $^+$. Anal. Calcd for $\text{C}_{58}\text{H}_{76}\text{O}_9$: C, 75.95; H, 8.35. Found: C, 75.57; H, 8.40.

Glycosidation of 11 with 15 The solution of **11** (1 g) and **15** (2.2 g) in

dry CH_2Cl_2 (20 ml) was added with trimethylsilyl trifluoromethanesulfonate (0.4 ml) and molecular sieve (4 Å, 600 mg). After stirring for 2 d at 50 °C, the mixture was centrifuged to give a supernatant which was poured into ice-water (100 ml) and extracted with CH_2Cl_2 (80 ml $\times$ 3). The combined organic extracts were washed successively with aqueous-saturated NaHCO_3 and water, dried over Na_2SO_4 , and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography (benzene–acetone, gradient up to 5%) to afford compound **21** (320 mg, 16%) and **22** (580 mg, 29%). ^1H -NMR spectrum of **21** (CDCl_3) δ : 0.73, 0.80, 0.90, 0.91, 1.10, 1.14, 1.35 (each 3H, s, CH_3), 1.93 (3H, s, Ac), 2.30 (1H, s, H-9), 2.77 (1H, br d, J = 10.3 Hz, H-18), 3.06 (1H, dd, J = 8.8, 4.1 Hz, H-3), 3.65 (1H, dd, J = 7.5, 7.3 Hz, H-3'), 3.72 (1H, t, J = 7.3 Hz, H-4'), 4.00 (1H, d, J = 7.3 Hz, H-5'), 4.53 (1H, d, J = 7.5 Hz, H-1'), 4.35–4.83 (6H, $-\text{CH}_2-$ $\times$ 3 on benzyl groups), 5.00 (1H, t, J = 7.5 Hz, H-2'), 5.68 (1H, s, H-12), 7.22–7.33 (15H, aromatic protons). FAB-MS m/z : 995 $[\text{M} + \text{Na}]^+$. *Anal.* Calcd for $\text{C}_{60}\text{H}_{76}\text{O}_{11}$: C, 74.04; H, 7.87. Found: C, 73.89; H, 8.01. ^1H -NMR spectrum of **22** (CDCl_3) δ : 0.80, 0.84, 0.90, 0.95, 1.00, 1.11, 1.14 (each 3H, s, CH_3), 2.00 (3H, s, Ac), 2.30 (1H, s, H-9), 2.69 (1H, br d, J = 10.3 Hz, H-18), 3.14 (1H, br t, J = 7.3 Hz, H-3), 3.36 (1H, H-4', overlapped with OCH_3), 3.98 (1H, t, J = 8.1 Hz, H-3'), 4.33 (1H, d, J = 9.9 Hz, H-5'), 4.46–4.86 (6H, $-\text{CH}_2-$ $\times$ 3 on benzyl groups), 4.76 (1H, dd, J = 8.1, 3.6 Hz, H-2'), 5.19 (1H, d, J = 3.6 Hz, H-1'), 5.66 (1H, s, H-12), 7.25–7.34 (15H, aromatic protons). FAB-MS m/z : 995 $[\text{M} + \text{Na}]^+$. *Anal.* Calcd for $\text{C}_{60}\text{H}_{76}\text{O}_{11}$: C, 74.04; H, 7.87. Found: C, 73.95; H, 7.90.

Deacetylation of 21 To a solution of **21** (200 mg) in MeOH (6 ml), 1.5 N NaOH–MeOH (1.1 ml) was added, and the mixture was allowed to stand for 20 h at room temperature. The mixture was poured into ice-water (20 ml) and extracted with CH_2Cl_2 (20 ml $\times$ 3). The combined organic extracts were washed with water, dried over Na_2SO_4 , and filtered. The filtrate was evaporated to give a residue which was purified by column chromatography (benzene–acetone, gradient up to 10%) to afford compound **24** (165 mg, 86%). ^1H -NMR (CDCl_3) δ : 0.81, 0.81, 1.01, 1.10, 1.11, 1.15, 1.36 (each 3H, s, CH_3), 2.37 (1H, s, H-9), 2.77 (1H, br d, J = 13.6 Hz, H-18), 3.14 (1H, t, J = 8.4 Hz, H-3), 3.57–3.65 (3H, H-2', 3', 4'), 3.68 (3H, s, OCH_3), 3.96 (1H, d, J = 8.4 Hz, H-5'), 4.43 (1H, d, J = 7.7 Hz, H-1'), 4.61–4.82 (6H, $-\text{CH}_2-$ $\times$ 3 on benzyl groups), 5.68 (1H, s, H-12), 6.80 (1H), 7.25–7.40 (14H) (aromatic protons). FAB-MS m/z : 953 $[\text{M} + \text{Na}]^+$. *Anal.* Calcd for $\text{C}_{58}\text{H}_{74}\text{O}_{10}$: C, 74.81; H, 8.01. Found: C, 74.77; H, 8.07.

Glycosidation of 23 with 26 To a solution of **23** (700 mg) and **26** (1 g) in dry CH_2Cl_2 (15 ml), silver trifluoromethanesulfonate (AgOTf) (700 mg) and 1,1,3,3-tetramethylurea (TMU) (0.5 ml) was added, and the mixture was stirred for 4 h at room temperature. The mixture was centrifuged, and the supernatant was poured into ice-water (50 ml) and extracted with CH_2Cl_2 (50 ml $\times$ 3). The combined organic extracts were washed successively with aqueous-saturated NaHCO_3 and H_2O , and dried over Na_2SO_4 , and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography (benzene–AcOEt, gradient up to 5%) to obtain a major product (795 mg, 85%). The product was further purified by preparative HPLC (solvent c) to afford pure compound **28** (495 mg, 53%). FAB-MS m/z : 1285 $[\text{M} + \text{K}]^+$. *Anal.* Calcd for $\text{C}_{72}\text{H}_{94}\text{O}_{18}$: C, 69.32; H, 7.60. Found: C, 69.30; H, 7.63.

Glycosidation of 23 with 27 The mixture of **23** (1 g), **27** (1.7 g), AgOTf (1 g), TMU (0.83 ml), and dry CH_2Cl_2 (20 ml) was stirred for 4 h at room temperature. The mixture was post-treated according to the preparation method of **28** to give a residue. Pure compound **29** (450 mg, 33%) was isolated by column chromatography (benzene–AcOEt, gradient up to 5%) followed by preparative HPLC (solvent b). FAB-MS m/z : 1285 $[\text{M} + \text{K}]^+$. *Anal.* Calcd for $\text{C}_{72}\text{H}_{94}\text{O}_{18}$: C, 69.32; H, 7.60. Found: C, 69.05; H, 7.69.

Glycosidation of 23 with 16 The mixture of **23** (500 mg), **16** (1.25 g), AgOTf (800 mg), TMU (0.56 ml) and dry CH_2Cl_2 (10 ml) was stirred for 4 h at room temperature. The mixture was post-treated according to the preparation method of **28** to give a residue which was purified by column chromatography (benzene–AcOEt, gradient up to 5%) followed by preparative HPLC (solvent b) to obtain compound **30** (360 mg, 54%). FAB-MS m/z : 1271 $[\text{M} + \text{K}]^+$. *Anal.* Calcd for $\text{C}_{71}\text{H}_{92}\text{O}_{18}$: C, 69.13; H, 7.52. Found: C, 69.10; H, 7.52.

Glycosidation of 25 with 27 The mixture of **25** (650 mg), **27** (1.2 g), AgOTf (710 mg), TMU (0.56 ml), and dry CH_2Cl_2 (15 ml) was stirred for 4 h at room temperature. The mixture was post-treated according to the preparation method of **28** to give a residue which was subjected to column chromatography to obtain a crude product (625 mg). The preparative HPLC (solvent c) of the product gave pure compound **33** (240 mg, 27%). FAB-MS m/z : 1285 $[\text{M} + \text{K}]^+$. *Anal.* Calcd for $\text{C}_{72}\text{H}_{94}\text{O}_{18}$: C, 69.32; H,

7.60. Found: C, 69.57; H, 7.73.

Glycosidation of 25 with 16 The mixture of **25** (500 mg), **16** (2 g), AgOTf (800 mg), TMU (0.56 ml), and dry CH_2Cl_2 (10 ml) was stirred for 5 h at room temperature. The mixture was post-treated according to the preparation method of **28** to give a residue which was subjected to column chromatography (benzene–AcOEt, gradient up to 5%) to obtain crude product **34** (380 mg). The preparative HPLC (solvent a) of the product gave pure compound **34** (260 mg, 39%). FAB-MS m/z : 1271 $[\text{M} + \text{K}]^+$. *Anal.* Calcd for $\text{C}_{71}\text{H}_{92}\text{O}_{18}$: C, 69.13; H, 7.52. Found: C, 68.89; H, 7.58.

Glycosidation of 24 with 26 The mixture of **24** (150 mg), **26** (300 mg), AgOTf (96 mg), TMU (0.1 ml), and dry CH_2Cl_2 (5 ml) was stirred for 8 h at room temperature. The mixture was post-treated according to the preparation method of **28** to give a residue which was subjected to column chromatography to obtain crude product **31** (150 mg). The preparative HPLC (solvent c) of the product gave pure **31** (100 mg, 49%). FAB-MS m/z : 1299 $[\text{M} + \text{K}]^+$. *Anal.* Calcd for $\text{C}_{72}\text{H}_{94}\text{O}_{18}$: C, 68.55; H, 7.35. Found: C, 68.35; H, 7.41.

Glycosidation of 24 with 27 The mixture of **24** (260 mg), **27** (350 mg), AgOTf (190 mg), TMU (0.2 ml), and dry CH_2Cl_2 (5 ml) was stirred for 6 h at room temperature. The mixture was post-treated according to the preparation method of **28** to give a residue which was subjected to column chromatography (benzene–AcOEt, gradient up to 5%) to obtain crude product **32** (156 mg). The preparative HPLC (solvent c) gave pure product **32** (110 mg, 31%). FAB-MS m/z : 1299 $[\text{M} + \text{K}]^+$. *Anal.* Calcd for $\text{C}_{72}\text{H}_{92}\text{O}_{19}$: C, 68.55; H, 7.35. Found: 68.09; H, 7.45.

Removal of the Protecting Groups of 28 To a solution of **28** (610 mg) in MeOH (20 ml), 1.5 N NaOMe–MeOH (4 ml) was added, then the mixture was allowed to stand for 12 h at room temperature. The mixture was neutralized with acetic acid, and evaporated to give a residue which was subjected to column chromatography ($\text{MeOH}-\text{CH}_2\text{Cl}_2$, 1:9) to afford a syrupy product. The product was dissolved in acetic acid (20 ml) and added with 10% Pd–charcoal (30 mg). The mixture was stirred in a hydrogen atmosphere for 20 h at room temperature and filtered. The filtrate was evaporated to give a residue. The residue was dissolved in 5% NaOH in H_2O –EtOH (1:1) (6.5 ml) and refluxed for 2 h. After cooling, the mixture was neutralized with acetic acid and evaporated to obtain a residue which was purified by column chromatography (CHCl_3 – $\text{MeOH}-\text{H}_2\text{O}$, 65:35:10) followed by preparative HPLC (solvent e) to yield glycoside **1** (125 mg, 32%). FAB-MS m/z : 817 $[\text{M} + \text{Na}]^+$. $[\alpha]_D^{25} + 64.1$ (c = 1.56, MeOH).

The Removal of the Protecting Groups of 29–34 The removal of the protecting groups of **29–34** was performed by the same method of **1** to obtain **2** (37%, FAB-MS m/z : 817 $[\text{M} + \text{Na}]^+$, $[\alpha]_D^{25} + 53.9$ (c = 1.52, MeOH)), **3** (30%, FAB-MS m/z : 831 $[\text{M} + \text{Na}]^+$, $[\alpha]_D^{25} + 50.7$ (c = 1.40, MeOH)), **4** (42%, FAB-MS m/z : 831 $[\text{M} + \text{Na}]^+$, $[\alpha]_D^{25} + 43.6$ (c = 1.56, MeOH)), **5** (40%, FAB-MS m/z : 831 $[\text{M} + \text{Na}]^+$, $[\alpha]_D^{25} + 55.7$ (c = 0.97, MeOH)), **6** (44%, FAB-MS m/z : 817 $[\text{M} + \text{Na}]^+$, $[\alpha]_D^{25} + 52.1$ (c = 0.96, MeOH)), and **7** (43%, FAB-MS m/z : 831 $[\text{M} + \text{Na}]^+$, $[\alpha]_D^{25} + 50.6$ (c = 1.42, MeOH)), respectively.

Methanolysis of 1–7 Each 5 mg of sample (**1–7**) was dissolved in 0.5 N HCl–MeOH (1 ml) and refluxed for 2 h. Each reaction mixture was extracted with AcOEt and washed with H_2O , dried over Na_2SO_4 , and filtered. The filtrate was evaporated to give a residue which was treated with diazomethane to obtain methylated aglycon. Each aglycon was purified by preparative TLC (benzene–acetone, 85:15). All aglycons obtained from **1–7** were identified with authentic **11** by HPLC (retention time: 38.4 min; solvent system, 30% H_2O –acetone; flow rate, 0.2 ml/min).

References and Notes

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