[Chem. Pharm. Bull. 28(1) 163—168 (1980)]

Constituents of the Chinese Crude Drug "Wujiapi". IX.[1] Structure of Glycoside H_2, a Potentiator of NGF-mediated Nerve Fiber Outgrowth

Seiichi Sakuma, Sachiko Kawanishi, and Junzo Shoji

School of Pharmaceutical Sciences, Showa University[2]

(Received June 29, 1979)

Glycoside H_2, $C_{56}H_{92}O_{25}$, $[\alpha]_D$ −25.9° (MeOH), which causes marked potentiation of the effect of NGF (nerve growth factor), was isolated from Bei-Wujiapi (the cortex of *Periploca sepium* Bge., Asclepiadaceae) and its chemical structure was established to be Δ^5-pregnene-3β,16α,20α-triol 3-O-[2-O-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranoside] 20-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl(1→2)-β-D-digitalopyranoside] (**1**).

Keywords——glycoside H_2; pregnane oligoside; Bei-Wujiapi; *Periploca sepium* Bge.; asclepiadaceae; potentiation of NGF; Δ^5-pregnene-3β,16α,20α-triol; cymarose; digitalose; 2-O-acetyldigitalose

Recently, Kasuya and Saito *et al.*[3] reported the potentiation of NGF-mediated nerve fiber outgrowth by saponins in organ cultures of chicken embryonic dorsal root and sympathetic ganglia. Among various saponins studied, marked potentiation of the effect of NGF was evident with glycoside K,[4] glycoside H_1[5] and glycoside H_2 of Bei-Wujiapi, the cortex of *Periploca sepium* Bunge (Asclepiadaceae). The structures of glycosides K and H_1 have been reported in our previous papers. In the present paper, the isolation and structure elucidation of glycoside H_2 (**1**) are described.

As described in the previous paper,[5] glycoside H, a mixture of glycosides H_1 and H_2, was obtained as a white powder. The mixture was subjected to column chromatography on silica gel, eluting with chloroform–methanol–ethyl acetate–water=2:2:5:1 (lower phase), and finally glycoside H_2 was obtained as a white powder.

The infrared (IR) and nuclear magnetic resonance (NMR) spectra of glycoside H_2(**1**), $C_{56}H_{92}O_{25}$, $[\alpha]_D$ −25.9° (in methanol), indicate the presence of one O-acetyl group (1740 cm^{-1}; 2.10 ppm, 3H, singlet), three O-methyl groups (3.45, 3.50, 3.52 ppm, each 3H, singlet), two tertiary C-methyl groups (0.64, 0.98 ppm, each 3H, singlet), four secondary C-methyl groups (1.42, 1.50 ppm, each 6H, doublet, J=6 Hz) and many hydroxyl groups (3400 cm^{-1}, broad).

On acid hydrolysis with 2N sulfuric acid-dioxane (1:1 v/v), **1** gave Δ^5-pregnene-3β,16α,20α-triol (**2**),[6] glucose and digitalose.

Identification of **2** by comparison with an authentic sample was carried out by mixed fusion and comparison of IR spectra, and the sugar components were detected by thin-layer chromatography (TLC) and gas liquid chromatography (GLC).

Partial hydrolysis of **1** with 0.05N sulfuric acid in 50% methanol gave three main products (**3**, **4**, and **5**). The reaction mixture was concentrated *in vacuo* and the aqueous residue was extracted with chloroform. The aqueous phase was neutralized with Amberlite IR-4B and extracted with butanol. The chloroform-soluble fraction was purified by column

1) Part VIII: S. Kawanishi, R. Kasai, S. Sakuma, and J. Shoji, *Chem. Pharm. Bull.*, **25**, 2055 (1977).
2) Location: *1-5-8, Hatanodai, Shinagawa-ku, Tokyo, 142, Japan.* Correspondence should be addressed to J. Shoji.
3) H. Saito, Y. Lee, and Y. Kasuya, *Jpn. J. Pharmacol.*, **27**, 114 (supplementum) 1977.
4) S. Sakuma, H. Ishizone, R. Kasai, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.*, **19**, 52 (1971).
5) S. Kawanishi, S. Sakuma, and J. Shoji, *Chem. Pharm. Bull.*, **20**, 469 (1972).
6) S. Sakuma, S. Kawanishi, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.*, **16**, 326 (1968).

chromatography on silica gel with ethyl acetate. The faster-moving component (**3**), colorless needles, mp 171°, $[\alpha]_D^{28}$ +21.3° (in methanol), gave cymarose and digitalose on acid hydrolysis. Based on the general properties and spectral data, **3** was identified as methyl 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-β-D-cymaropyranoside[7]; it was confirmed to be identical with an authentic sample (mixed fusion, TLC and IR spectra). The second component (**4**), colorless needles, mp 176—177°, was identical with an authentic sample of 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-D-cymarose (mixed fusion, TLC and IR spectra). The third product (**5**), $C_{40}H_{66}O_{17}$, mp 280—282°, obtained from the butanol extract of the foregoing hydrolysate, was hydrolyzed with 2N sulfuric acid–dioxane (1: 1 v/v) to give Δ^5-pregnene-3β,16α,20α-triol (**2**), glucose and digitalose.

On treatment with 0.5N sodium hydroxide **1** affords desacetyl glycoside H_2(**6**), a crystalline powder, $C_{54}H_{90}O_{24}$, which does not show any acetyl absorption band in the IR spectrum. A per-O-methyl ether (**7**), a white powder, IR ν_{max}^{Nujol} cm^{-1}: OH (nil), which was prepared by Hakomori methylation[8] of **6**, was hydrolyzed with 2N sulfuric acid in 50% methanol to afford **8**, $C_{22}H_{36}O_3$, colorless needles, together with 2,3,4,6-tetra-O-methylglucose, 2,3,4-tri-O-methylglucose, 2,4-di-O-methyldigitalose and 4-O-methyldigitalose. Each O-methyl derivative was identified by TLC and GLC, while **8** was established to be Δ^5-pregnene-3β,16α,20α-triol 16-O-methyl ether as follows. The IR spectrum of **8** shows a hydroxyl absorption band at 3550 cm^{-1} (broad) and the presence of one O-methyl group was indicated by the NMR spectrum (δ 3.34 ppm, 3H, singlet). To confirm the location of the methoxyl group, **8** was subjected to Oppenauer oxidation to afford **9**, $C_{22}H_{32}O_3$, colorless needles. The NMR spectrum of **9** revealed the presence of one tertiary C-methyl group adjacent to a carbonyl group at δ 2.24 ppm (singlet) and one olefinic proton conjugated with a carbonyl group at δ 5.82 ppm (singlet). The IR and UV spectra both supported the presence of an α,β-unsaturated ketone system in **9**. The results of the foregoing experiments suggested that **9** was identical with 16α-methoxyprogesterone,[9] and the two glycosyl linkages of **1** must be present at the C-3 and C-20 hydroxyl groups of the aglycone.

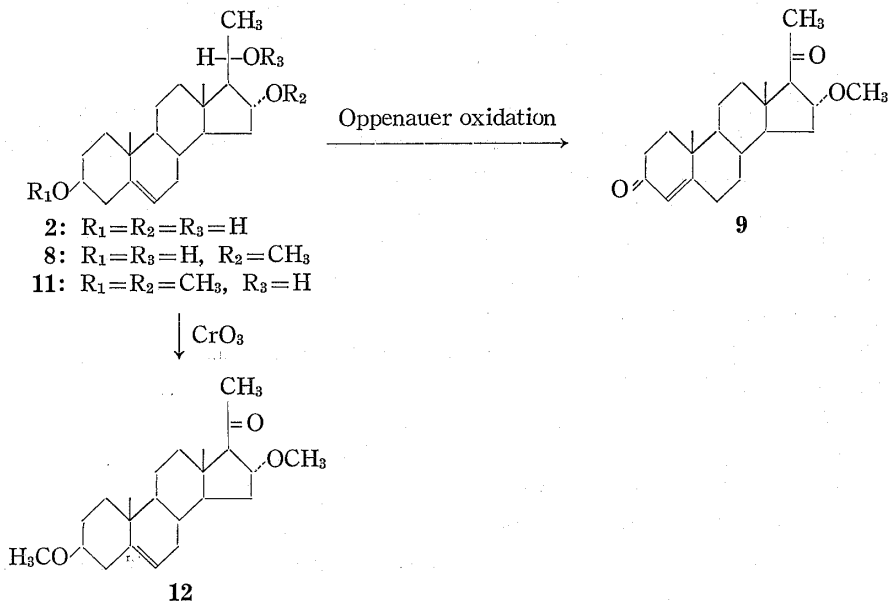

Chart 1

7) S. Kawanishi, S. Sakuma, H. Okino, and J. Shoji, *Chem. Pharm. Bull.*, **20**, 93 (1972).
8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
9) *a*) G. Cooley, B. Ellis, and V. Petrow, *J. Chem. Soc.*, 1813 (1954); *b*) D.K. Fukushima and T.F. Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951); *c*) R.N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 2820 (1952).

The structure of **5** was established by following method. A per-O-methyl derivative (**10**) prepared by Hakomori methylation of **5** was hydrolyzed with 2N sulfuric acid–50% methanol to afford 2,3,4,6-tetra-O-methylglucose, 2,3,4-tri-O-methylglucose, 4-O-methyldigitalose and an aglycone (**11**). The NMR spectrum indicated the presence of two O-methyl groups in **11**, which was oxidized with Jones reagent[10] to afford a ketonic compound (**12**). In the NMR spectrum, the doublet signal (δ 1.25, J=6 Hz) of **11**, corresponding to a secondary C-methyl group, was not found in **12**, but a new signal due to a tertiary C-methyl group adjacent to a carbonyl

Table I

Glucose→Glucose	$[M]_{D\cdot 5}$ —$[M]_{D\cdot 13}$	−38.5°	β
Glucose→Digitalose	$[M]_{D\cdot 13}$—$[M]_{D\cdot 14}$	−61.0°	β
Digitalose→Aglycone	$[M]_{D\cdot 14}$—$[M]_{D\cdot 2}$	−4.5°	β
	NMR anomer H of **14** δ=4.62 J=8 Hz		β
Methyl α-D-glucopyranoside		$[M]_D$=+307°	
Methyl β-D-glucopyranoside		$[M]_D$= −68°	
Methyl α-D-digitalopyranoside		$[M]_D$=+240°	
Methyl β-D-digitalopyranoside		$[M]_D$=−100°	

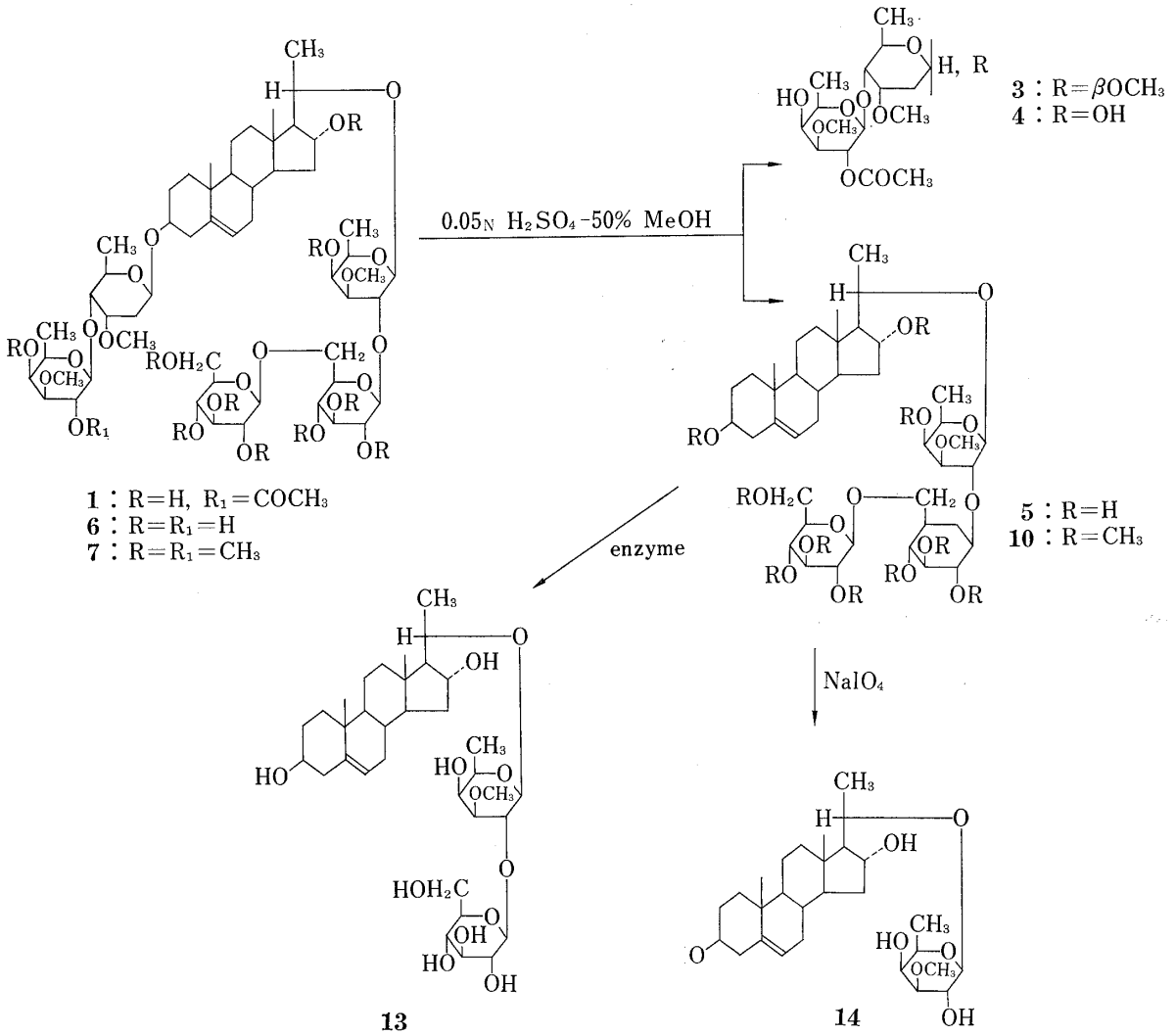

Chart 2

10) C. Djerassi, R.R. Engle, and A. Boers, *J. Org. Chem.*, **21**, 1547 (1956).

group was observed at δ 2.15. Consequently, **5** has a glycosyl moiety consisting of glucose and digitalose at the C-20 hydroxyl group and the disaccharide 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-D-cymaropyranose in **1** should be linked at the C-3 hydroxyl group of the aglycone.

To clarify the sugar sequence of glucose and digitalose, **5** was hydrolyzed with takadiastase-A to afford **13**, $C_{34}H_{56}O_{12}$. On hydrolysis with 2N sulfuric acid–dioxane (1:1 v/v), **13** gave **2**, glucose and digitalose.

Using the degradation method described in the previous paper,[4] **5** was oxidized with sodium metaperiodate and the product was reduced with sodium borohydride. The reaction mixture was extracted with chloroform and the extractive was hydrolyzed with 0.05N hydrogen chloride in 50% methanol to give Δ^5-pregnene-3β,16α,20α-triol monodigitaloside (**14**), $C_{28}H_{46}O_7$. On hydrolysis with 2N sulfuric acid in 50% dioxane, **14** gave **2** and digitalose.

Based on the coupling constant of the anomeric proton signal (δ 4.90 ppm) in the NMR spectrum of **7**, the configuration of D-cymarose was assigned as β, and the configurations of all the monosaccharides of **5** were assigned as β by comparison of the chemical shifts and the coupling constants (7 Hz) of anomeric protons of **10**. In addition, the application of Klyne's rule[11] to **2**, **14**, **13** and **5** also supported β-configurations of digitalose and glucose.

These experimental data established the structure of **1** as Δ^5-pregnene-3β,16α,20α-triol 3-O-[2-O-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranoside] 20-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl(1→2)-β-D-digitalopyranoside].

The potentiation of NGF-mediated nerve fiber outgrowth by glycosides K, H_1 and H_2 in organ cultures of chicken embryonic dorsal root and sympathetic ganglia has already been described. Among these glycosides, glycoside H_2 shows the most marked potentiation of the effect of NGF. The results will be reported in detail shortly.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Yanagimoto OR-50 automatic polarimeter. IR spectra were obtained with a Hitachi EPI-2 machine and NMR spectra were taken at 90 MHz with a Hitachi R-22 high-resolution NMR spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. GLC was run on a Shimadzu GC-6A unit with a flame ionization detector. TLC was performed on Kieselgel H (Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating.

Isolation and Properties of Glycoside H_2 (1)——As described in the previous paper,[5] glycoside H was obtained as a white powder from the butanol-soluble fraction of the methanol extract of Bei-Wujiapi (cortex of *Periploca sepium* Bge., Asclepiadaceae). The crude glycoside H (8 g) was subjected to column chromatography on silica gel, eluting with $CHCl_3$–MeOH–AcOEt–H_2O (2:2:5:1 lower phase), to afford a white powder (2.4 g). Recrystallization from dilute EtOH furnished glycoside H_2 (**1**) as a white powder, (mp 191—192°), $[\alpha]_D^{30}$ −25.9° (c=1.93, MeOH), *Anal.* Calcd for $C_{56}H_{92}O_{25}\cdot H_2O$: C, 56.84; H, 8.01. Found: C, 56.68; H, 8.26. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1740, 1250 ($OCOCH_3$). NMR (C_5D_5N) δ: 0.64 (3H, s, C$\underline{H}_3$), 0.98 (3H, s, C$\underline{H}_3$), 1.42 (6H, d, J=6 Hz, C$\underline{H}_3\times$2), 1.50 (6H, d, J=6 Hz, C$\underline{H}_3\times$2), 2.10 (3H, s, OCOC$\underline{H}_3$), 3.45 (3H, s, OC$\underline{H}_3$), 3.50 (3H, s, OC$\underline{H}_3$), 3.52 (3H, s, OC$\underline{H}_3$), 5.55 (1H, m, >C=C$\underline{H}$).

Hydrolysis of 1 with 2N H_2SO_4——Compound **1** (220 mg) was refluxed with 2N H_2SO_4-dioxane (1:1 v/v, 32 ml) on a water bath for 1 hr. The reaction mixture was diluted with water and extracted with $CHCl_3$, then the $CHCl_3$ solution was washed with water and dried over anhyd. Na_2SO_4. After removal of the solvent, the residue was recrystallized from AcOEt to give **2**, colorless needles (43 mg), mp 251°, $[\alpha]_D^{30}$ −66.1° (c=0.61, MeOH). *Anal.* Calcd for $C_{21}H_{34}O_3$: C, 75.42; H, 9.99. Found: C, 75.41; H, 9.86. The aglycone, **2**, was identical with an authentic sample of Δ^5-pregnene-3β,16α,20α-triol[6] (mixed fusion and IR spectra).

The aqueous layer was neutralized with Amberlite IR-4B and evaporated to dryness *in vacuo*. The residue was examined by TLC (solvent, $CHCl_3$–MeOH–H_2O=7:3:0.5; Rf 0.15 glucose; 0.42 digitalose) and GLC (column, 5% SE-52 on Chromosorb W; 3 mm×1 m; column temp., 155°; carrier gas, N_2 1.2 kg/cm²; sugar samples, TMS derivatives; t_R (min), 4.0, 4.5, 5.4 digitalose; 18.3, 29.3 glucose).

Hydrolysis of 1 with 0.05N H_2SO_4-50% MeOH——Compound **1** (900 mg) was refluxed with 0.05N H_2SO_4–50% MeOH (40 ml) on a water bath for 30 min. The reaction mixture was diluted with 20 ml of water and MeOH was removed *in vacuo* at room temperature. The residue was extracted with $CHCl_3$, washed with water and dried over anhyd. Na_2SO_4. After removal of the solvent, the residue was separated into

11) W. Klyne, *Biochem. J.*, **47**, xli (1950).

two major products (TLC: solvent AcOEt; *Rf* 0.28 and 0.16) by column chromatography on silica gel with AcOEt.

The faster-moving component was recrystallized from AcOEt to give **3** (180 mg), colorless needles, mp 171°, $[\alpha]_D^{28}$ +21.3° (*c*=0.83, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3550 (OH), 1735, 1240 ($OCOCH_3$), NMR ($CDCl_3$) δ: 1.20 (3H, d, *J*=6 Hz, C$\underline{H}_3$), 1.38 (3H, d, *J*=6 Hz, C$\underline{H}_3$), 2.00 (3H, s, OCOC$\underline{H}_3$), 3.45 (3H, s, OC$\underline{H}_3$), 3.48 (6H, s, 2×OC$\underline{H}_3$), 4.42 (1H, d, *J*=8 Hz, anomer $\underline{H}$), 4.68 (1H, q, J_1=2 Hz, J_2=9 Hz, anomer $\underline{H}$), 5.15 (1H, q, J_1=9 Hz, J_2=8 Hz, $\underline{H}$-C-OAc). **3** was identical with an authentic sample of methyl 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-β-D-cymaropyranoside[7] (mixed fusion, TLC and IR spectra).

The slower-moving component was recrystallized from dilute MeOH to afford **4** (60 mg), colorless needles, mp 176—177°; this compound was identical with an authentic sample of 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-D-cymarose[7] (mixed fusion, TLC and IR spectra).

The aqueous layer was neutralized with Amberlite IR-4B and extracted with BuOH. The BuOH extract was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7:3:0.5) to give **5** (573 mg), colorless needles from dilute MeOH, mp 280—282°, $[\alpha]_D^{30}$ −39.6° (*c*=1.31, MeOH). *Anal.* Calcd for $C_{40}H_{66}O_{17}$: C, 58.66; H, 8.12. Found: C, 58.39; H, 8.04. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH).

Hydrolysis of 3 and 4 with 0.05N H_2SO_4-50% MeOH——Compounds **3** and **4** (10 mg each) were each refluxed with 0.05N H_2SO_4–50% MeOH (6 ml) on a water bath for 1 hr. The reaction mixture was neutralized with Amberlite IR-4B and evaporated to dryness *in vacuo*. Each residue was examined by TLC (solvent, $CHCl_3$–MeOH=9:1; *Rf* 0.40 cymarose; 0.11 digitalose) and GLC (column, 5% SE-52 on Chromosorb W; 3 mm×1 m; column temp., 120°; carrier gas, N_2 1.25 kg/cm^2; sugar samples, TMS derivatives; t_R (min), 5.1, 5.8 cymarose; 12.7, 16.7 digitalose).

Hydrolysis of 5 with 2N H_2SO_4——Compound **5** (55 mg) was refluxed with 2N H_2SO_4–dioxane (1:1 v/v, 8 ml) on a water bath for 1 hr. The reaction mixture was treated as usual and the products were identified as **2**, glucose and digitalose.

Deacetylation of 1 with 0.5N NaOH——A solution of **1** (620 mg) in 0.5N NaOH (40 ml) was warmed at 50° for 2 hr with stirring under an N_2 gas flow. The reaction mixture was neutralized with Amberlite IR-200 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with $CHCl_3$–MeOH–H_2O (7:3:0.5), to give a white powder (**6**, 564 mg), which was recrystallized from diluted EtOH to afford a crystalline powder, mp 202—203°, $[\alpha]_D^{30}$ −30.4° (*c*=2.30, MeOH), *Anal.* Calcd for $C_{54}H_{90}O_{24}\cdot H_2O$: C, 56.83; H, 8.13. Found: C, 56.79; H, 8.24, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), acetyl(nil).

Methylation of 6 by Hakomori's Method——NaH (350 mg) was stirred with dimethylsulfoxide (DMSO 15 ml) at 65° for 1 hr under an N_2 gas flow. A solution of **6** (500 mg) in DMSO (8 ml) was then added to this reagent and the mixture was stirred for 20 min at room temperature under an N_2 gas flow. CH_3I (8 ml) was added to the solution and the reaction mixture was stirred at room temperature for 5 hr. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic phase was washed with water, dried and evaporated to a syrup (540 mg). The residue was chromatographed on a column of silica gel with benzene–acetone (5:1) to afford **7** (420 mg), a white powder from *n*-hexane, (mp 121—123°), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), NMR ($CDCl_3$) δ: 0.76 (3H, s, C$\underline{H}_3$), 1.02 (3H, s, C$\underline{H}_3$), 1.25 (6H, d, *J*=6 Hz, 2×C$\underline{H}_3$), 1.38 (3H, d, *J*=6 Hz, C$\underline{H}_3$), 1.42 (3H, d, *J*=6 Hz, C$\underline{H}_3$), 3.34—3.66 (3H, each s, 14×OC$\underline{H}_3$), 4.23 (1H, d, *J*=7 Hz, anomer $\underline{H}$), 4.25 (2H, d, *J*=7 Hz, 2×anomer $\underline{H}$), 4.77 (1H, d, *J*=7 Hz, anomer $\underline{H}$), 4.90 (1H, q, J_1=2 Hz, J_2=8 Hz, anomer $\underline{H}$), 5.40 (1H, m, >C=C$\underline{H}$).

Acid Hydrolysis of 7 with 2N H_2SO_4——A solution of **7** (400 mg) in MeOH (10 ml) was hydrolyzed with 4N H_2SO_4 (10 ml) for 1 hr under reflux on a water bath. The reaction mixture was diluted with water (10 ml) and MeOH was removed *in vacuo* at room temperature. The aqueous residue was extracted with $CHCl_3$ and the $CHCl_3$ layer was washed with water. After drying over anhyd. Na_2SO_4, the solvent was removed to give **8**, a white powder, which was recrystallized from diluted MeOH as colorless needles (86 mg), mp 204—205°, $[\alpha]_D^{26}$ −103.7° (*c*=0.63, MeOH), *Anal.* Calcd for $C_{22}H_{36}O_3$: C, 75.81; H, 10.41. Found: C, 75.61; H, 10.63. IR ν_{max}^{Nujol} cm^{-1}: 3550 (OH). NMR ($CDCl_3$) δ: 0.70 (3H, s, C$\underline{H}_3$), 1.02 (3H, s, C$\underline{H}_3$), 1.22 (3H, d, *J*=7 Hz, C$\underline{H}_3$), 3.34 (3H, s, OC$\underline{H}_3$), 3.55 (1H, m, $\underline{H}$-C(OH)–O–), 3.90 (2H, m, 2×$\underline{H}$-C(OH)–O–), 5.40 (1H, m, >C=C$\underline{H}$).

The aqueous layer was neutralized with Amberlite IR-4B and the solution was concentrated *in vacuo*. The residue was examined by TLC (solvent, $CHCl_3$–MeOH=9:1; *Rf* 0.46 2,3,4,6-tetra-O-methylglucose; 0.35 2,4-di-O-methyldigitalose; 0.23 2,3,4-tri-O-methylglucose; 0.16 4-O-methyldigitalose) and GLC (column, 3% SE-52 on Chromosorb W; 3 mm×1 m; column temp., 120°; carrier gas, N_2 1.2 kg/cm^2; sugar samples, TMS derivatives; t_R (min), 2.1, 2.9 2,4-di-O-methyldigitalose; 3.9, 4.7 4-O-methyldigitalose; 5.0 2,3,4,6-tetra-O-methylglucose; 7.8, 8.5 2,3,4-tri-O-methylglucose).

Oppenauer Oxidation of 8——A solution of **8** (80 mg) and Al (iso-Pro)$_3$ (1.3 g) in dry toluene (30 ml) and cyclohexane (9 ml) was heated on an oil bath under reflux for 40 min. After cooling, the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel with AcOEt to afford **9**, colorless needles (46 mg) from aq. MeOH, mp 134—135°, $[\alpha]_D^{30}$ +103.6° (*c*=1.60, MeOH), *Anal.* Calcd for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.90; H, 9.51. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1700 (C=O), 1660 (>C=C–CO), UV λ_{max}^{EtOH} nm (log ε): 241 (4.24), NMR ($CDCl_3$) δ: 0.70 (3H, s, C$\underline{H}_3$), 1.22 (3H, s, C$\underline{H}_3$), 2.24 (3H, s, C$\underline{H}_3$), 2.60 (1H, d, *J*=6 Hz, C-17 α-$\underline{H}$), 4.24 (1H, t, d, J_1=6 Hz, J_2=2 Hz, C-16 β-$\underline{H}$), 5.82 (1H, s,

C-4 H).

Methylation of 5 by Hakomori's Method——Compound **5** (350 mg) was methylated by Hakomori's method and the reaction mixture was worked up as usual. The product was purified by column chromatography on silica gel with benzene–acetone (5: 1) to afford **10**, a white powder from *n*-hexane, (mp 114—116°), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), NMR ($CDCl_3$) δ: 0.76 (3H, s, CH$_3$), 1.03 (3H, s, CH$_3$), 1.32 (3H, d, J=6 Hz, CH$_3$), 1.38 (3H, d, J=7 Hz, CH$_3$), 3.32—3.60 (3H, each s, 11×OCH$_3$), 4.26 (2H, d, J=7 Hz, 2×anomer H), 4.78 (1H, d, J=7 Hz, anomer H), 5.40 (1H, m, –C=CH).

Acid Hydrolysis of 10——A solution of **10** (300 mg) in 2N H_2SO_4-50% MeOH (20 ml) was refluxed for 1 hr. The reaction mixture was treated by the procedure described above. From the residue of the aqueous layer, 4-O-methyldigitalose, 2,3,4-tri-O-methylglucose and 2,3,4,6-tetra-O-methylglucose were identified by TLC and GLC. The $CHCl_3$ extract gave a white powder which was recrystallized from MeOH to afford **11** (62 mg), colorless needles, mp 102—103°, $[\alpha]_D^{30}$ −97.1° (c=1.37, MeOH), *Anal.* Calcd for $C_{23}H_{38}O_3$: C, 76.19; H, 10.56. Found: C, 76.16; H, 10.49. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), NMR ($CDCl_3$) δ: 0.72 (3H, s, CH$_3$), 1.04 (3H, s, CH$_3$), 1.25 (3H, d, J=6 Hz, CH$_3$), 3.38 (3H, s, OCH$_3$), 3.42 (3H, s, OCH$_3$), 3.92 (3H, m, 3×H–C–O–), 5.42 (1H, m, >C=CH).

Oxidation of 11 with Chromium Trioxide——A solution of **11** (42 mg) in acetone (6 ml) was treated with 0.3 ml of the Jones reagent (CrO_3 2 g, H_2SO_4 3 g, H_2O 15 ml) dropwise with stirring at 0°, and the reaction mixture was kept at room temperature for 30 min. After the addition of 0.6 ml of MeOH, the reaction mixture was diluted with 50 ml of water and the solution was extracted with benzene. The benzene layer was washed with 5% $NaHCO_3$ and water, and dried over anhyd. Na_2SO_4. The benzene solution was concentrated *in vacuo* to give a white powder, which was recrystallized from diluted EtOH to afford **12**, colorless needles (28 mg), mp 123—124°, $[\alpha]_D^{18}$ −19.0° (c=0.86, MeOH). *Anal.* Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.59; H, 10.11. IR ν_{max}^{Nujol} cm^{-1}: 1700 (C=O). NMR ($CDCl_3$) δ: 0.64 (3H, s, CH$_3$), 1.00 (3H, s, CH$_3$), 2.15 (3H, s, CH$_3$), 2.52 (1H, d, J=6 Hz, C-17 α-H), 3.20 (3H, s, OCH$_3$), 3.35 (3H, s, OCH$_3$), 3.75 (1H, m, H–C–OH), 4.35 (1H, t, d, J_1=6 Hz, J_2=2 Hz, H–C–O–), 5.35 (1H, m, –C=CH).

Enzymatic Hydrolysis of 5——Takadiastase A (3 g) and toluene (1 ml) were added to a solution of **5** (500 mg) in water (30 ml), and the mixture was kept at 34° for 10 days. The reaction mixture was extracted with BuOH and the BuOH solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7: 3: 0.5) to afford a single product. The product was recrystallized from diluted EtOH to afford **13** (320 mg), colorless needles, mp 284—285°, $[\alpha]_D^{30}$ −40.9° (c=0.66, MeOH). *Anal.* Calcd for $C_{34}H_{56}O_{12}$: C, 62.17; H, 8.59. Found: C, 61.98; H, 8.42. The aqueous layer of the reaction mixture was concentrated under reduced pressure and the residue was shown by TLC and GLC to contain glucose.

Acid Hydrolysis of 13 with 2N H_2SO_4——A solution of **13** (30 mg) in 2N H_2SO_4-dioxane (1: 1) was refluxed on a water bath for 1 hr. The reaction mixture was treated by the procedure described above. The aglycone, **2**, was found in the $CHCl_3$ extract, while digitalose and glucose were detected in the aqueous layer.

Degradation of 5 with $NaIO_4$——A solution of $NaIO_4$ (560 mg) in H_2O (8 ml) was added to a solution of **5** (340 mg) in 95% EtOH (60 ml) with stirring at room temperature for 1 hr. After removing the precipitate by filtration, EtOH was evaporated off under reduced pressure and the aqueous solution was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. A solution of the residue in 95% MeOH (40 ml) was treated with $NaBH_4$ (240 mg) in small portions at room temperature with stirring. The reaction mixture was stirred at room temperature for 1 hr, then the reaction mixture was neutralized with 5% AcOH, and concentrated *in vacuo* under 50°. The residue was extracted with $CHCl_3$ and the $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in 0.05N HCl–50% MeOH (10 ml) and the solution was refluxed for 30 min. The reaction mixture was neutralized with 0.1N $KHCO_3$ and, after addition of H_2O (5 ml), the solution was concentrated *in vacuo*. The residue was extracted with $CHCl_3$ and the $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with $CHCl_3$–MeOH (9: 1 v/v), to give a white powder (**14**). On recrystallization from EtOH, **14** was obtained as colorless needles, mp 278—279°, $[\alpha]_D^{30}$ −45.6° (c=1.51, MeOH), *Anal.* Calcd for $C_{28}H_{46}O_7$: C, 67.98; H, 9.37. Found: C, 67.78; H, 9.32. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), NMR (C_5D_5N) δ: 0.70 (3H, s, CH$_3$), 1.00 (3H, s, CH$_3$), 1.48 (6H, d, J=6 Hz, 2×CH$_3$), 3.42 (3H, s, OCH$_3$), 4.62 (1H, d, J=8 Hz, anomer H), 5.50 (1H, m, >C=CH). On hydrolysis with 2N H_2SO_4–50% dioxane under reflux for 1 hr, **14** gave **2** and digitalose.

Acknowledgement The authors are grateful to Prof. T. Reichstein, Institute für Organische Chemie der Universität Basel for providing an authentic sample and to Dr. H. Okazaki, Research Laboratory of Sankyo Co., Ltd. for supplying takadiastase A. We are grateful to Prof. Y. Kasuya and Dr. H. Saito, Faculty of Pharmaceutical Sciences, University of Tokyo, for valuable discussions on the potentiation of NGF-mediated nerve fiber outgrowth by saponins. Thanks are also due to the staff of the Analytical Laboratory of the School of Pharmaceutical Sciences, Showa University for elemental analysis and NMR spectral measurement. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged.