Phytochemistry, 1981, Vol. 20, pp. 139–142. © Pergamon Press Ltd. Printed in England. 0031-9422/81/0101-0139 $02.00/0

THE STRUCTURE OF PROSAPOGENIN OBTAINED FROM THE SAPONIN OF *GLEDITSIA JAPONICA*

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Key Word Index—*Gleditsia japonica*; Leguminosae; saponins; bisdesmoside; echinocystic acid derivatives; *Gleditsia* saponin C.

Abstract—Prosapogenin was obtained by alkaline hydrolysis of *Gleditsia* saponin GS-C (echinocystic acid 3, 28-*O*-bisdesmoside), a new triterpenoid saponin isolated from *Gleditsia japonica*. Prosapogenin was shown on the basis of chemical and physicochemical data to be echinocystic acid 3-*O*-β-D-xylopyranosyl-(1-2)-α-L-arabinopyranosyl-(1-6)-β-D-glucopyranoside.

INTRODUCTION

Gleditsia japonica Miquel (Leguminosae, Japanese name Saikachi) is a plant widely distributed in Japan, and its dried fruits (Sokyo in Japanese), have long been known in oriental medicine as a kind of saponin drug, and used, for example, as a diuretic and expectorant. There have been a number of studies [1–3] on a mixture of saponins and sapogenins from this plant but the structures of these compounds were not established. Recently, Hashimoto *et al.* [4] have reported that echinocystic acid was isolated as one of the sapogenins.

Our investigations revealed that there are two types of *G. japonica* Miq. in Japan; one of them contains a great deal of saponin, and the other contains, if any, amounts too small to be utilized as a source of saponin. In their external morphology, however, no remarkable differences in leaf, spine or legume were observed. Therefore the type of the plant used in the present work is here distinguished as *Gleditsia japonica* cv Saponifera.

This paper describes the isolation from *G. japonica* cv Saponifera of three new triterpenoid saponins which we have named *Gleditsia* saponin B, C and D (GS-B, GS-C and GS-D), and gives details of the structure elucidation of their prosapogenin (**1**).

RESULTS AND DISCUSSION

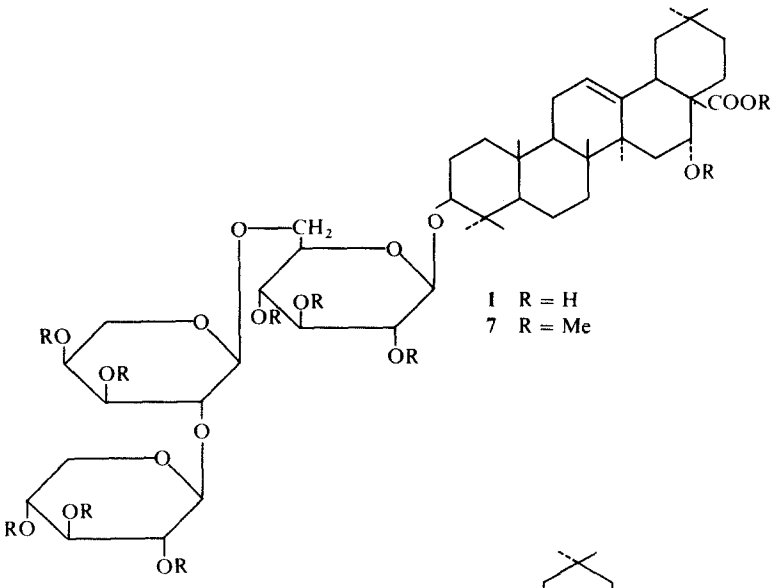
The fruit of *Gleditsia japonica* cv Saponifera was extracted with hot 50% MeOH and the extract was treated as described in the experimental. The crude saponin mixture was separated by chromatography over Si gel using $CHCl_3$–MeOH–H_2O (65:35:10; lower layer). A GS-C rich fraction was rechromatographed in the same manner several times, further purified by gel filtration on Sephadex LH20, and reprecipitated with MeOH–Et_2O to give a chromatographically pure saponin, GS-C. GS-B and GS-D were obtained in a similar manner.

The IR spectrum indicated many hydroxyl groups (3400–3600 cm^{-1}) and ester groups (1725 cm^{-1}). Hydrolysis of GS-C with 2 N H_2SO_4 in EtOH gave aglycone (**2**) and the four sugars (glucose, arabinose, xylose and rhamnose) which were identified by preparative and TLC. The aglycone (**2**) was presumed to be echinocystic acid from its physical data. Compound **2** was methylated with CH_2N_2 to give the methyl ester (**3**) and it was reduced with $LiAlH_4$ to a substance which was identified as primulagenin A (**4**) through comparison by mmp, TLC and IR spectrum with an authentic sample of primulagenin A derived from the saponin of *Primula sieboldi* [5].

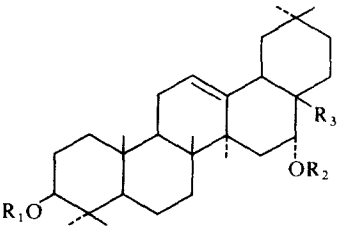
Alkaline hydrolysis of GS-B, GS-C and GS-D with 20% KOH in EtOH afforded the same prosapogenin (**1**) which had the composition $C_{46}H_{74}O_{17}$. Prosapogenin (**1**) gave aglycone (**2**) and three sugars (glucose, arabinose and xylose) by acid hydrolysis with 2 N H_2SO_4 in EtOH. Partial hydrolysis of **1** with 0.6 N H_2SO_4 afforded compounds **5** and **6**. Compounds **5** and **6** gave by acid hydrolysis with 2 N H_2SO_4, respectively, genin (**2**), glucose and arabinose, and genin (**2**) and glucose.

The ^{13}C NMR chemical shift values of prosapogenin (**1**), compounds **5** and **6** are shown in Table 1. The signal of the glucose C-6 shifted to lower field + 8.9 ppm, (63.1 ppm of **6** to 72.0 of **1** and **5**), and the glucose C-5 shifted to an upper field (77.9 ppm of **6** to 75.8 of **1** and 76.3 of **5**). The signal of the arabinose C-2 shifted to a lower field (69.7 ppm of **5** to 80.0 of **1**) and arabinose C-1 and C-3 shifted upfield (104.9 ppm of **5** and 74.0 of **5** to 102.2 of **1** and 72.4 of **1**, respectively). These glycosidation shifts show [6] that prosapogenin (**1**) had the sugar moiety xylosyl-(1-2)-arabinosyl-(1-6)-glucoside.

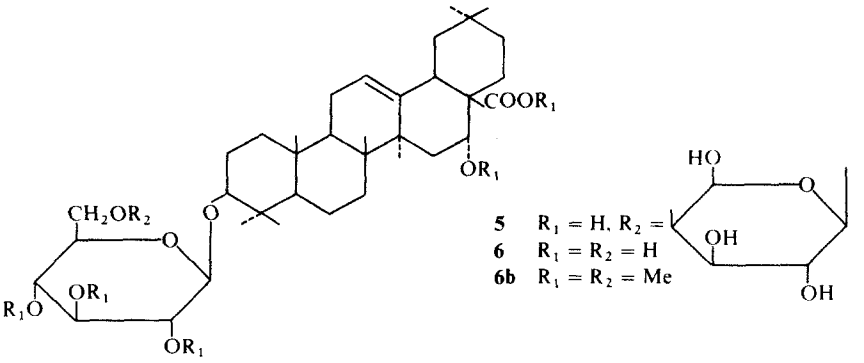
Prosapogenin (**1**) was methylated by Hakomori's method [7] to afford the permethylate (**7**), and methanolysis of **7** with 6% HCl in dried MeOH gave three methylated sugars, methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside, methyl 3,4-di-*O*-methyl-L-arabinopyranoside and methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside and compound **8**. These *O*-methylated monosaccharides were identified by TLC and GLC with authentic samples, and this confirmed the conclusion derived from the ^{13}C NMR spectra.



1 R = H
7 R = Me



2 $R_1 = R_2 = H$, $R_3 = COOH$
3 $R_1 = R_2 = H$, $R_3 = COOMe$
4 $R_1 = R_2 = H$, $R_3 = CH_2OH$
8 $R_1 = H$, $R_2 = Me$, $R_3 = COOMe$



5 $R_1 = H$, $R_2 =$
6 $R_1 = R_2 = H$
6b $R_1 = R_2 = Me$

The configuration of each sugar was deduced as follows. The ^1H NMR signals of three anomeric protons, δ 4.25 (*d*, $J = 7$ Hz), 4.47 (*d*, $J = 7$ Hz) and 4.60 (*d*, $J = 5$ Hz), were observed, and the molecular rotation differences [8] between **2** and **6**, **5** and **6**, and between **5** and **1**, were $-112°$, $-120°$ and $-24°$, respectively. These facts suggest that glucose and xylose were both in the β-form, and arabinose was in the α-form.

The MS of compound **8** showed a strong fragment ion peak at *m/e* 292, which arose from a typical retro Diels–Alder fragmentation [9]. Therefore the oligosaccharide moiety of prosapogenin (**1**) must be attached to the C-3 hydroxyl group of echinocystic acid, and not to the C-16 hydroxyl group. The structure of prosapogenin (**1**) was therefore established as echinocystic acid 3-*O*-β-D-xylopyranosyl-(1-2)-α-L-arabinopyranosyl-(1-6)-β-D-glucopyranoside, and this prosapogenin was common to GS-B, GS-C and GS-D.

EXPERIMENTAL

Mps were uncorr. The ^1H NMR spectra were measured in $CDCl_3$, and the ^{13}C NMR spectra were measured in C_5D_5N, with TMS as an int. standard. GLC was carried out on a 200 × 0.3 cm 15% NEGS on chromosorb W, column temp. 175°, carrier gas N_2 (30 ml/min).

Extraction and isolation. *G. japonica* fruits were collected in November 1978, at Hikone, Shiga Prefecture, Japan. The crushed

Table 1. ^{13}C NMR chemical shift values (ppm) of compounds **1**, **5** and **6**.

	Prosapogenin **1**	**5**	**6**
Glucose C-1	106.4	106.6	106.6
C-2	75.4	75.4	75.7
C-3	78.0	78.3	78.6
C-4	71.9	71.7	71.9
C-5	75.8	76.3	77.9
C-6	72.0	72.0	63.1
Arabinose C-1	102.2	104.9	
C-2	80.0	69.7	
C-3	72.4	74.0	
C-4	67.4	68.7	
C-5	64.2	65.9	
Xylose C-1	105.8		
C-2	75.0		
C-3	77.5		
C-4	70.6		
C-5	66.9		

dried fruits (2 kg) were extracted with 50% hot MeOH (10 l.) and evapd under red. pres., the residue was dissolved in H_2O, and extracted with EtOAc. The aq. layer was extracted with *n*-BuOH, and the organic layer was concentrated. This extract was dissolved in MeOH and treated with active charcoal to afford the crude saponins (yield *ca* 45 g). The crude saponin fraction was examined by TLC on Si gel using $CHCl_3$–MeOH–H_2O (65:35:10, lower layer) as developing solvent. The fraction contained more than ten saponins, and the major saponin (R_f, 0.15) was named *Gleditsia* saponin C. The crude saponin was chromatographed over Si gel using the same solvent. The saponin C-rich fraction was repeatedly rechromatographed using $CHCl_3$–MeOH–H_2O (8:3:1, lower layer). Chromatographically pure GS-C was obtained (yield *ca* 1.5 g) by filtration on Sephadex LH20 using MeOH as eluants, followed by repeated precipitations with MeOH–Et_2O. GS-C was a colourless powder, mp 192–193°, $[\alpha]_D^{19}$ − 24.2° (MeOH, c = 1.02), IR ν_{max}^{KBr} cm^{-1}: 3400–3600 (OH), 1725 (COOR).

Hydrolysis of GS-C. To the soln of GS-C (200 mg) in 10 ml EtOH was added 10 ml 2 N H_2SO_4 and the mixture was refluxed for 5 hr. The soln was concd to 10 ml under red. pres., and the residue was extracted with Et_2O. The aq. layer was neutralized with Amberlite IR 45, and concd to 1 ml. The residue was found to be mixture of glucose, arabinose, xylose and rhamnose by TLC and PC. TLC: EtOAc–*i*-PrOH–H_2O (32:12:6), detection with aniline hydrogen phthalate. PC: *i*-PrOH–*n*-BuOH–H_2O (7:1:2), detection was effected with the same reagent as TLC. The organic layer was washed with H_2O, dried ($MgSO_4$) and evapd. The residue was repeatedly recrystallized from MeOH to give **2** colourless needles (30 mg), mp 308–309°, $[\alpha]_D^{19}$ + 39° (95% EtOH, c = 1.0), IR ν_{max}^{KBr} cm^{-1}: 3400–3500 (OH), 1685 (COOH). ^1H NMR (C_5D_5N): δ 0.95, 1.02, 1.06, 1.19, 1.22, 1.82 (total 21 H, Me × 7), 5.23 (1H, *br. s*), 5.65 (1H, *br. s*), 8.69 (1H, *s*, COOH). MS *m/e*: 472 (M^+), 264, 246, 201. (Calc. for $C_{30}H_{48}O_4 \cdot 1/2\,H_2O$: C, 75.11; H, 10.29. Found: C, 75.26; H, 10.31%).

Methylation of **2**. To the soln of **2** (50 mg) in MeOH (20 ml) was added an ethereal CH_2N_2 soln. The mixture was kept standing for 1 day, and worked up by the usual method to give 35 mg of colourless needles of **3**, mp 217–219° (from MeOH). $[\alpha]_D^{19}$ + 33° (MeOH, c = 0.50). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1725 (COOMe). ^1H NMR ($CDCl_3$): δ 0.74, 0.79, 0.93, 1.00, 1.36 (total 21H, Me × 7), 3.61 (3H, *s*, COOMe), 4.50 (1H, *br. s*), 5.37 (1H, *t*, J = 4 Hz), MS *m/e*: 486 (M^+), 468, 278, 260, 201. (Calc. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.37; H, 10.49%).

Reduction of methyl ester **3**. To the soln of the methyl ester (**3**) (30 mg) in THF (30 ml) was added, in portions, 80 mg of $LiAlH_4$ and the reaction mixture was refluxed for 5 hr. Excess $LiAlH_4$ was decomposed with wet Et_2O and the reaction mixture was extracted with Et_2O. The extract was washed with H_2O, dried ($MgSO_4$) and evapd. The residue was repeatedly recrystallized from Me_2CO–hexane to give 12 mg of silky needles, mp 242–244°, identified with an authentic sample of primulagenin A (**4**) by comparison of IR spectrum and TLC and by mmp.

Hydrolysis of GS-C. A soln of GS-C (300 mg) in 20% KOH (30 ml) and EtOH (30 ml) was refluxed for 3 hr. The reaction mixture was cooled to room temp. and neutralized with 10% H_2SO_4 under ice-cooling. The neutral soln was concd under red. pres. and residue was extracted with EtOAc. The extract was dried ($MgSO_4$) and evapd to leave an oily substance (110 mg). This oil was chromatographed on a Si gel column using $CHCl_3$–MeOH–H_2O (65:35:10, lower layer), and the eluant was reprecipitated from MeOH–Et_2O to give **1**, colourless powder (70 mg), mp 219–222, $[\alpha]_D^{16}$ − 11.7° (MeOH, c = 1.01). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (COOH). (Calc. for $C_{46}H_{74}O_{17} \cdot 5/2\,H_2O$: C, 58.52; H, 8.43. Found: C, 58.52; H, 8.40%).

Hydrolysis of **1** *with* 2 N H_2SO_4. A soln of **1** (20 mg) in 2 N H_2SO_4–EtOH (10 ml) was refluxed for 2 hr and extracted with EtOAc. Echinocystic acid was obtained from the organic layer by treatment in a similar manner to that described above. From the aq. layer, glucose, arabinose and xylose were obtained and characterized by TLC and PC.

Permethylation of **1**. According to Hakomori's method, NaH (500 mg) was stirred with DMSO (30 ml) at 80° for 30 min under N_2 gas. To this reagent (10 ml), **1** (60 mg) in DMSO (10 ml) was added and the reaction mixture was stirred for 1 hr at room temp. under N_2 gas. MeI (10 ml) was added and the reaction mixture

was stirred for 3 hr at room temp. The reaction mixture was poured into ice-water and extracted with EtOAc. The organic layer was washed with 5% $Na_2S_2O_3$ soln and H_2O, dried ($MgSO_4$) and evapd under red. pres. to give 65 mg of oily product. This oil was separated by PLC using C_6H_6–Me_2CO (3:1) to afford **7**, colourless powder (42 mg), $[\alpha]_D^{18}$ − 29.5° (MeOH, c = 1.00), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (COOMe). ^{1}H NMR ($CDCl_3$): δ 0.17, 0.84, 0.87, 0.93, 0.95, 1.02, 1.28 (each 3H, *s*, Me), 3.31, 3.43, 3.46, 3.47, 3.52, 3.58, 3.59, 3.62 (each 3H, *s*, OMe), 3.60 (6H, *s*, OMe × 2), 4.25 (1H, *d*, J = 7 Hz, amomeric H), 4.47 (1H, *d*, J = 7 Hz, anomeric H), 4.60 (1H, *d*, J = 5 Hz, anomeric H). (Calc. for $C_{56}H_{94}O_{17}$: C, 64.71; H, 9.12. Found: C, 64.47; H, 9.06%).

Methanolysis of **7**. A soln of **7** (30 mg) in methanolic 6% HCl (4 ml) was refluxed for 2 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evapd under red. pres. and the residue was separated by PLC (C_6H_6–Me_2CO, 5:2). Each *O*-methylated sugar was identified with the authentic sample by TLC and GLC. R_t: 2′12″, 2′35″ (methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside), 6′25″, 12′00″ (methyl 3,4-di-*O*-methyl-L-arabino-pyranoside), 8′18″, 10′57″ (methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside). Methyl 16-*O*-methylechinocystate (**8**) was obtained by the method described above. It was recrystallized from MeOH–H_2O to give colourless needles, mp 167–168°, $[\alpha]_D^{21}$ + 12.4 ($CHCl_3$, c = 0.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1725 (COOMe). ^{1}H NMR ($CDCl_3$): δ 0.71, 0.78, 0.85, 0.92, 0.95, 0.98, 1.29 (each 3H, *s*, Me), 3.30 (3H, *s*, OMe), 3.58 (3H, *s*, COOMe), 3.92 (1 H, *br. s*), 5.33 (1 H, *t*, J = 3 Hz); MS *m/e*: 500 (M^+), 468, 440, 292, 260, 201. (Calc. for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.54; H, 10.73%).

Partial hydrolysis of **1**. A soln of **1** (800 mg) in 120 ml 0.6 N H_2SO_4–EtOH (1:1) was refluxed for 2.5 hr. The reaction mixture was cooled to room temp. and diluted with 50 ml H_2O. This soln was neutralized with 5% NaOH soln and concd under red. pres. The residue was extracted with *n*-BuOH and the organic layer was washed with satd. NaCl soln and evapd to give an oily product. This oil was fractionated by chromatography over Si gel using $CHCl_3$–MeOH–H_2O [9:1:0.1 (a), 8:3:1 (b), 13:7:2 (c)]. Echinocystic acid (53 mg) was eluted with solvent (a). The fraction eluted with solvent (b) was recrystallized from MeOH to give **6**, colourless needles (120 mg), mp 267–268°, $[\alpha]_D^{19}$ + 6.1° (MeOH, c = 1.00). IR ν_{max}^{KBr} cm^{-1}: 3400–3600 (OH), 1670 (COOH). (Calc. for: $C_{36}H_{58}O_9 \cdot 1/2\ H_2O$: C, 67.15; H, 9.24. Found: C, 67.00; H, 9.30%). The fraction eluted with solvent (c) was recrystallized from MeOH–H_2O to give **5**, 165 mg colourless needles, mp 225–227°, $[\alpha]_D^{19}$ − 10.7 (MeOH, c = 1.03). IR ν_{max}^{KBr} cm^{-1}: 3600 (OH), 1670 (COOH). (Calc. for $C_{41}H_{66}O_{13} \cdot 2H_2O$: C, 61.32; H, 8.79. Found: C, 61.17; H, 9.04%).

Hydrolysis of **5** *and* **6**. Compound **5** was hydrolysed with 2 N H_2SO_4–EtOH in the same way as for GS-C to give echinocystic acid, glucose and arabinose. These sugars were identified by TLC and PC. Compound **6** was hydrolysed as described above to give echinocystic acid and glucose.

Permethylation and methanolysis of **6**. Compound **6** was methylated by Hakomori's method as for prosapogenin (**1**). The crude product was separated by PLC (C_6H_6–Me_2CO, 3:1) to give 70 mg of pentamethyl ether (**6b**), $[\alpha]_D^{16}$ − 11.8 (MeOH, c = 1.00). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH (nil), 1725 (COOMe). ^{1}H NMR ($CDCl_3$): δ 0.70, 0.84, 0.87, 0.92, 0.95, 1.03, 1.29 (each 3H, *s*, Me), 3.30, 3.39, 3.52, 3.58, 3.60, 3.63 (each 3H, *s*, OMe), 3.93 (1H, *br. s*), 4.27 (1H, *d*, J = 7 Hz, anomeric H), 5.33 (1H, *t*). Compound **6b** was methanolysed by the same method as for **7** to give methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside. This methyl glucoside was identified with an authentic sample by TLC and GLC. R_t: 3′36″ and 4′54″.

Acknowledgements—Our thanks are due to Emeritous Professor S. Kitamura, Kyoto University, for identifying and naming the species. We thank Professor I. Kitagawa of Osaka University for the generous gifts of primulagenin A and methylated sugars, and his helpful discussion and suggestions. We are also grateful to Dr. Kuroda of Kyoto University and to Mr. Fujiwara of this College for the ^{1}H NMR and ^{13}C NMR spectra.

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