Chemistry of Natural Compounds, Vol. 31, No. 5, 1995

TRITERPENE GLYCOSIDES OF *Sophora japonica* SEEDS

V. I. Grishkovets and L. A. Gorbacheva

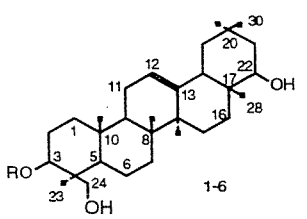
UDC 547.918:543.422

We have isolated from the seeds of Sophora japonica *the known soyasaponogenol B 3-[O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranuronoside] (adzukisaponin II), soyasapogenol B [3-O-β-galactopyranosyl-(1→2)-O-β-D-glucopyranuronoside] (soyasaponin III), soyasapogenol B 3-[O-α-L-rhamnopyranosyl-(1 → 2)-O-β-L-glucopyranosyl-(1→2)-O-β-D-glucopyranuronoside] (adzukisaponin V), soyasapogenol B 3-[O-α-D-rhamnopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→2)-O-β-D-glucopyranuronoside] (soyasaponin I), and the new glycoside (1) — soyasapogenol B 3-[O-β-D-glucopyranuronoside]. The structure of this glycoside has been established on the basis of the results of enzymatic, complete, and partial hydrolyses and ^{13}C NMR spectra.*

We have previously established the immunological activity of extracts of the fruit of the Japanese pagoda tree (*Sophora japonica*, fam. Fabaceae) [1], and in the present paper we give the results of a study of the glycoside composition of the fruit of this plant.

Preparative TLC analysis of the pericarp (flesh of the fruit) and seeds, taken separately, showed the presence of a large amount of triterpene glycosides in the seeds and trace amounts in the pericarp, together with a large amount of phenolic glycosides in both parts of the fruit. The plant seeds were subjected to more detailed study.

To isolate the triterpene glycosides, the seeds were ground and were defatted with hexane, and the glycosides were extracted with water-saturated butanol. TLC analysis of the butanol extract showed the presence of at least five glycosides, designated as (**1**)-(**5**) in order of increasing polarity. After evaporation, the butanol extract was subjected to a preliminary chromatographic separation on silica gel L into three fractions, F-1 – F-3 with gradient elution by a chloroform – ethanol – water solvent system. According to TLC, fraction F-1 contained mainly glycoside (**1**), fraction F-2 a combination of (**2**) and (**3**), and F-3 a combination of (**4**) and (**5**) together with a large amount of phenolic compounds.



1-6

	R
1	β-D-GlcUA$_p$ →
2	β-D-Glc$_p$ $\xrightarrow{2}$ β-D-GlcUA$_p$ →
3	β-D-Gal$_p$ $\xrightarrow{2}$ β-D-GlcUA$_p$ →
4	α-L-Rha$_p$ $\xrightarrow{2}$ β-D-Glc$_p$ $\xrightarrow{2}$ β-D-GlcUA$_p$ →
5	α-L-Rha$_p$ $\xrightarrow{2}$ β-D-Gal$_p$ $\xrightarrow{2}$ β-D-GlcUA$_p$ →
6	H

The fractions were purified on silica gel with elution by the chloroform – ethanol – aqueous ammonia solvent system, which permitted the elimination of a large part of the phenolic compounds, these being converted into phenolates and retained in the column under the alkaline conditions prevailing. The separation of pairs of glycosides with close mobilities — (**2**) and (**3**), and (**4**) and (**5**) — was achieved by chromatography on Silpearl microspherical silica gel, with elution by the chloroform – ethanol – aqueous ammonia system, which enabled the necessary efficiency of the column to be created.

The complete acid hydrolysis of glycosides (**1**)-(**5**) led to one and the same aglycon, identified by TLC, physical constants, and ^{13}C NMR as soyasapogenol B (**6**), which is typical for glycosides from plants of the Fabaceae family [2].

Simferopol' State University and Kursk State Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 709-713, September-October, 1995. Original article submitted March 3, 1995.

0009-3130/95/3105-0596$12.50

TABLE 1. Chemical Shifts of the ^{13}C Atoms of the Aglycon Moieties of Glycosides (1-5) (δ, ppm, 0 — TMS, C_5D_5N)

C Atom	Compound					
	1	2	3	4	5	6
1	38.6	38.6	38.5	38.6	38.5	39.1
2	26.7	26.5	26.7	26.6	26.7	28.4
3	90.9	90.9	90.8	90.9	91.0	80.2
4	43.8	43.7	43.8	43.8	43.9	43.2
5	56.2	56.3	56.4	56.2	56.2	56.4
6	18.6	18.7	18.7	19.0	19.0	19.2
7	33.3	33.3	33.2	33.3	33.4	33.6
8	40.0	40.0	40.0	40.1	40.0	40.1
9	47.8	47.7	47.8	47.8	47.9	48.1
10	36.5	36.5	36.6	36.7	36.8	37.3
11	24.1	24.1	24.0	24.1	24.0	24.1
12	122.4	122.5	122.5	122.5	122.6	122.5
13	144.9	144.9	145.0	144.9	144.8	144.9
14	42.4	42.4	42.5	42.6	42.5	42.4
15	26.5	26.5	26.6	26.5	26.7	26.5
16	28.7	28.7	28.8	28.7	28.9	28.7
17	38.0	38.0	38.1	38.0	38.1	38.2
18	45.3	45.4	45.5	45.4	45.4	45.4
19	46.8	46.9	46.9	46.9	46.8	46.8
20	30.9	30.9	31.0	30.9	31.1	30.9
21	42.4	42.4	42.5	42.6	42.5	42.3
22	75.6	75.6	75.6	75.7	75.5	75.5
23	23.0	23.0	23.1	23.0	23.2	23.6
24	63.4	63.4	63.5	63.4	64.0	64.7
25	15.7	15.8	15.7	15.9	16.0	16.3
26	17.0	17.1	17.0	17.2	17.1	17.2
27	25.8	25.8	25.7	25.8	25.7	25.7
28	28.7	28.6	28.7	28.8	28.7	28.7
29	33.3	33.4	33.3	33.4	33.3	33.3
30	21.2	21.2	21.1	22.0	21.2	21.1

In acid and enzymatic hydrolyzates of (**1**), glucuronic acid was identified. The position of the glucuronic acid residue at the C-3 atom of the aglycon followed from a comparison of the ^{13}C NMR spectra of (**1**) and (**6**) (Table 1). This showed a large positive α-effect at the C-3 atom (+11 ppm) and β-effects on the neighboring atoms C-2 (−2.1 ppm) and C-4 (+0.6 ppm). The chemical shifts of the signals of the other C-atoms of the aglycon moiety of (**1**) and (**6**) were practically identical, and the remaining six signals in the carbohydrate moiety agreed with literature figures for a β-D-glucuronic acid residue [3]. Consequently glycoside (**1**) was soyasapogenol B 3-O-β-*D*-glucopyranuronoside, a new triterpene glycoside.

According to the results of full acid hydrolysis the carbohydrate composition of (**2**) was represented by glucose and glucuronic acid. The ^{13}C NMR spectrum of (**2**) revealed, in addition to the signals of the aglycon moiety, practically identical with those of (**1**), 12 signals of carbohydrate C-atoms. Consequently, (**2**) was a bioside of soyasapogenol B. Partial hydrolysis of (**2**) gave glucose and (**1**). The signals of the glucose residue in the ^{13}C NMR spectrum of (**2**) were found by comparison with literature figures [4] for a terminal (unsubstituted) β-*D*-glucopyranoside. A comparison of the spectra of (**2**) and (**1**) revealed an α-effect (+5.5 ppm) on the C-2 atom of the glucuronic acid residue and β-effects (−3.7 and −2.5 ppm) on C-1 and C-3, which showed a 1→2 type of bond between the monosaccharide residues and the definitive structure of (**2**) as soyasapogenol B 3-[O-β-*D*-glucopyranosyl-(1→2)-O-β-*D*-glucopyranuronoside], identical in structure to adzukisaponin-II from seeds of *Vigna angularis* [5]. The epigeal part of *Galega officinalis* [6], and buds of *Sophora japonica* [7].

The acid hydrolysis of (**3**), as compared with that of (**2**), gave galactose in place of glucose. The structure of (**3**) was established in a similar manner to that of (**2**), as soyasapogenol B 3-[O-β-*D*-galactopyranosyl-(1→2)-O-β-*D*-glucopyranuronoside], identical in structure with soyasaponin III, isolated previously from *Glycine max* beans (soybeans) [8] and *Sophora japonica* buds [7].

The acid hydrolysis of (**4**) yielded rhamnose, glucose, and glucuronic acid, and that of (**5**) gave rhamnose, galactose, and glucuronic acid. Partial acid hydrolysis of (**4**) and (**5**) formed rhamnose, which was, therefore, the terminal monosaccharide, and progenins identical with (**2**) and (**3**), respectively. The types of bonds between the terminal rhamnose residues, the signals of which were identical with those given in the literature for an unsubstituted α-*L*-rhamnopyranoyl residue [4], and glucose or galactose, respectively (in (**4**) or (**5**)), were determined by comparing the ^{13}C NMR spectra of (**4**) with (**2**) and of (**5**) with (**3**) and by an analysis of glycosylation effects, as described for (**2**) and (**3**). This showed the 1→2 type of bond between the rhamnose and glucose or galactose residues. Thus, (**4**) was soyasapogenol B 3-[O-α-*L*-rhamnopyranosyl-(1→2)-O-β-*D*-glucopyranosyl-(1→2)-glucopyranuronoside], identical in structure with adzukisaponin-V from

TABLE 2. Chemical Shifts of the ^{13}C Atoms of the Carbohydrate Moieties of Glycosides (1-5) (δ, ppm, 0 — TMS, C_5D_5N)

C atom	Compound				
	1	2	3	4	5
	GlcUA	GlcUA	GlcUA	GlcUA	GlcUA
1′	107.2	105.0	104.9	104.8	104.6
2′	75.5	81.8	80.9	81.0	80.8
3′	78.1	75.7	75.6	75.8	76.3
4′	73.5	73.0	73.4	73.1	72.5
5′	78.0	78.3	77.0	76.6	76.3
6′	172.1	172.0	171.9	172.0	172.1
		Glc	Gal	Glc	Gal
1″		104.6	105.3	103.7	103.0
2″		76.3	72.5	84.0	84.4
3″		78.4	75.6	78.1	74.4
4″		71.0	70.9	71.4	70.3
5″		78.1	77.0	78.0	77.7
6″		62.7	62.5	62.8	62.4
				Rha	Rha
1‴				102.8	102.8
2‴				72.5	72.3
3‴				72.7	72.6
4‴				73.9	73.7
5‴				70.4	70.6
6‴				18.6	18.4

Vigna angularis seeds [5] and *Sophora japonica* buds [7], while (**5**) was soyasapogenol B 3-[O-α-*L*-rhamnopyranosyl-(1→2)-O-β-*D*-galactopyranosyl-(1→2)-glucopyranuronoside], identical in structure with soyasaponin I from *Sophora japonica* buds [7], *Glycine max* beans [9] and *Sophora flavescens* roots [10].

Trace amounts of (**4**) and (**5**) were identified in the fruit of *Sophora japonica* by TLC.

EXPERIMENTAL

NMR spectra were recorded on a Bruker HX-90E instrument using solutions in deuteropyridine (C_5D_5N) at 40°C. Specific rotations were measured on a SU-4 saccharimeter at 589 nm (the sodium D-line).

TLC was conducted on Silufol plates in the solvent systems chloroform–methanol–water (100:30:5) and (100:40:7) and chloroform–methanol–25% aqueous ammonia (100:40:10) and (100:45:15) for the glycosides; chloroform–methanol–25% aqueous ammonia (100:50:17) for the sugars; and benzene–acetone (8:1) for the aglycons. To detect the spots of the glycosides of the aglycons, we used a 20% solution of tungstophosphoric acid in chloroform–ethanol (4:1), and, to detect the sugars, acid aniline phthalate (10% solution in ethanol), followed by the heating of the chromatograms.

Complete acid hydrolysis of the glycosides was accomplished with 2 N CF_3COOH in water–dioxane (1:1) at 100°C for 2 h. Partial acid hydrolysis was performed with 1 N CF_3COOH in water–dioxane (1:1) at 100°C for 30 min, in order to split out glucose or galactose, or 15 min, to split out rhamnose. Enzymatic hydrolysis was carried out with β-glucuronidase (EC 3.2.1.31) at pH 7 (10 mg of enzyme to 10 mg of glycoside in 1 ml of water) for 10 h.

Isolation of Glycosides (1-5). *Sophora japonica* seeds (400 g) were ground and were defatted with hexane (3 × 1.5 liter), and the glycosides were extracted with water-saturated butanol (4 × 1 liter) at 60-80°C. The combined butanol extracts were evaporated to dryness, giving 48 g of residue. According to TLC, the glycosides obtained in this way contained — in addition to phenolic compounds (yellow-brown spots on revelation with tungstophosphoric acid) — triterpene glycosides (violet coloration of the spots) designated as compounds (**1**)-(**5**) in order of increasing polarity.

The total amount of glycosides, 48 g, was separated on 2.5 kg of silica gel L with gradient elution by the water-saturated chloroform–ethanol (10:1 → 1:1) solvent system. This gave glycoside fractions F-1 (3.0 g), F-2 (3.5 g), and F-3 (12.0 g). Fractions F-1 to F-3 were freed from phenolic compounds chromatographically on 0.5 kg of silica gel L with gradient elution by the (25% aqueous ammonia)-saturated chloroform–ethanol (2:1 → 1:1) system. Fraction F-1 yielded 100 mg of (**1**); F-2, 1.0 g of (**2**) + (**3**); and F-3, 2.5 g of (**4**) + (**5**). The separation of the purified fractions F-2 and F-3 into the individual glycosides (**2**)-(**5**) was conducted on 250 g of Silpearl silica gel with elution by the (25% aqueous ammonia)-saturated chloroform–ethanol (2:1 → 1:1) system. This gave 250 mg of (**2**), 370 mg of (**3**), 800 mg of (**4**), and 1.2 g of (**5**).

The final purification of (**2**)-(**5**) was carried out chromatographically on silica gel L with elution by a water-saturated chloroform–ethanol (2:1 → 1:1) system. This gave 50 mg of (**1**), 0.2 g of (**2**), 0.3 g of (**3**), 0.6 g of (**4**), and 0.9 g of (**5**). The ^{13}C NMR spectra of (**1**)-(**5**) are given in Tables 1 and 2.

Acid Hydrolysis of (1)-(5). The complete acid hydrolysis of (**1**) gave glucuronic acid and the aglycon (**6**); (**2**) gave glucose, glucuronic acid, and (**6**); (**3**) — galactose, glucuronic acid, and (**6**); (**4**) — rhamnose, glucose, glucuronic acid, and (**6**); (**5**) — rhamnose, galactose, glucuronic acid, and (**6**). After its extraction from the hydrolysates with chloroform, the aglycon (**6**) was identified by TLC with an authentic specimen of soyasapogenol B. As a result of additional chromatography, with elution by the carbon tetrachloride–acetone (10:1) system, pure (**6**) was obtained, with mp 257-260°C $[\alpha]_D$ +85° (*c* 2.0, chloroform). Lit. [11]: mp 260-261°C, $[\alpha]_D$ +90° (chloroform). The ^{13}C NMR spectrum of (**6**) was identical with that of soyasapogenol B [12].

Enzymatic Hydrolysis of (1)-(5). The enzymatic hydrolysis of (**1**) gave glucuronic acid and (**6**). Partial acid hydrolysis of (**2**) and (**3**) gave (**1**) and glucose and galactose, respectively. Partial acid hydrolysis of (**4**) and (**5**) led to rhamnose and (**2**) and (**3**), respectively.

REFERENCES

1. G. A. Drozd and L. A. Gorbacheva, Farmatsiya, No. 1, 34 (1994).
2. V. Ya. Chirva and T. V. Sergienko, Rast. Res., **24**, No. 2, 281 (1988).
3. H. Kizu, S. Kitayama, F. Nakatani, T. Tomimori, and T. Namba, Chem. Pharm. Bull., **33**, 3324 (1985).
4. S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, J. Am. Chem. Soc., **100**, 3331 (1978).
5. I. Kitagawa, H. K. Wang, M. Saito, and M. Yoshikawa, Chem. Pharm. Bull., **31**, 664, 674, 683 (1983).
6. T. Fukunaga, K. Nishiya, K. Takeya, and H. Itokawa, Chem. Pharm. Bull., **35**, 1610 (1987).
7. I. Kitagawa, T. Taniyama, W. W. Hong, K. Hori, and M. Yoshikawa, J. Pharm. Soc. Jpn., **108**, 538 (1988).
8. I. Kitagawa, M. Yoshikawa, and I. Yosioka, Chem. Pharm. Bull., **24**, 121 (1976).
9. I. Kitagawa, M. Yoshikawa, H. K. Wang, M. Saito, V. Tosirisuk, T. Fujiwara, and K. Tomita, Chem. Pharm. Bull, **30**, 2294 (1982).
10. M. Yoshikawa, H. K. Wang, H. Kayakiri, T. Taniyama, and I. Kitagawa, Chem. Pharm. Bull., **33**, 4267 (1985).
11. R. Tschesche and G. Wulff, Fortschr. Chem. Org. Naturst., **30**, 461 (1973)
12. J. Kinjo, I. Miyamoto, K. Murakami, K. Kida, T. Tomimatsu, M. Yamasaki, and T. Nohara, Chem. Pharm. Bull, **33**, 1293 (1983).