Medicinal Flowers. XII.[1] New Spirostane-Type Steroid Saponins with Antidiabetogenic Activity from *Borassus flabellifer*

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The methanolic extract from the male flowers of *Borassus flabellifer* was found to inhibit the increase of serum glucose levels in sucrose-loaded rats at a dose of 250 mg/kg, *p.o*. From the methanolic extract, six new spirostane-type steroid saponins, borassosides A—F (1—6), were isolated together with 23 known constituents. The structures of borassosides (1—6) were elucidated on the basis of chemical and physicochemical evidences. In addition, the principal steroid saponin, dioscin (13), inhibited the increase of serum glucose levels in sucrose-loaded rats at a dose of 50 mg/kg, *p.o*.

Key words *Borassus flabellifer*; antidiabetogenic activity; borassoside; 22β-*O*-spirostane-type steroid saponin; dioscin

The Palmae plant, *Borassus flabellifer* L. (palmyra palm in English), is widely distributed and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, *etc*. The fruit pulp of *B. flabellifer* has been used in traditional dishes and the sap, which was trapped from the flower part, has been used as a sweetener for diabetic patients. In the previous studies, several steroidal saponins,[2—4] a polysaccharide,[5] and a triterpene[6] constituents were isolated from the fruit pulp, seeds, and young shoot of *B. flabellifer*. However, the chemical and pharmacologic studies for the flower parts of this medicinal food were left uncharacterized. In the course of our characterization studies on Thai medicinal foods,[7—20] we found that the methanolic extract from the male flowers of *B. flabellifer* was found to inhibit the increase of serum glucose levels in sucrose-loaded rats. From the methanolic extract, six new spirostane-type steroid saponins, borassosides A—F (**1**—**6**), were isolated together with 20 known steroidal glycosides (**7**—**25** and β-sitosterol 3-*O*-β-D-glucopyranoside) and three known steroids (**26**, **27**, and β-sitosterol). This paper deals with the structure elucidation of borassosides A—F (**1**—**6**) from the male flowers of *B. flabellifer* as well as the inhibitory effects of the methanolic extract and a principal constituent, dioscin (**13**), on the increase of serum glucose levels in sucrose-loaded rats.

Extraction and Isolation The male and female flowers of *B. flabellifer* (collected in Thailand) were extracted with methanol to give methanolic extracts (12.2% from the male flowers; 11.3% from the female flowers). As shown in Table 1, the methanolic extract from the male flowers of *B. flabellifer* was found to inhibit the increase of serum glucose levels in sucrose-loaded rats at a dose of 250 mg/kg, *p.o.* However,

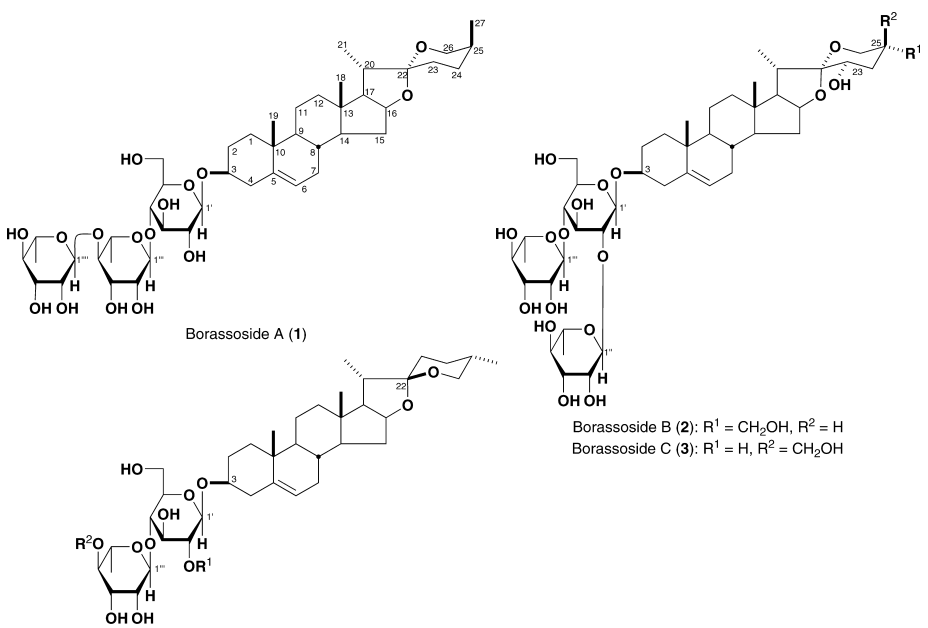

Chart 1

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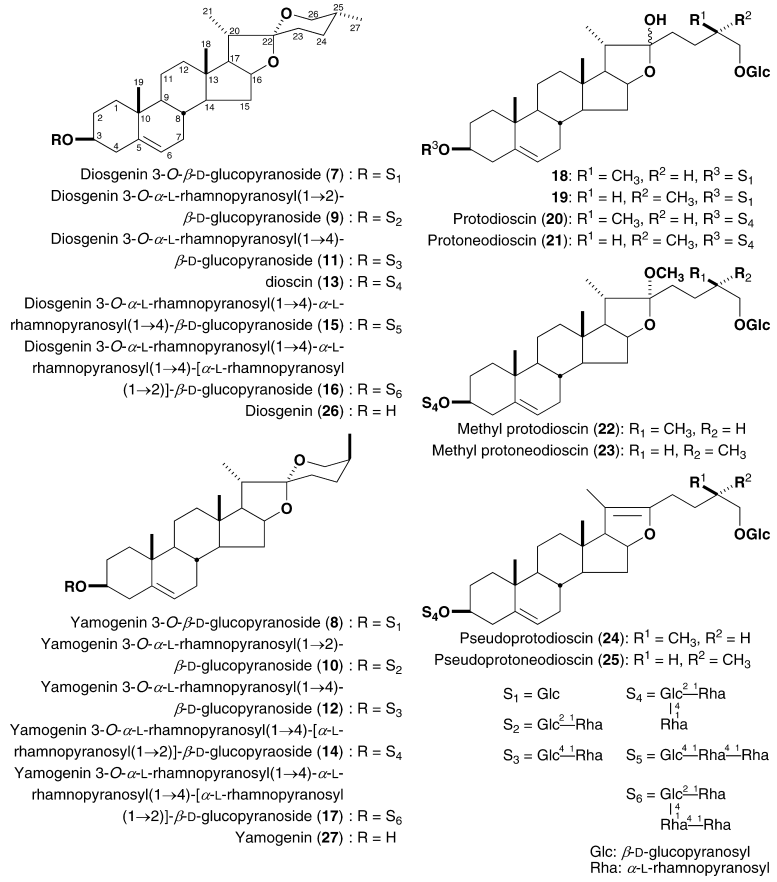

Chart 2

Table 1. Inhibitory Effects of the Methanolic Extracts from the Male and Female Flowers of *B. flabellifer* on the Increase in Serum Glucose Levels in Sucrose-Loaded Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose level (mg/dl)		
			0.5 h	1.0 h	2.0 h
Normal[a)]	—	5	69.2±4.6**	83.0±7.9**	93.4±12.5**
Control[b)]	—	6	175.8±2.8	156.0±6.3	134.9±4.1
MeOH Ext.[c)]					
Male flowers	250	5	147.1±6.6*	156.3±3.4	135.6±4.5
	500	5	123.6±10.0**	128.7±10.0**	142.5±8.4
Female flowers	250	5	167.0±7.4	149.1±3.0	128.4±2.6
	500	5	154.7±5.1	140.1±6.3	126.4±4.0

a) Acacia solution (5 ml/kg) and water (5 ml) were administrated orally. *b*) Acacia solution (5 ml/kg) and 20% sucrose solution (5 ml) were administrated orally. *c*) Test sample suspended in acacia solution (5 ml/kg) and 20% sucrose solution (5 ml) were administrated orally. Values represent the means±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

the methanolic extract from the female flowers did not show significant inhibition for the increase of serum glucose levels. The methanolic extract from the male flowers was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish the EtOAc-soluble fraction and an aqueous layer. The aqueous layer was extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH and H_2O-soluble fractions. From the *n*-BuOH-soluble fraction, borassosides A (**1**, 0.0010%), B (**2**, 0.0013%), C (**3**, 0.00072%), D (**4**, 0.00097%), E (**5**, 0.012%), and F (**6**, 0.00069%), diosgenin 3-*O*-β-D-glucopyranoside[21] (**7**, 0.00071%), yamogenin 3-*O*-β-D-glucopyranoside[22] (**8**, 0.00056%), diosgenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside[23] (**9**, 0.00087%) and yamogenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside[24] (**10**, 0.0012%), diosgenin 3-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranoside[21] (**11**, 0.013%), yamogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranoside[25] (**12**, 0.012%), diosgenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside[23] (**13**, 0.16%), yamogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside[26] (**14**, 0.13%), diosgenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranoside[27] (**15**, 0.0016%), diosgenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside[27] (**16**, 0.018%), yamogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-

rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside[26] (**17**, 0.012%), 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-β-D-glucopyranoside[28] (**18**, 0.00088%), 26-*O*-β-D-glucopyranosyl-(25*S*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-β-D-glucopyranoside[29] (**19**, 0.0020%), protodioscin[30] (**20**, 0.015%), protoneodioscin[31] (**21**, 0.0079%), methyl protodioscin[32] (**22**, 0.15%), methyl protoneodioscin[32] (**23**, 0.11%), pseudoprotodioscin[33] (**24**, 0.017%), pseudoprotoneodioscin[33] (**25**, 0.010%), diosgenin[34] (**26**, 0.00085%), yamogenin[34] (**27**, 0.00074%), β-sitosterol[35] (0.010%), and β-sitosterol 3-*O*-β-D-glucopyranoside[36] (0.0042%).

Structures of Borassosides A (1), B (2), C (3), D (4), E (5), and F (6) Borassoside A (**1**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{23}$ −89.6° in MeOH). The IR spectrum of **1** showed absorption bands at 3435, 1073, and 1060 cm^{-1} ascribable to hydroxyl and ether functions. In the positive- and negative-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 891 $(M+Na)^+$ and 867 $(M-H)^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **1** to be $C_{45}H_{72}O_{16}$. The acid hydrolysis of **1** with 2.0 M hydrochloric acid (HCl)–1,4-dioxane (1 : 1, v/v) liberated D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector.[37—39] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Tables 2, 3) spectra of **1**, which were assigned by various NMR experiments,[40] showed signals assignable to four methyls [δ 0.83, 0.92 (3H each, both s, 18, 19-H_3), 1.08, 1.15 (3H each, both d, *J*=6.9 Hz, 27, 21-H_3)], a methylene and two methines bearing an oxygen function {δ[3.37 (1H, br d, *J*=*ca.* 11 Hz), 4.06 (1H, dd, *J*=2.8, 11.0 Hz), 26-H_2], 3.88 (1H, m, 3-H), 4.51 (1H, m, 16-H)}, an olefin [δ 5.32 (1H, br d, *J*=*ca.* 5 Hz, 6-H)] together with a glucopyranosyl [δ 4.97 (1H, d, *J*=7.6 Hz, 1′-H)] and two rhamnopyranosyl moieties [δ 1.62, 1.70 (3H each, both d, *J*=6.2 Hz, 6‴, 6⁗-H_3), 5.86, 6.34 (1H each, both br s, 1‴, 1⁗-H)]. The proton and carbon signals due to the aglycon part in the ^{1}H- and ^{13}C-NMR data of **1** were superimposable on those of yamogenin glycosides (**8**—**14**), whereas the proton and carbon signals assigned to the trisaccharide moiety were very similar to those of **15**. As shown in Fig. 1, the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) experiment on **1** indicated the presence of partial structures written in bold lines, and the carbon skeleton and the positions of the oligoglycoside linkage were characterized by the heteronuclear multiple-bond correlations (HMBC) experiment, which showed long-range correlations between the following protons and carbons (1-H_2 and 10-C; 4-H_2 and 3, 5, 10-C; 6-H and 4, 10-C; 9-H and 10-C; 12-H_2 and 13-C; 17-H and 13-C; 18-H_3 and 12—14, 17-C; 19-H_3 and 1, 5, 9, 10-C; 20-H and 22, 23-C; 21-H_3 and 17, 20, 22-C; 23-H_2 and 22-C; 26-H_2 and 22-C; 27-H_3 and 24—26-C; 1′-H and 3-C; 1‴-H and 4″-C; 1⁗-H and 4‴-C). Next, the stereostructure of **1** was characterized by rotating Frame nuclear Overhauser and exchange spectroscopy (ROESY) experiment, which showed the NOE correlations between the following proton pairs [1α-H and 3-H; 1β-H and 19-H_3; 3-H and 4α-H; 4β-H and 19-H_3; 8-H and 18, 19-H_3; 14-H and 16-H; 16-H and 17-H, 26_{ax}-H (δ 4.06), 21-H_3; 17-H and 21-H_3; 18-H_3 and 20-H; 24_{ax}-H (δ 2.14) and 25-H, 26_{ax}-H; 26_{eq}-H (δ 3.37) and 27-H_3] (Fig. 1). On the basis of this evidence, the structure of borassoside A was determined to be yamogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranoside (**1**).

Borassosides B (**2**) and C (**3**) were also obtained as white powders with negative optical rotations (**2**: $[\alpha]_D^{23}$ −102.3°, **3**: $[\alpha]_D^{22}$ −91.1° both in MeOH). The IR spectrum of **2** and **3** showed similar absorption bands (**2**: 3652, 3569, 1070, 1052 cm^{-1}; **3**: 3453, 1073, 1051 cm^{-1}), ascribable to hydroxyl, and ether functions. The same molecular formula, $C_{45}H_{72}O_{18}$, of **2** and **3** were determined from the positive- and negative-ion FAB-MS [*m/z* 923 $(M+Na)^+$ and 899 $(M-H)^-$] and by high-resolution positive-ion FAB-MS. The acid hydrolysis of **2** and **3** with 2.0 M HCl–1,4-dioxane (1 : 1, v/v) liberated D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector, respectively.[37—39] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Tables 2, 3) spectra[40] of **2** showed signals assignable to three methyls [δ 0.98, 1.01 (3H each, both s, 19, 18-H_3), 1.21 (3H, d, *J*=6.9 Hz, 21-H_3)], two methylenes and three methines bearing an oxygen function [δ 3.69, 3.75 (1H each, both m, 27-H_2), 3.85 (1H, m, 3-H), 3.90, 4.09 (1H each, both d, *J*=*ca.* 11 Hz, 26-H_2), 3.97 (1H, dd, *J*=4.4, 11.3 Hz, 23-H), 4.65 (1H, m, 16-H)], an olefin [δ 5.27 (1H, br d, *J*=*ca.* 5 Hz, 6-H)] together with a glucopyranosyl [δ 4.95 (1H, d, *J*=7.6 Hz, 1′-H)] and two rhamnopyranosyl moieties [δ 1.62, 1.75 (3H each, both d, *J*=6.2 Hz, 6‴, 6⁗-H_3), 5.86, 6.40 (1H each, both br s, 1‴, 1⁗-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR data of **2** were superimposable on those of **13**, except for the signals due to the F ring in the aglycon part. As shown in Fig. 2, the ^{1}H–^{1}H COSY experiment on **2** indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons (1-H_2 and 10-C; 4-H_2 and 3, 5, 10-C; 6-H and 4, 10-C; 9-H and 10-C; 12-H_2 and 13-C; 16-H and 22-C; 17-H and 13-C; 18-H_3 and 12—14, 17-C; 19-H_3 and 1, 5, 9, 10-C; 20-H and 22, 23-C; 21-H_3 and 17, 20, 22-C; 23-H and 22-C; 26-H_2 and 22-C; 27-H_3 and 24—26-C; 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 4′-C). Thus the planar structure of the aglycon having the 23- and 27-hydroxyl groups and the triglycoside structure including the position of oligosugar linkage in **2** were clarified. The stereostructure of **2** was characterized by ROESY experiment, which showed the NOE correlations between the following proton pairs [16-H and 26_{ax}-H (δ 3.90); 20-H and 23_{ax}-H (δ 3.97); 21-H_3 and 23_{ax}-H; 23_{ax}-H and 24_{eq}-H (δ 2.31), 25-H; 24_{ax}-H (δ 2.04) and 27-H_2 (δ 3.69), 26_{ax}-H] as shown in Fig. 2. On the basis of the above-mentioned evidence, the structure of borassoside B was determined to be 23α,27-dihydroxydioscin (**2**).

The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Tables 2, 3) spectra[40] of **3** showed signals assignable to three methyls [δ 0.98, 0.99 (3H each, both s, 19, 18-H_3), 1.16 (3H, d, *J*=7.1 Hz, 21-H_3)], two methylenes and three methines bearing an oxygen function {δ 3.86 (1H, m, 3-H), [3.99 (1H, br d, *J*=*ca.* 11 Hz), 4.09 (1H, dd, *J*=2.9, 11.2 Hz), 26-H_2], 4.00, 4.18 (1H each, both m, 27-H_2), 4.07 (1H, m, 23-H), 4.62 (1H, m, 16-H)}, an olefin [δ 5.28 (1H, br d, *J*=*ca.* 5 Hz, 6-H)] together with a glucopyranosyl [δ 4.94 (1H, d, *J*=7.6 Hz, 1′-H)] and two rhamnopyranosyl moieties [δ 1.62, 1.75 (3H each, both d, *J*=6.2 Hz, 6‴, 6⁗-H_3), 5.86, 6.40 (1H each, both br s, 1‴, 1⁗-H)], which were very similar to those of **14**, except for the signals due to the F ring in the aglycon part. The planer

Table 2. ^{1}H- and ^{13}C-NMR (Pyridine-d_5) Data of Borassosides A—F (**1**—**6**) (Aglycon Part)

Position	**1**		**2**		**3**		**4**		**5**		**6**	
	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C
1	0.98 (m)	37.5	0.94 (m)	37.5	0.95 (m)	37.5	0.94 (m)	37.4	0.98 (m)	37.5	0.98 (m)	37.6
	1.70 (m)		1.70 (m)		1.70 (m)		1.67 (m)		1.72 (m)		1.72 (m)	
2	1.70 (m)	30.2	1.83 (m)	30.1	1.83 (m)	30.2	1.70 (m)	30.2	1.84 (m)	30.2	1.83 (m)	30.2
	2.07 (m)		2.07 (m)		2.05 (m)		2.05 (m)		2.07 (m)		2.07 (m)	
3	3.88 (m)	78.2	3.85 (m)	78.1	3.86 (m)	78.1	3.86 (m)	78.2	3.86 (m)	78.2	3.86 (m)	78.1
4	2.46 (dd, 11.6, 13.2)	39.3	2.69 (dd, 11.3, 13.0)	39.0	2.69 (dd, 11.9, 13.4)	39.0	2.43 (dd, 11.3, 13.4)	39.3	2.70 (dd, 11.5, 13.2)	39.0	2.70 (dd, 11.5, 13.2)	39.1
	2.71 (dd, 2.2, 13.2)		2.77 (dd, 2.4, 13.0)		2.78 (dd, 2.6, 13.4)		2.69 (dd, 2.4, 13.4)		2.79 (dd, 2.6, 13.2)		2.79 (dd, 3.8, 13.2)	
5		140.9		140.7		140.8		140.9		141.0		141.0
6	5.32 (br d, *ca.* 5)	121.8	5.27 (br d, *ca.* 5)	121.8	5.28 (br d, *ca.* 5)	121.8	5.31 (br d, *ca.* 5)	121.7	5.33 (br d, *ca.* 5)	121.8	5.33 (br d, *ca.* 5)	121.8
7	1.42 (m)	32.2[a)]	1.45 (m)	32.2[a)]	1.46 (m)	32.1[a)]	1.46 (m)	32.3	1.50 (m)	32.4	1.51 (m)	32.5
	1.84 (m)		1.83 (m)		1.82 (m)		1.84 (m)		1.89 (m)		1.89 (m)	
8	1.54 (m)	31.7	1.50 (m)	31.6	1.51 (m)	31.6	1.52 (m)	31.4	1.53 (m)	31.5	1.52 (m)	31.6
9	0.88 (m)	50.3	0.87 (m)	50.3	0.87 (m)	50.3	0.86 (m)	50.4	0.92 (m)	50.5	0.89 (m)	50.5
10		37.1		37.1		37.1		37.1		37.2		37.2
11	1.40 (m)	21.1	1.38 (m)	21.1	1.38 (m)	21.1	1.37 (m)	21.1	1.43 (m)	21.2	1.44 (m)	21.2
12	1.10 (m)	39.9	1.11 (m)	40.2	1.09 (m)	40.2	1.07 (m)	40.2	1.10 (m)	40.3	1.08 (m)	40.4
	1.69 (m)		1.72 (m)		1.71 (m)		1.62 (m)		1.68 (m)		1.69 (m)	
13		40.4		41.0		41.0		41.0		41.0		41.1
14	1.04 (m)	56.7	1.06 (m)	56.6	1.05 (m)	56.6	0.96 (m)	55.8	0.98 (m)	55.9	0.97 (m)	55.9
15	1.48 (m)	32.3[a)]	1.46 (m)	32.3[a)]	1.47 (m)	32.3[a)]	1.47 (m)	33.2	1.48 (m)	33.3	1.47 (m)	33.3
	2.00 (m)		2.01 (m)		2.02 (m)		1.99 (m)		2.03 (m)		2.02 (m)	
16	4.51 (m)	81.2	4.65 (m)	81.7	4.62 (m)	81.7	4.29 (m)	80.8	4.33 (m)	80.9	4.31 (m)	80.9
17	1.78 (dd, 6.2, 8.2)	62.7	1.89 (dd, 6.9, 8.6)	62.5	1.86 (dd, 7.0, 8.8)	62.4	1.57 (dd, 6.2, 8.9)	62.5	1.61 (dd, 6.2, 8.6)	62.7	1.59 (dd, 7.3, 8.3)	62.7
18	0.83 (s)	16.4	1.01 (s)	16.5	0.99 (s)	16.5	0.95 (s)	16.7	0.97 (s)	16.7	0.97 (s)	16.7
19	0.92 (s)	19.4	0.98 (s)	19.3	0.98 (s)	19.4	0.88 (s)	19.4	1.04 (s)	19.4	1.04 (s)	19.4
20	1.91 (m)	42.5	3.06 (m)	35.8	2.99 (m)	36.1	2.31 (dq, 6.8, 7.2)	42.1	2.32 (m)	42.2	2.32 (m)	42.2
21	1.15 (d, 6.9)	14.9	1.21 (d, 6.9)	14.7	1.16 (d, 7.1)	14.6	1.00 (d, 7.2)	16.7	1.02 (d, 7.2)	16.7	1.02 (d, 6.9)	16.8
22		109.7		112.1		112.5		110.6		110.6		110.7
23	1.36 (m)	26.2	3.97 (dd, 4.4, 11.3)	67.6	4.07 (m)	63.9	1.67 (2H, m)	28.2	1.67 (2H, m)	28.3	1.68 (2H, m)	28.4
	1.90 (m)											
24	1.44 (m)	26.4	2.04 (m)	33.6	2.32 (m)	30.0	1.56 (2H, m)	28.1	1.55 (2H, m)	28.2	1.57 (2H, m)	28.2
	2.14 (m)		2.31 (m)		2.39 (m)							
25	1.59 (m)	27.6	2.29 (m)	40.5	2.15 (m)	39.4	1.63 (m)	30.7	1.64 (m)	30.4	1.63 (m)	30.8
26	3.37 (br d, *ca.* 11)	65.1	3.90 (br d, *ca.* 11)	63.2	3.99 (br d, *ca.* 11)	59.7	3.68 (2H, d-like)	69.6	3.68 (2H, d-like)	69.6	3.68 (2H, d-like)	69.7
	4.06 (dd, 2.8, 11.0)		4.09 (br d, *ca.* 11)		4.09 (dd, 2.9, 11.2)							
27	1.08 (d, 6.9)	16.3	3.69 (m)	64.0	4.00 (m)	62.3	0.67 (d, 6.5)	17.3	0.70 (d, 6.3)	17.3	0.70 (d, 6.3)	17.3
			3.75 (m)		4.18 (m)							

a) May be interchangeable within the same column.

Table 3. ^{1}H- and ^{13}C-NMR (Pyridine-d_5) Data of Borassosides A—F (**1**—**6**) (Sugar Part)

Position	**1**		**2**		**3**		**4**		**5**		**6**	
	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C
3-*O*-Glc-1′	4.97 (d, 7.6)	102.5	4.95 (d, 7.6)	100.3	4.94 (d, 7.6)	100.3	4.97 (d, 7.6)	102.5	4.91 (d, 6.6)	100.3	4.92 (d, 6.6)	100.5
2′	4.00 (dd, 7.6, 8.9)	75.7	4.21 (m)	78.0	4.20 (m)	78.0	3.99 (dd, 7.6, 9.2)	75.5	4.18 (m)	77.9	4.18 (m)	78.2
3′	4.23 (m)	76.6	3.60 (m)	76.9	3.62 (m)	76.9	4.22 (m)	76.7	3.63 (m)	76.9	3.60 (m)	77.0
4′	4.49 (dd, 9.2, 9.2)	77.7	4.39 (dd, 9.2, 9.2)	78.6	4.39 (dd, 9.2, 9.2)	78.6	4.47 (dd, 9.3, 9.3)	78.3	4.30 (m)	79.0	4.35 (m)	78.2
5′	3.70 (m)	77.2	4.21 (m)	77.8	4.21 (m)	77.8	3.72 (m)	77.2	4.17 (m)	77.9	4.20 (m)	77.8
6′	4.11 (m)	61.5	4.07 (m)	61.3	4.07 (m)	61.3	4.13 (m)	61.5	4.07 (br d, *ca.* 11)	61.4	4.04 (br d, *ca.* 12)	61.4
	4.25 (m)		4.19 (m)		4.18 (m)		4.26 (br d, *ca.* 11)		4.20 (m)		4.18 (m)	
2′-*O*-Rha-1″			6.40 (br s)	102.0	6.40 (br s)	102.0			6.31 (br s)	102.0	6.34 (br s)	102.2
2″			4.82 (m)	72.5	4.83 (m)	72.5			4.79 (m)	72.5	4.80 (m)	72.5
3″			4.61 (dd, 2.9, 9.2)	72.7	4.61 (dd, 2.7, 9.1)	72.8			4.57 (dd, 2.0, 9.2)	72.7	4.59 (dd, 2.6, 9.5)	72.9
4″			4.36 (m)	73.9	4.35 (m)	73.9			4.32 (m)	73.9	4.32 (m)	74.2
5″			4.93 (m)	69.5	4.92 (m)	69.5			4.90 (m)	69.5	4.89 (m)	69.5
6″			1.75 (d, 6.2)	18.6	1.75 (d, 6.2)	18.6			1.73 (d, 6.3)	18.6	1.74 (d, 6.0)	18.6
4′-*O*-Rha-1‴	5.86 (br s)	102.2	5.86 (br s)	102.9	5.86 (br s)	102.9	5.91 (br s)	102.7	5.78 (br s)	102.9	5.78 (br s)	102.3
2‴	4.59 (br s)	72.9	4.67 (m)	72.6	4.68 (br s)	72.6	4.71 (br s)	72.6	4.63 (m)	72.5	4.51 (br s)	72.9
3‴	4.62 (m)	73.5	4.53 (dd, 2.9, 9.2)	72.8	4.53 (dd, 2.7, 9.1)	72.8	4.59 (dd, 2.8, 9.3)	72.8	4.48 (dd, 2.0, 8.9)	72.8	4.52 (m)	73.3
4‴	4.49 (m)	80.3	4.33 (m)	74.1	4.33 (m)	74.2	4.35 (dd, 9.3, 9.3)	74.0	4.30 (m)	74.2	4.40 (m)	80.4
5‴	5.07 (m)	68.3	4.91 (m)	70.4	4.91 (m)	70.4	5.03 (m)	70.4	4.82 (m)	70.5	4.85 (m)	68.4
6‴	1.70 (d, 6.2)	18.9	1.62 (d, 6.2)	18.5	1.62 (d, 6.2)	18.5	1.74 (d, 6.2)	18.6	1.60 (d, 6.3)	18.4	1.57 (d, 6.6)	18.9
4‴-*O*-Rha-1⁗	6.34 (br s)	103.2									6.22 (br s)	103.3
2⁗	4.91 (br s)	72.7									4.85 (m)	72.7
3⁗	4.55 (m)	73.1									4.47 (m)	72.9
4⁗	4.33 (m)	74.1									4.27 (m)	74.1
5⁗	4.41 (m)	70.4									4.34 (m)	70.4
6⁗	1.62 (d, 6.2)	18.5									1.59 (d, 7.2)	18.4

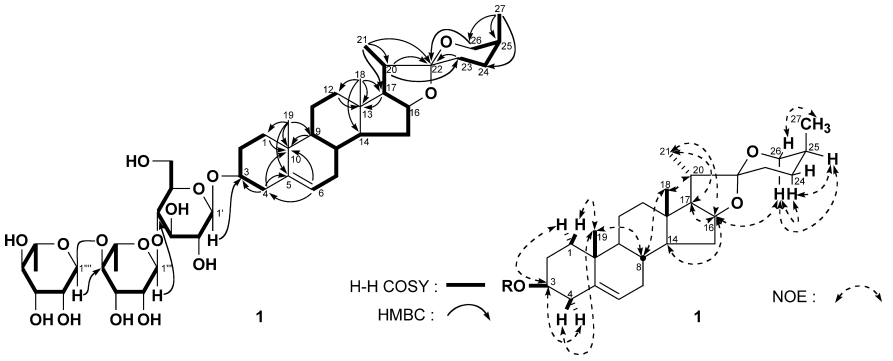

Fig. 1

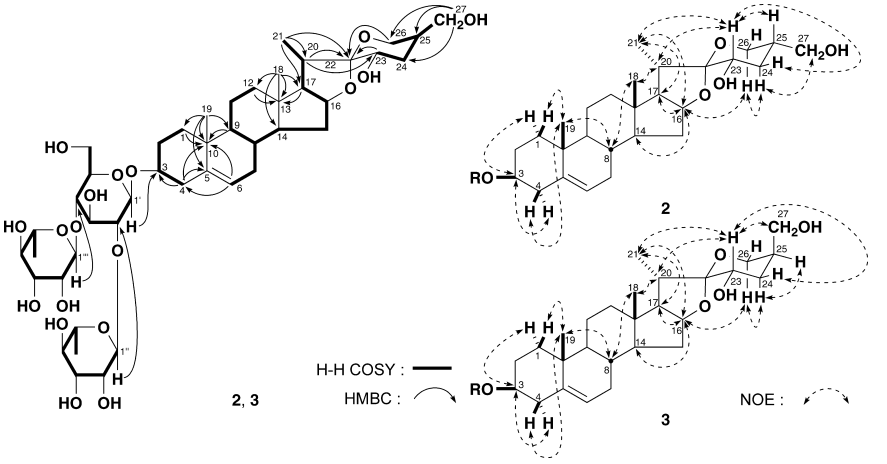

Fig. 2

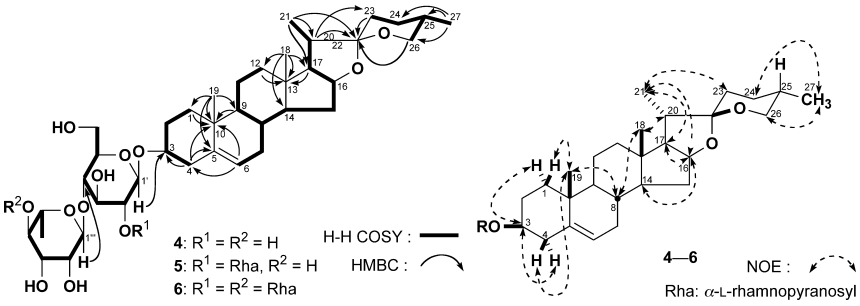

Fig. 3

structure of **3** was clarified by the ^{1}H–^{1}H COSY and HMBC experiments as shown in Fig. 2, which was the same as that of **2**. The stereostrucutre of **3** was elucidated by the ROESY experiment, whose NOE correlations were observed between the following proton pairs [16-H and 26$_{ax}$-H (δ 4.09); 20-H and 23$_{ax}$-H (δ 4.07); 21-H$_3$ and 23$_{ax}$-H; 23$_{ax}$-H and 24$_{eq}$-H (δ 2.39), 27-H$_2$; 24$_{ax}$-H (δ 2.32) and 25$_{eq}$-H (δ 2.15), 26$_{ax}$-H] as shown in Fig. 2. On the basis of above-mentioned findings, the stereostructure of borassoside C was determined to be as the 23α,27-dihydroxyyamogenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (**3**).

Borassoside D (**4**) was obtained as a white powder with negative optical rotation ([α]$_D^{26}$ −59.7° in MeOH). The IR spectrum of **4** showed absorption bands at 3432, 1096, 1060, and 1040 cm^{-1} ascribable to hydroxyl and ether functions. In the positive- and negative-ion FAB-MS of **4**, quasimolecular ion peaks were observed at *m/z* 745 $(M+Na)^+$, and 721 $(M-H)^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **4** to be $C_{39}H_{62}O_{12}$. The acid hydrolysis of **4** with 2.0 M HCl–1,4-dioxane (1 : 1, v/v) liberated D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector.[37—39] The proton and carbon signals in the ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Tables 2, 3) spectra[40] of **4** resembled those for **11** very closely, except for the signal due to the F ring in the aglycon part, which showed four methyls [δ 0.67 (3H, d, J=6.5 Hz, 27-H$_3$), 0.88, 0.95 (3H each, both s, 19, 18-H$_3$), 1.00 (3H, d, J=7.2 Hz, 21-H$_3$)], a methylene and two methines bearing an oxygen function [δ 3.68 (2H, d-like, 26-H$_2$), 3.86 (1H, m, 3-H), 4.29 (1H, m, 16-H)], an olefin [δ 5.31 (1H, br d, J=*ca.* 5 Hz, 6-H)] together with a glucopyranosyl [δ 4.97 (1H, d, J=7.6 Hz, 1′-H)] and a rhamnopyranosyl moieties [δ 1.74 (3H, d, J=6.2 Hz, 6‴-H$_3$), 5.91 (1H, br s, 1‴-H)]. The ^{1}H–^{1}H COSY experiment on **4** indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons (1-H$_2$ and 10-C; 4-H$_2$ and 3, 5, 10-C; 6-H and 4, 10-C; 9-H and 10-C; 12-H$_2$ and 13-C; 16-H and 22-C; 17-H and 13-C; 18-H$_3$ and 12—14, 17-C; 19-H$_3$ and 1, 5, 9, 10-C; 20-H and 22, 23-C; 21-H$_3$ and 17, 20, 22-C; 23-H$_2$ and 22-C; 26-H$_2$ and 22-C; 27-H$_3$ and 24—26-C; 1′-H and 3-C; 1‴-H and 4′-C) (Fig. 3). Thus, the planar structure of the aglycon and diglycoside structure in **4** were clarified.

Borassosides E (**5**) and F (**6**) were also obtained as white powders with negative optical rotations (**5**: [α]$_D^{23}$ −50.2°; **6**: [α]$_D^{23}$ −47.5°, both in MeOH). The molecular formula, (**5**: $C_{45}H_{72}O_{16}$ and **6**: $C_{51}H_{82}O_{20}$) were determined from the positive- and negative-ion FAB-MS [**5**: *m/z* 891 $(M+Na)^+$ and 867 $(M-H)^-$ and **6**: *m/z* 1037 $(M+Na)^+$ and 1013 $(M-H)^-$] and by high-resolution FAB-MS, which were the same as those of **13** and **16**, respectively. The acid hydrolysis of **5** and **6** with 2.0 M HCl–1,4-dioxane (1 : 1, v/v) liberated D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector, respectively.[37—39] The ^{1}H-(pyridine-d_5) and ^{13}C-NMR (Tables 2, 3) spectra[40] of **5** and **6** indicated the presence of the following functions: an aglycon part {**5**: four methyls [δ 0.70 (3H, d, J=6.3 Hz, 27-H$_3$), 0.97, 1.04 (3H each, both s, 18, 19-H$_3$), 1.02 (3H, d, J=7.2 Hz, 21-H$_3$)], a methylene and two methines bearing an oxygen function [δ 3.68 (2H, d-like, 26-H$_2$), 3.86 (1H, m, 3-H), 4.33 (1H, m, 16-H)], an olefin [δ 5.33 (1H, br d, J=*ca.* 5 Hz, 6-H)]; **6**: δ 0.70 (3H, d, J=6.3 Hz, 27-H$_3$), 0.97, 1.04 (3H each, both s, 18, 19-H$_3$), 1.02 (3H, d, J=6.9 Hz, 21-H$_3$), 3.68 (2H, d-like, 26-H$_2$), 3.86 (1H, m, 3-H), 4.31 (1H, m, 16-H), 5.33 (1H, br d, J=*ca.* 5 Hz, 6-H)} and oligosaccharide moieties {**5**: a glucopyranosyl [δ 4.91 (1H, d, J=6.6 Hz, 1′-H) and two rhamnopyranosyl moieties [δ 1.60, 1.73 (3H each, both d, J=6.3 Hz, 6‴, 6″-H$_3$), 5.78, 6.31 (1H each, both br s, 1‴, 1″-H)]; **6**: a glucopyranosyl [δ 4.92 (1H, d, J=6.6 Hz, 1′-H) and three rhamnopyranosyl moieties [δ 1.57 (3H, d, J=6.6 Hz, 6‴-H$_3$), 1.59 (3H, d, J=7.2 Hz, 6⁗-H), 1.74 (3H, d, J=6.0 Hz, 6″-H$_3$), 5.78, 6.22, 6.34 (1H each, all br s, 1‴, 1⁗, 1″-H)]. The proton and carbon signals due to the aglycon part in the ^{1}H- and ^{13}C-NMR data of **5** and **6** were superimposable on those for **4**. The structure and linkaged positions of oligosugar moieties in **5** and **6** were determined from the HMBC experiment, which showed long-range correlations between the following proton and carbon pairs: **5**: 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 4′-C; **6**: 1′-H and 3-C, 1″-H and 2′-C, 1‴-H and 4′-C, 1⁗-H and 4‴-C.

Next, the stereostructure of the aglycon part in **4**—**6** was characterized by ROESY experiment, in which the NOE correlations were observed as shown in Fig. 3. Furthermore, by comparison of the ^{13}C-NMR data for **4**—**6** with those for 22-α-*O*-spirostanol oligoglycosides (**11**, **13**, **16**), the signals due to the 23- and 24-carbons in **4**—**6** were observed at higher field compared with those of corresponding 22-α-*O*-spirostanol oligoglycosides, while the signals due to the 26-carbon in **4**—**6** were observed at lower field compared with those of corresponding 22α-*O*-spirostanol oligoglycosides as shown in Fig. 4. The same trends were observed in 22α- and 22β-*N*-spirostanol oligoglycosides, solamarine (**28**) and solamargine (**29**).[41] On the basis of above-mentioned evidence, the stereostructures of brssosides D—F were determined to

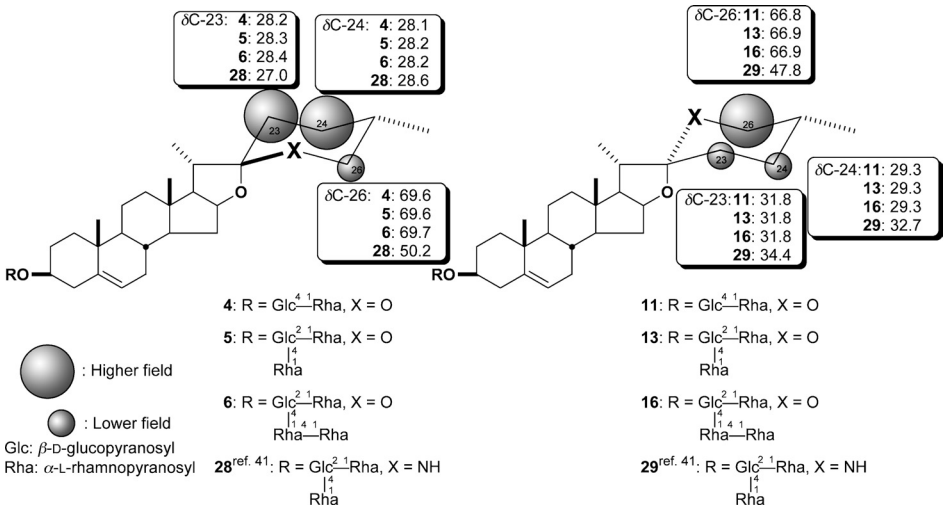

Fig. 4. Comparison for the ^{13}C-NMR Data of 22β- and 22α-*O*- or -*N*-spirostanol Glycosides (in Pyridine-d_5)

Table 4. Inhibitory Effects of Dioscin (**13**), Tolbutamide, and Metformin on the Increase in Serum Glucose Levels in Sucrose-Loaded Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose level (mg/dl)		
			0.5 h	1.0 h	2.0 h
Normal[a)]	—	5	77.0±5.2**	84.2±6.4**	76.8±2.5**
Control[b)]	—	7	160.3±3.6	148.0±3.7	109.2±3.1
Dioscin (**13**)[c)]	12.5	5	160.2±6.1	147.8±4.3	115.0±2.8
	25	5	145.2±9.7	135.9±8.7	127.8±1.5
	50	5	130.4±8.0**	124.5±8.1*	112.3±4.6
Normal[a)]	—	6	81.4±2.8**	80.8±2.2**	84.7±2.4**
Control[b)]	—	7	169.6±6.7	138.6±3.7	118.5±3.0
Tolbutamide[c)]	12.5	6	152.6±2.8*	130.5±4.0	114.9±3.8
	25	6	138.1±3.5**	106.3±3.5**	99.5±2.1**
Normal[a)]	—	5	85.6±3.6**	88.9±4.1**	87.1±2.9**
Control[b)]	—	6	159.6±7.0	145.0±6.9	110.9±5.4
Metformin hydrochloride[c)]	125	5	134.8±4.3*	136.0±5.1	115.5±4.7
	250	5	124.1±5.5**	116.3±5.5**	126.4±1.9

a) Acacia solution (5 ml/kg) and water (5 ml) were administrated orally. *b*) Acacia solution (5 ml/kg) and 20% sucrose solution (5 ml) were administrated orally. *c*) Test sample suspended in acacia solution (5 ml/kg) and 20% sucrose solution (5 ml) were administrated orally. Values represent the means±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

be as shown (**4**—**6**).

Inhibitory Effect of Dioscin (13) on the Increase in Serum Glucose Level in Sucrose-Loaded Rats Previously, we reported that several triterpene saponin constituents were found to inhibit the increase of serum glucose level in sucrose-loaded rat.[42)] As a continuing study of the antidiabetogenic constituents from the medicinal foods, effects of the principal constituent of *B. flabellifer*, dioscin (**13**) on the increase of serum glucose level in sucrose-loaded rat were examined. There are a few reports about hypoglycemic effects of steroid saponins, but they were applied intraperitoneally (i.p.) but not orally (*p.o.*).[43,44)] As shown in Table 4, compound **13** inhibited the increase of serum glucose levels in this model at a dose of 50 mg/kg, *p.o.* In this experimental model, an insulin-secretion agent, tolbutamide,[45)] exhibited substantial inhibition, and metformin hydrochloride, which shows an inhibitory effect on intestinal glucose absorption as well as improving of peripheral insulin sensitivity,[46)] exhibited moderate inhibition. In the present study, the effect of **13** was stronger than that of metformin hydrochloride.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JNM-ECA600 (600 MHz) spectrometer; ^{13}C-NMR spectra, JNM-ECA600 (150 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV–VIS detectors. HPLC column, COSMOSIL 5C_{18}-PAQ (Nacalai Tesque, 250×4.6 mm and 250×20 mm i.d.), YMC-Pack ODS-AL (YMC, 250×4.6 mm and 250×20 mm i.d.), and Chiral CD-Ph (Shiseido, 250×4.6 mm) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh) and Diaion HP-20 (Nippon Rensui); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The male and female flowers of *B. flabellifer* were pur-

chased in Thailand in July 2003, and identified by one of authors (Yutana Pongpiriyadacha). A voucher specimen (No. T-37) is on file in our laboratory.

Extraction and Isolation The dried male flowers of *B. flabellifer* (15.0 kg) were powdered and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (1826 g, 12.2% from the dried flowers), and an aliquot (775 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (136 g, 2.1%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (368 g, 5.8%) and an H_2O-soluble fraction (272 g, 4.3%). The *n*-BuOH-soluble fraction (50.0 g) was subjected to Diaion HP-20 column chromatography (2.0 kg, H_2O→MeOH) to give H_2O- and MeOH-eluted fractions (9.7 g, 1.1% and 39.8 g, 4.6%), respectively.

The MeOH-eluted fraction (35.5 g) was subjected to ordinary-phase silica gel column chromatography [1.2 kg, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give six fractions [Fr. 1 (1.37 g), Fr. 2 (0.99 g), Fr. 3 (4.63 g), Fr. 4 (2.56 g), Fr. 5 (9.29 g), and Fr. 6 (14.03 g)]. Fraction 1 (1.37 g) was subjected to reversed-phase silica gel column chromatography [45 g, MeOH–H_2O (30 : 70→45 : 55→60 : 40→75 : 25→90 : 10, v/v)→MeOH] to give 12 fractions [Fr. 1-1 (81 mg), Fr. 1-2 (48 mg), Fr. 1-3 (27 mg), Fr. 1-4 (47 mg), Fr. 1-5 (52 mg), Fr. 1-6 (76 mg), Fr. 1-7 (65 mg), Fr. 1-8 (75 mg), Fr. 1-9 (26 mg), Fr. 1-10 (251 mg), Fr. 1-11 (96 mg), and Fr. 1-12 (270 mg)]. Fraction 1-6 (76 mg) was further purified by HPLC [COSMOSIL 5C_{18}-PAQ, CH_3CN–H_2O (50 : 50, v/v) and Chiral CD-Ph, MeOH–H_2O (85 : 15, v/v)] to give diosgenin 3-*O*-*β*-D-glucopyranoside (**7**, 5.5 mg, 0.00071%) and yamogenin 3-*O*-*β*-D-glucopyranoside (**8**, 4.3 mg, 0.00056%). Fraction 1-7 (65 mg) was also purified by HPLC [COSMOSIL 5C_{18}-PAQ, MeOH–H_2O (90 : 10, v/v) and Chiral CD-Ph, MeOH–H_2O (85 : 15, v/v)] to give diosgenin (**26**, 6.6 mg, 0.00085%) and yamogenin (**27**, 5.7 mg, 0.00074%). Fr. 1-8 (75 mg) was separated by HPLC [COSMOSIL 5C_{18}-PAQ, MeOH–H_2O (90 : 10, v/v)] to give *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (32.6 mg, 0.0042%). Fr. 1-10 (251 mg) was purified by HPLC [COSMOSIL 5C_{18}-PAQ, MeOH–H_2O (95 : 5, v/v)] to give *β*-sitosterol (80.3 mg, 0.010%). Fraction 2 (0.99 g) was subjected to reversed-phase silica gel column chromatography [30 g, MeOH–H_2O (15 : 85→30 : 70→45 : 55→60 : 40→75 : 25→90 : 10, v/v)→MeOH] to afford 12 fractions [Fr. 2-1 (20 mg), Fr. 2-2 (62 mg), Fr. 2-3 (32 mg), Fr. 2-4 (58 mg), Fr. 2-5 (37 mg), Fr. 2-6 (25 mg), Fr. 2-7 (49 mg), Fr. 2-8 (19 mg), Fr. 2-9 (343 mg), Fr. 2-10 (71 mg), Fr. 2-11 (18 mg), and Fr. 2-12 (57 mg)]. Fraction 2-9 (343 mg) was separated by HPLC [YMC-Pack ODS-AL, MeOH–H_2O (80 : 20, v/v) and Chiral CD-Ph, MeOH–H_2O (85 : 15, v/v)] to give borassosides A (**1**, 8.0 mg, 0.0010%) and D (**4**, 7.5 mg, 0.00097%), diosgenin 3-*O*-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranoside (**9**, 6.7 mg, 0.00087%) and yamogenin 3-*O*-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranoside (**10**, 9.0 mg, 0.0012%), diosgenin 3-*O*-*α*-L-rhamnopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranoside (**15**, 12.5 mg, 0.0016%), diosgenin 3-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranoside (**11**, 99.6 mg, 0.013%), and yamogenin 3-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranoside (**12**, 94.1 mg, 0.012%). Fraction 3 (4.63 g) was subjected to reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30→85 : 15, v/v)→MeOH] to afford 10 fractions [Fr. 3-1 (101 mg), Fr. 3-2 (206 mg), Fr. 3-3 (89 mg), Fr. 3-4 (63 mg), Fr. 3-5 (52[6[mg), Fr. 3-6 (33 mg), Fr. 3-7 (84 mg), Fr. 3-8 (152 mg), Fr. 3-9 (3200 mg), and Fr. 3-10 (111 mg)]. Fraction 3-7 (84 mg) was further purified by HPLC [CH_3CN–H_2O (30 : 70, v/v)] to give 26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22*ξ*,26-triol 3-*O*-*β*-D-glucopyranoside (**18**, 6.8 mg, 0.00088%) and 26-*O*-*β*-D-glucopyranosyl-(25*S*)-furost-5-ene-3*β*,22*ξ*,26-triol 3-*O*-*β*-D-glucopyranoside (**19**, 15.1 mg, 0.0020%). Fraction 3-9 (1000 mg) was purified by HPLC [COSMOSIL 5C_{18}-PAQ, MeOH–H_2O (75 : 25, v/v) and Chiral CD-Ph, MeOH–H_2O (80 : 20, v/v)] to give borassoside E (**5**, 29.4 mg, 0.012%), dioscin (**13**, 380.0 mg, 0.16%), and yamogenin 3-*O*-*α*-L-rhamnopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranoside (**14**, 302.8 mg, 0.13%). Fraction 4 (2.56 g) was subjected to reversed-phase silica gel column chromatography [75 g, MeOH–H_2O (30 : 70→45 : 55→60 : 40→75 : 25, v/v)→MeOH] to afford 12 fractions [Fr. 4-1 (240 mg), Fr. 4-2 (56 mg), Fr. 4-3 (89 mg), Fr. 4-4 (83 mg), Fr. 4-5 (16 mg), Fr. 4-6 (96 mg), Fr. 4-7 (416 mg), Fr. 4-8 (212 mg), Fr. 4-9 (579 mg), Fr. 4-10 (40 mg), Fr. 4-11 (55 mg), and Fr. 4-12 (232 mg)]. Fraction 4-7 (416 mg) was separated by HPLC [COSMOSIL 5C_{18}-PAQ, MeOH–H_2O (60 : 40, v/v)] to give borassosides B (**2**, 10.0 mg, 0.0013%) and C (**3**, 5.6 mg, 0.00072%). Fraction 4-9 (579 mg) was separated by HPLC [COSMOSIL 5C_{18}-PAQ, CH_3CN–H_2O (45 : 55, v/v) and Chiral CD-Ph, MeOH–H_2O (80 : 20, v/v)] to give borassoside F (**6**, 5.3 mg, 0.00069%), diosgenin 3-*O*-*α*-L-rhamnopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→4)-[*α*-L-rhamnopyranosyl(1→2)]-*β*-D-glucopyranoside (**16**, 136.9 mg, 0.018%), and yamogenin 3-*O*-*α*-L-rhamnopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→4)-[*α*-L-rhamnopyranosyl(1→2)]-*β*-D-glucopyranoside (**17**, 93.1 mg, 0.012%). Fraction 5 (8.5 g) was subjected to reversed-phase silica gel column chromatography [250 g, MeOH–H_2O (30 : 70→45 : 55→65 : 35, v/v)→MeOH] to afford eight fractions [Fr. 5-1 (23 mg), Fr. 5-2 (120 mg), Fr. 5-3 (213 mg), Fr. 5-4 (86 mg), Fr. 5-5 (367 mg), Fr. 5-6 (6.767 g), Fr. 5-7 (776 mg), and Fr. 5-8 (117 mg)]. Fraction 5-6 (700 mg) was further purified by HPLC [COSMOSIL 5C_{18}-PAQ, CH_3CN–H_2O (30 : 70, v/v)] to give methyl protodioscin (**22**, 107.0 mg, 0.15%) and methyl protoneodioscin (**23**, 80.3 mg, 0.11%). Fraction 5-8 (776 mg) was also purified by HPLC [COSMOSIL 5C_{18}-PAQ, CH_3CN–H_2O (30 : 70, v/v)] to give protodioscin (**20**, 109.1 mg, 0.015%), protoneodioscin (**21**, 56.0 mg, 0.0079%), pseudoprotodioscin (**24**, 116.7 mg, 0.017%), and pseudoprotoneodioscin (**25**, 71.5 mg, 0.010%). The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS] with reported values.[21—36] On the other hand, the dried female flowers of *B. flabellifer* (400 g) were also extracted with the same procedure to give a methanolic extract (45 g, 11.3%).

Borassoside A (**1**): A white powder, $[\alpha]_D^{23}$ −89.6° (*c*=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{45}H_{72}O_{16}Na$ $(M+Na)^+$: 891.4718. Found: 891.4724. IR (KBr): 3435, 1141, 1073, 1060, 996, 927, 902, 850 cm^{-1}. ^{1}H- and ^{13}C-NMR: given in Tables 2 and 3. Positive-ion FAB-MS *m/z* 891 $(M+Na)^+$. Negative-ion FAB-MS *m/z* 867 $(M-H)^-$, 721 $(M-C_6H_{11}O_4)^-$, 575 $(M-C_{12}H_{21}O_8)^-$.

Borassoside B (**2**): A white powder, $[\alpha]_D^{23}$ −102.3° (*c*=0.80, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{45}H_{72}O_{18}Na$ $(M+Na)^+$: 923.4616. Found: 923.4612. IR (KBr): 3652, 3569, 2935, 1145, 1070, 1052, 996, 927, 902, 850 cm^{-1}. ^{1}H- and ^{13}C-NMR data: given in Tables 2 and 3. Positive-ion FAB-MS *m/z* 923 $(M+Na)^+$. Negative-ion FAB-MS *m/z* 899 $(M-H)^-$, 753 $(M-C_6H_{11}O_4)^-$.

Borassoside C (**3**): A white powder, $[\alpha]_D^{22}$ −91.1° (*c*=0.30, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{45}H_{72}O_{18}Na$ $(M+Na)^+$: 923.4616. Found: 923.4621. IR (KBr): 3453, 2934, 1141, 1073, 1051, 988, 922, 905, 846 cm^{-1}. ^{1}H- and ^{13}C-NMR: given in Tables 2 and 3. Positive-ion FAB-MS *m/z* 923 $(M+Na)^+$. Negative-ion FAB-MS *m/z* 899 $(M-H)^-$, 753 $(M-C_6H_{11}O_4)^-$.

Borassoside D (**4**): A white powder, $[\alpha]_D^{26}$ −59.7° (*c*=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{39}H_{62}O_{12}Na$ $(M+Na)^+$: 745.4139. Found: 745.4135. IR (KBr): 3432, 2936, 1132, 1096, 1060, 1040, 982, 920, 901, 866 cm^{-1}. ^{1}H- and ^{13}C-NMR: given in Tables 2 and 3. Positive-ion FAB-MS *m/z* 745 $(M+Na)^+$. Negative-ion FAB-MS *m/z* 721 $(M-H)^-$, 575 $(M-C_6H_{11}O_4)^-$.

Borassoside E (**5**): A white powder, $[\alpha]_D^{23}$ −50.2° (*c*=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{45}H_{72}O_{16}Na$ $(M+Na)^+$: 891.4718. Found: 891.4725. IR (KBr): 3410, 2932, 1132, 1055, 1044, 994, 914 cm^{-1}. ^{1}H- and ^{13}C-NMR: given in Tables 2 and 3. Positive-ion FAB-MS *m/z* 891 $(M+Na)^+$. Negative-ion FAB-MS *m/z* 867 $(M-H)^-$, 721 $(M-C_6H_{11}O_4)^-$.

Borassoside F (**6**): A white powder, $[\alpha]_D^{23}$ −47.5° (*c*=0.40, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{51}H_{82}O_{20}Na$ $(M+Na)^+$: 1037.5297. Found: 1037.5294. IR (KBr): 3453, 2934, 1132, 1076, 1051, 982, 923, 901, 850 cm^{-1}. ^{1}H- and ^{13}C-NMR: given in Tables 2 and 3. Positive-ion FAB-MS *m/z* 1037 $(M+Na)^+$. Negative-ion FAB-MS *m/z* 1013 $(M-H)^-$, 867 $(M-C_6H_{11}O_4)^-$, 721 $(M-C_{12}H_{21}O_8)$.

Acid Hydrolysis of Borassosides A—F (1—6) A solution of **1**—**6** (2.0 mg each) in 2 M HCl–1,4-dioxane (1 : 1, v/v, 0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH_3CN–H_2O (85 : 15, v/v); flow rate 0.8 ml/min; column temperature, room temperature. Identification of L-rhamnose (**i**) and D-glucose (**ii**) from **1**—**6** present in the aqueous layer was carried out by comparison of their retention time and optical rotation with those of authentic samples. t_R: (**i**) 7.8 min (negative optical rotation); (**ii**) 13.9 min (positive optical rotation).

Bioassay

Animals Male Wistar rats weighing 130—170 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were

fasted for 24—26 h prior to the beginning of the experiment, but were allowed free access to tap water. All of the experiments were performed with conscious rats unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Effects on Increase of Serum Glucose Levels in Oral Surcose-Loaded Rats Experiments on the effects of the methanolic extracts and dioscin (**13**) from the male and female flowers of *B. flabellifer* on increase of serum glucose levels in sucrose-loaded rats were performed according to the method reported previously.[42,47] The test samples were suspended in 5% acacia solution (5 ml/kg), and then orally administered to the rats. Thirty minutes thereafter, a water solution (5 ml/kg) of sucrose (1.0 g/kg) was orally administered. Blood (*ca*. 0.4 ml) was collected from infraorbital venous plexus under light ether anesthesia at 0.5, 1.0, and 2.0 h after administration of sugar and the serum glucose concentration was assayed by the glucose-oxidase method (Glucose CII-test Wako, Wako Pure Chemical Industries, Ltd.). Tolubutamide was obtained from Wako Pure Chemical Industries, Ltd. Metformin hydrochloride from Sigma.

Statistics Values were expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis.

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