Studies on *Balanites aegyptiaca* Fruits, an Antidiabetic Egyptian Folk Medicine

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An aqueous extract of mesocarps of the fruits of *Balanites aegyptiaca* exhibited a prominent antidiabetic activity by oral administration in streptozotocin induced diabetic mice. From one of the active fractions of this extract, two new steroidal saponins were isolated, and their structures were determined as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22,26-triol 3-*O*-[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside and its 22-methyl ether. In addition, two known saponins, 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22,26-triol 3-*O*-(2,4-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside and its methyl ether were isolated and identified. It was revealed that the individual saponins did not show antidiabetic activity, while the recombination of these saponins resulted in significant activity. From an ethanolic extract of the epicarps, two known flavonol glycosides, isorhamnetin-3-*O*-robinobioside and isorhamnetin-3-*O*-rutinoside were isolated and identified.

Keywords *Balanites aegyptiaca*; Balanitaceae; Egyptian medicinal plant; steroidal glycosides; flavonoidal glycoside; antidiabetic activity

Balanites aegyptiaca DEL. (Balanitaceae) is widely distributed in Africa and has a variety of uses as a folk medicine in many countries of Africa.[1] In Egyptian folk medicine, the fruits (after removal of the epicarp) are used as an oral antidiabetic drug. This paper describes the antidiabetic activities of an aqueous extract of the mesocarps and its fractions as well as isolation, characterization and biological activity of the steroidal glycosides from one of the active fractions. In addition, the isolation and identification of the major flavonol glycosides from an ethanolic extract of the epicarps is also reported.

The mesocarps were extracted with water. To an aqueous solution of the extract, was added an excess of ethanol to give precipitates and supernatant. The precipitate was dialyzed against water to yield a non-dialyzed fraction tentatively named polysaccharide fraction. The antidiabetic activity test in streptozotocin (STZ) induced diabetic mice (Table I) revealed that oral administration of the aqueous extract, as well as the polysaccharide fraction and the supernatant, exhibited prominent antidiabetic actions in 6 h; −33%, −53% and −53%, respectively. In order to identify the active principles, the supernatant was chromatographed on a highly porous synthetic resin (Diaion HP-20) by eluting with water, 40% methanol (MeOH), 80% MeOH, MeOH and acetone, successively. The activity of each eluate was tested orally in STZ diabetic mice. As shown in Table II, the most remarkable decrease (−57%) in the blood glucose levels was observed for the 80% MeOH eluate 6 h after administration.

The active 80% MeOH eluate was separated by chromatography on silica gel to give four fractions tentatively designated as F-1—4 in order of increasing polarity. Of these fractions, F-2 was proved to be remarkably active for its antidiabetic effect (Table II), so that this fraction was further chromatographed on a column of LiChroprep RP-8 and finally purified by high-performance liquid chromatography (HPLC) to give four compounds, **1**—**4** together with several minor compounds, **5**—**8**. Compounds **1**—**4** were assumed to be furostanol saponins on the basis of the positive coloration with Ehrlich reagent.[2] Acid hydrolysis of **1** yielded L-rhamnose, D-glucose and diosgenin. Methylation analysis of **1** revealed the presence of a 2,4-linked glucosyl unit together with terminal glucosyl and rhamnosyl units. Inspection of the carbon-13 and proton nuclear magnetic resonance (^{13}C- and ^{1}H-NMR) spectra (Tables III, IV), as well as the negative fast atom bombardment mass spectrum (FAB-MS), led to the formulation of **1** as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22,26-triol 3-*O*-(2,4-di-*O*-α-L-rhamnopy-

TABLE I. Antidiabetic Effect of Aqueous Extract, Polysaccharide Fraction and Supernatant on Blood Glucose Levels in STZ Diabetic Mice

Sample[a]	0		4 h		6 h	
	m±S.E. (mg%)	%	m±S.E. (mg%)	%	m±S.E. (mg%)	%
Control	299±21.1	100	299± 7.2	100	290±4.6	97
Extract	299± 5.1	100	268±11.4	90	194±7.2	65
Polysaccharide fraction	273± 2.7	100	192±16.0	70	137±3.5	50
Supernatant	223± 5.5[b]	100	173± 6.7[c]	78	137±5.0	61

a) Dose: 80 mg/kg, orally and $n=5$ (m, mean; S.E., standard error). *b*) $p<0.01$. *c*) $0.1>p>0.05$.

TABLE II. Antidiabetic Effect of Eluates and Fractions of Supernatant Chromatography (Diaion HP-20 and Silica Gel) on Blood Glucose Levels in STZ Diabetic Mice

Sample[a]	0		4 h		6 h	
	m±S.E. (mg%)	%	m±S.E. (mg%)	%	m±S.E. (mg%)	%
Exp. 1						
Control	286± 2.5	100	168± 4.9	59	194± 7.3	68
40% MeOH eluate	303± 4.5[b]	100	259± 5.5	85	169±21.1	56
80% MeOH eluate	305±18.1[c]	100	134± 5.9	44	83± 2.7	27
MeOH eluate	279±26.9	100	167± 8.2	60	128± 5.1	46
Exp. 2						
Control	366±18.0	100	395± 3.3	108	337± 4.4	103
F-2	380±11.1	100	315± 4.6	83	107± 7.7[d]	28
F-3	383± 7.6	100	380±14.7	99	373± 7.6	97

a) Dose: 80 mg/kg, orally and $n=5$ (m, mean; S.E., standard error). *b*) $p<0.01$. *c*) $p<0.05$. *d*) $p<0.001$.

TABLE III. ^{13}C-NMR Data of the Sugar Moieties of Compounds **1**—**4** (100 MHz, in C_5D_5N)

Carbon No.	1	2	3	4	Carbon No.	1	2	3	4
26-Glucosyl					3	72.7	72.8	72.7	72.7
1	104.9[a]	105.1[a]	104.5[a]	104.5[a]	4	74.9[b]	74.9[b]	74.9[b]	75.1[b]
2	75.2[b]	75.0[b]	75.3[b]	75.2[b]	5	69.4	69.4	69.4	69.4
3	78.6[c]	78.6[c]	78.6[c]	78.2[c]	6	18.6	18.6	18.6	18.6
4	71.7	71.8	71.2	71.7	1′	100.1	100.0	99.9	99.9
5	78.3[c]	78.2[c]	78.2[c]	78.1[c]	2′	71.3	71.2	71.7	71.7
6	62.9	62.9	62.1	62.8	3′	72.4	72.4	72.5	72.4
3-Glucosyl					4′	74.1	74.1	74.1	74.1
1	105.1[a]	105.1[a]	104.9[a]	105.1[a]	5′	69.4	69.4	69.0	69.0
2	82.0	82.0	82.5	81.5	6′	18.6	18.6	18.6	18.6
3	76.2	76.2	87.0	87.3	Xylosyl				
4	78.4[c]	77.7[c]	78.8[c]	78.6[c]	1			106.2	106.2
5	77.4	77.4[c]	77.6	77.6	2			75.4[b]	75.2[b]
6	63.8	63.8	62.8	62.8	3			78.0[c]	78.0[c]
Rhamnosyl					4			70.9	70.9
1	101.7	101.8	101.7	101.7	5			67.3	67.3
2	71.7	71.7	72.4	72.4					

a—*c*) Signals may be interchangeable in each column.

TABLE IV. ^{1}H-NMR Spectral Data of Compounds **3**, **4** and **9** (400 MHz, in C_5D_5N)

Proton No.	3	9	4
3		3.53, br t (8.9)	
18	0.79, s		0.73, s
19	1.02, s		1.02, s
21	1.30, d (6.6)		1.22, d (6.5)
27	0.88, d (7.0)		0.82, d (6.8)
Glc-1	4.82, d (6.8)	4.12, d (8.1)	4.80, d (7.1)
Glc-2		4.32, dd (8.1, 9.7)	
Glc-3		4.27, dd (9.7, 9.7)	
Glc-4		4.15, dd (9.7, 9.8)	
Glc-5		4.06, ddd (2.7, 6.5, 9.8)	
Glc-6a		4.40, dd (2.7, 9.8)	
Glc-6b		4.30, dd (6.5, 9.8)	
Glc-1′	5.09, d (6.4)		5.09, d (7.1)
Rha-1	6.20, br s	4.95, br s	6.18, br s
Rha-1′	6.20, br s	5.03, br s	6.18, br s
Xyl-1	5.20, d (7.2)	4.47, d (8.0)	5.20, d (7.7)
OCH_3			3.22, s

Coupling constants (*J* in Hz) are in parenthesis.

ranosyl)-β-D-glucopyranoside.

Acid hydrolysis of **2** yielded the same sugars as those of **1**. Boiling **2** with aqueous acetone yielded **1**, and on boiling with methanol, **1** regenerated **2**.[3] The ^{13}C- and ^{1}H-NMR spectra (Tables IV, V) revealed the presence of a methoxyl group at C-22,[4] leading to the formulation of **2** as a 22-*O*-methyl derivative of **1**. Compounds **1** and **2** have already been isolated from rhizomes of *Dioscorea gracillima* by Kawasaki *et al.*[5] The identification was substantiated by comparison of the NMR spectra with the reported data.

Acid hydrolysis of **3** produced D-xylose, L-rhamnose and D-glucose together with aglycone, diosgenin. A peak at *m/z* 1179.1779 $[M-H]^-$ in the negative FAB-MS of **3** is consistent with the molecular formula $C_{56}H_{92}O_{26}$. The following significant FAB-MS fragment peaks were observed; $[M-H-Xyl]^-$ at *m/z* 1047, $[M-H-Xyl-Glc]^-$ at *m/z* 885 and $[M-H-Xyl-Glc-Rha]^-$ at *m/z* 739. The carbon signals due to the aglycone moeity of **3** appeared at almost the same positions as those of **1** (Table V), indicating that **3** is also a 3,26-*O*-bisdesmosidic furostanol saponin with the same aglycone as **1**.[6,7]

The ^{13}C- and ^{1}H-NMR signals due to the sugar moiety of **3** (Tables III, IV) revealed the presence of five sugar units; one terminal β-glucopyranosyl, one substituted β-glucopyranosyl, one terminal β-xylopyranosyl and two terminal α-rhamnopyranosyl units. The structure of the sugar moiety of **3** was established by ^{1}H–^{1}H two-dimensional nuclear Overhauser effect (NOE) correlation spectroscopy (2D NOESY, Chart 1) of the acetate of **3** (**9**) based on the assignment of the sugar proton signals (Table IV) by two-dimensional correlation spectroscopy (2D COSY). The NOE were observed between H-1 of the xylosyl unit and H-3 of the glucosyl unit. The presence of NOE between one of two H-1 of rhamnosyl units and H-2 of the glucosyl unit, as well as between another H-1 of rhamnosyl units and H-4 of the same glucosyl unit, was also noticed. These results led to the formulation of the sugar moiety as illustrated in Chart 1. The location of this glycoside group at the 3β-hydroxyl group of the aglycone was established by the presence of the NOE between H-1 of the 2,3,4-trisubstituted glucosyl unit and H-3 of the aglycone moiety. Consequently, the structure of **3** was assigned as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22,26-triol 3-*O*-[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside.

The carbon signals due to the aglycone moiety of **4** (Table V) were very similar to that of **2**. Acid hydrolysis of **4** gave D-xylose, L-rhamnose and D-glucose in addition to diosgenin, and the ^{13}C- and ^{1}H-NMR signals of the sugar moeity of **4** are almost superimposable over those of **3**. As in the case of **1** and **2**, **3** and **4** are interconvertible by boiling with MeOH (from **3** to **4**) and with aqueous acetone (from **4** to **3**).[3] Accordingly, **4** was formulated as the 22-methyl ether of **3**.

Preliminary NMR inspection suggested that the other minor compounds, **5**—**8** may be tetrahydroxycholestadiene glycosides, biogenetic precursors of **1**—**4**. However, owing to the very low yields and exhaustion of the samples for the pharmacological test, chemical characterization of these

TABLE V. ^{13}C-NMR Spectral Data of the Aglycone Moieties of Compounds **1**—**4** (100 MHz, in C_5D_5N)

Carbon No.	1	2	3	4	Carbon No.	1	2	3	4
1	37.5	37.5	37.5	37.4	15	33.2[b]	32.8[b]	32.8[b]	32.7[b]
2	30.1	30.2	30.1	29.9	16	81.1	81.1	81.5	81.3
3	78.4	78.4	78.4	78.4	17	62.1	61.9	61.9	61.5
4	38.9[a]	39.0[a]	38.9[a]	38.9[a]	18	16.4	16.3	16.4	16.4
5	140.8	140.9	140.8	140.8	19	19.4	19.4	19.3	19.3
6	121.8	121.8	121.8	121.7	20	40.7	40.5	40.7	40.5
7	32.3[b]	32.3[b]	32.2[b]	32.3[b]	21	16.4	16.3	16.0	16.2
8	31.7	31.7	31.7	31.6	22	110.7	112.7	110.7	112.5
9	50.4	50.4	50.3	50.3	23	37.1	31.0	37.1	31.4
10	37.1	37.3	37.1	37.1	24	28.3	28.3	28.9	28.3
11	21.1	21.1	21.1	21.1	25	34.3	34.5	34.3	34.2
12	39.8[a]	39.8[a]	39.8[a]	39.8[a]	26	75.2	75.2	75.2	75.1
13	40.8	40.8	40.1	40.7	27	17.4	17.5	17.4	17.2
14	56.6	56.6	56.5	56.5	OCH_3		47.4		47.2

a, *b*) Signals may be interchangeable in each column.

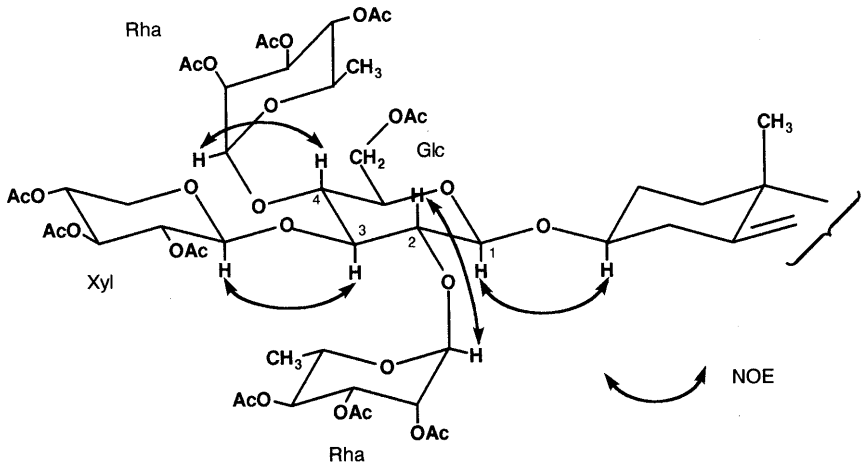

Chart 1. NOE Correlation of **9**

TABLE VI. Antidiabetic Effect of Isolated Compounds on Blood Glucose Levels in STZ Diabetic Mice

Sample[a]	0		4 h		6 h	
	m±S.E. (mg%)	%	m±S.E. (mg%)	%	m±S.E. (mg%)	%
Exp. 1						
Control	400±20.4	100	311±21.1	78	272± 5.4	68
Compds**1**—**8**	400± 4.1	100	333± 2.8	83	150± 9.1	38
Exp. 2						
Control	400± 8.2	100	400± 2.4	100	283± 5.3	71
Compds **1**—**4**	368±11.4[b]	100	247± 3.9[c]	67	196± 4.8	53
Exp. 3						
Control	389± 7.4	100	358± 2.0	92	395± 2.9	102
Compds **1**, **2**	370±23.0	100	389±12.9	105	390± 1.3	105
Compd **1**	388±11.0	100	393± 4.0[b]	101	369±18.2	95
Compd **2**	340±24.5[b]	100	377±13.3	111	391± 5.8	115
Exp. 4						
Control	400±10.8	100	363±15.9	91	293± 4.6	73
Compds **3**, **4**	400±12.4	100	350± 7.6	88	289± 2.5	72
Compd **3**	390± 5.4	100	372± 2.5	95	295±10.0	76
Compd **4**	400± 5.0	100	364±14.2	91	287±13.3	71

a) Doses: 80 mg/kg (orally), $n=5$ (m, mean; S.E., standard error). *b*) $0.1>p>0.05$. *c*) $p<0.05$.

compounds has not been achieved as yet. Properties and structures of these compounds will be reported in the near future.

TABLE VII. Effect of Polysaccharide Fraction and Saponin Mixture on Hexokinase Activity in Mouse Liver

Sample[a]	Activity (IU/mg)	%
Control	$5.27\times10^{-5}\pm3.8$	100
Polysaccharide fraction	$6.23\times10^{-5}\pm2.5$	118
Saponin mixture[b]	$5.52\times10^{-5}\pm5.1$	105

a) Doses: 80 mg/kg (orally), $n=4$. The activity was measured 6 h after administration. *b*) Mixture of compounds **1**—**4** (1 : 1 : 1 : 1).

The biological test revealed that no antidiabetic activity was observed for individual saponins **1**—**4**, while it was disclosed that recombined mixture of **1**—**4** (1 : 1 : 1 : 1) and a recombined mixture of **1**—**8** (1 : 1 : 1 : 1 : 0.5 : 0.5 : 0.5 : 0.5) exhibited significant activity, as shown in Table VI. A mixture of **1**+**2** and of **3**+**4** showed no activity.

With regard to the mechanism of the antidiabetic action of the saponin mixture and polysaccharide fraction, the following tests were conducted. Liver hexokinase is an important enzyme in the metabolism of carbohydrates. It was found that the saponin mixture (compounds **1**—**4**) and polysaccharide fraction did not exhibit a significant effect on its activity (Table VII). No effect on glucose transport through membranes of Ehrlich ascites tumor cells was also observed for the saponin mixture and the polysaccharide fraction (Table VIII). As shown in Table II, the antidiabetic

TABLE VIII. Effect of Polysaccharide Fraction and Saponin Mixture on Glucose Transport through Membranes of Ehrlich Ascites Tumor Cells (EATC)

Sample[a)]	Activity (pmol/10^6 cells)	%
Exp. 1		
Control	310±13.0	100
P. (oral)	140± 7.7	45
P. (i.p.)	330±17.5	106
Exp. 2		
Control	6000± 5.9	100
P. (*vitro*)	2800± 4.6	47
Exp. 3		
Control	106±16.0	100
S. (oral)	99±22.8	93
Exp.4		
Control	5800± 9.1	100
S. (i.p.)	3800±10.9	66
Exp. 5		
Control	252±10.4	100
S. (*vitro*)	190± 5.2	75

P., polysaccharide fraction; S., saponin mixture (compds **1**—**4**, 1:1:1:1). Doses: 80 mg/kg (orally and i.p.), 0.4 mg/20 μl cell suspension (*vitro*) and $n=4$.

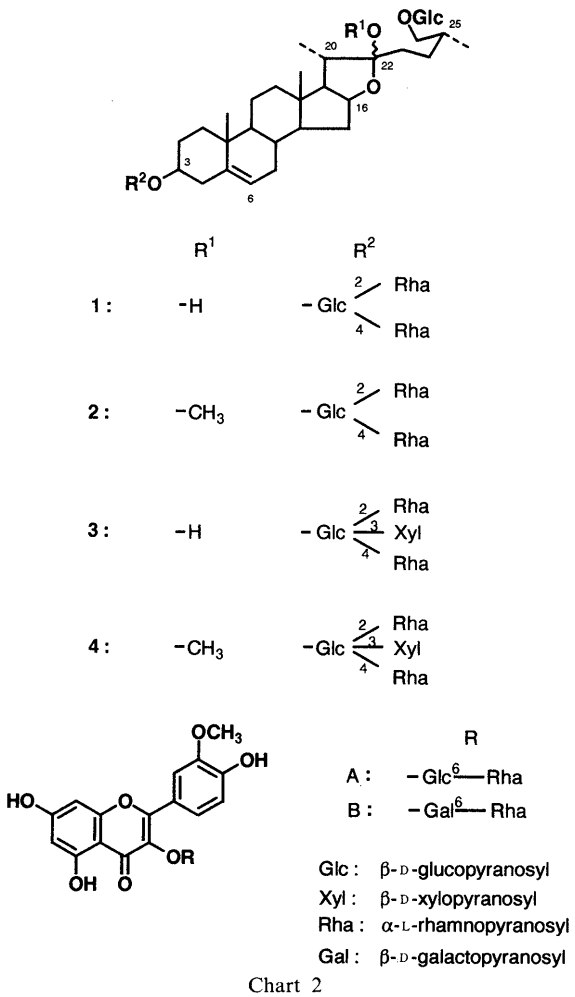

Chart 2

activity of F-2 is stronger than the saponin mixtures (*vide supra*). This suggests the presence of additional compounds which promote the activity in this active fraction.

The ethanolic extract of the epicarps was subjected to column chromatography on a Diaion HP-20 followed by chromatography on RP-8 and subsequent reverse phase HPLC, affording two flavonol glycosides A and B (Chart 2). Acid hydrolysis of A produced D-glucose and L-rhamnose in addition to isorhamnetin. Inspection of the ^{13}C- and ^{1}H-NMR spectra, as well as the FAB-MS, led to the structure of A as isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (isorhamnetin-3-*O*-rutinoside), which has already been isolated from *Cucurbita pepo* L. by Itokawa *et al.*,[8] and the identification was confirmed by comparison of the ^{13}C-NMR spectrum with that reported in literature.[9]

Acid hydrolysis of B yielded D-galactose, L-rhamnose and isorhamnetin. The inspection of the ^{13}C- and ^{1}H-NMR spectra, as well as the FAB-MS, revealed that B is isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (isorhamnetin-3-*O*-robinobioside) previously isolated from *Gomphena martiana* by Pomilio *et al.*[10]

Experimental

Optical rotations were taken on a Union PM-1 digital polarimeter. NMR spectra were recorded on a JEOL JNM GX 400 spectrometer (400 MHz for proton and 100 MHz for carbon-13) using tetramethylsilane (TMS) as an internal standard. FAB-MS were taken on a JEOL JMS-SX 102 spectrometer. For steroidal glycosides, preparative HPLC was carried out on a column of TSK-gel ODS-120T (21.5 mm i.d. × 30 cm) (detection: refraction index (RI) by a Tosoh RI-8 differential refractometer). For flavonol glycosides, preparative HPLC was carried out on a column of Amide-80 (21.5 mm i.d. × 30 cm) (detection: ultraviolet (UV) 254 nm by a Tosoh UV-8 detector). Flow rate of the mobile phase in both the cases: 6 ml/min.

Thin layer chromatography (TLC) was carried out on a precoated silica gel plate (Kieselgel 60 F_{254}, Merck). For column chromatography, Silica gel G (Merck), LiChroprep RP-8 (40—63 μm, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. Solvent systems: (a) $CHCl_3$–MeOH–H_2O (65:35:7, homogeneous); (b) $CHCl_3$–MeOH–H_2O (6:4:1, homogeneous); (c) 0.05% trifluoroacetic acid (TFA) in 67% MeOH; (d) 0.05% TFA in 70% MeOH; (e) 50% MeOH; (f) 90% CH_3CN and (g) $CHCl_3$–MeOH–H_2O (65:35:5, homogeneous). Spray reagents, 10% H_2SO_4 unless otherwise stated.

Extraction and Isolation The fruits (3 kg) of *B. aegyptiaca* were collected from the western desert of Egypt. The epicarps were removed and the mesocarps (2.9 kg) were extracted with hot water. After removal of water by evaporation, the extract (91 g) was dissolved in H_2O (200 ml) and to the aqueous solution was added EtOH (1.5 l) to yield precipitates and supernatant. The precipitates were dissolved in H_2O and dialyzed against running water through seamless cellulose tubing for 24 h. The non-dialysate, a brown powder after lyophilization (tentatively named polysaccharide fraction) was tested for antidiabetic activity. The supernatant was chromatographed on a column of Diaion HP-20 with H_2O, 40% MeOH, 80% MeOH, MeOH and Me_2CO. The 80% MeOH eluate (19.5 g) was chromatographed on a column of silica gel with solvent (a) to give four fractions. Fraction 2 was subjected to chromatography on LiChroprep RP-8 with solvents (c) and (d), and finally purified by preparative HPLC with solvent (c) to produce eight compounds **1**—**8**. Compound **1** (109 mg): A white powder from MeOH, $[\alpha]_D^{19}$ +60° ($c=0.22$, MeOH). TLC *Rf* 0.22 (solvent a). HPLC t_R 36 min (solvent c). Acid hydrolysis yielded L-rhamnose, D-glucose and diosgenin. FAB-MS *m/z*: 1047 $[M-H]^-$, 885 $[M-H-Glc]^-$, 739 $[M-H-Glc-Rha]^-$, 593 $[M-H-Glc-2Rha]^-$, 415 $[M-H-Glc-2Rha-(O-Glc)]^-$. ^{1}H-NMR (C_5D_5N): δ 0.87 (3H, s, H-18), 1.02 (3H, s, H-19), 0.96 (3H, d, $J=6.8$ Hz, H-27), 1.33 (3H, d, $J=6.8$ Hz, H-21), 1.75 (6H each d, $J=6.1$ Hz, H-6 and -6′ of Rha), 4.79 (1H, d, $J=7.7$ Hz, H-1 of Glc), 5.12 (1H, d, $J=7.7$ Hz, H-1′ of Glc), 6.21 (2H each, br s, H-1 and -1′ of Rha). Compound **2** (85 mg): A white powder from MeOH, $[\alpha]_D^{26}$ −70° ($c=0.35$, MeOH). TLC *Rf* 0.34 (solvent a). HPLC t_R 45.5 min (solvent c). Acid hydrolysis produced L-rhamnose, D-glucose and diosgenin. ^{1}H-NMR (C_5D_5N): δ 0.79 (3H, s, H-18), 1.01 (3H, s, H-19), 0.91 (3H, d, $J=7.5$ Hz, H-27), 1.14 (3H, d, $J=6.8$ Hz, H-21), 1.69 (6H each, d, $J=6.0$ Hz, H-6 and -6′ of Rha), 6.19 (2H each, br s, H-1 and -1′ of Rha), 3.22 (3H, s, OCH_3). Compound **3** (22 mg): A white powder from MeOH, $[\alpha]_D^{19}$ +25.2° ($c=0.15$, MeOH). TLC *Rf* 0.20 (solvent a). HPLC t_R 33.5 min (solvent c). FAB-MS $[M-H]^-$ Calcd for $C_{56}H_{92}O_{26}-H$: 1179.1779. Found:

1179.1794. Acid hydrolysis produced D-xylose, L-rhamnose, D-glucose and diosgenin. **3** (15 mg) was acetylated with acetic anhydride and pyridine for 12 h at room temp. The solvent was removed and the acetylated sample (**9**) was subjected to 2D NMR analysis. Compound **4** (26 mg): A white powder from MeOH, $[\alpha]_D^{19}$ −14.6° (c=0.1, MeOH). TLC Rf 0.3 (solvent a). HPLC t_R 40.5 min (solvent c). Ehrlich reagent test: positive. Acid hydrolysis produced D-xylose, L-rhamnose and D-glucose as well as diosgenin. Compound **5** (11 mg): A white powder from MeOH, $[\alpha]_D^{20}$ −43° (c=0.3, MeOH). TLC Rf 0.21 (solvent a). HPLC t_R 64.0 min (solvent c). Ehrlich reagent test: negative. Acid hydrolysis produced L-rhamnose and D-glucose. FAB-MS m/z: 1047 $[M-H]^-$, 885 $[M-H-Glc]^-$. Compound **6** (10 mg): A white powder from MeOH. $[\alpha]_D^{20}$ −123° (c=0.07, MeOH). TLC Rf 0.17 (solvent a). HPLC t_R 61.5 min (solvent c). Ehrlich reagent test: negative. Acid hydrolysis produced L-rhamnose, D-glucose and D-xylose. FAB-MS m/z: 1179 $[M-H]^-$, 1047 $[M-H-Xyl]^-$, 885 $[M-H-Xyl-Glc]^-$ and 739 $[M-H-Xyl-Glc-Rha]^-$. Compound **7** (9 mg): A white powder from MeOH, $[\alpha]_D^{20}$ −49° (c=0.2, MeOH). TLC Rf 0.33 (solvent a). HPLC t_R 71 min (solvent c). Ehrlich reagent test: negative. Acid hydrolysis produced L-rhamnose and D-glucose. FAB-MS m/z: 1047 $[M-H]^-$, 885 $[M-H-Glc]^-$. Compound **8** (10 mg): A white powder from MeOH, $[\alpha]_D^{20}$ −93° (c=0.13, MeOH). TLC Rf 0.28 (solvent a). HPLC t_R 67 min (solvent c). Ehrlich reagent test: negative. Acid hydrolysis produced D-xylose, L-rhamnose and D-glucose. FAB-MS m/z: 1179 $[M-H]^-$, 1047 $[M-H-Xyl]^-$, 885 $[M-H-Xyl-Glc]^-$.

Methylation Analysis of Compound 1 To a solution of compound **1** (5 mg) in dimethyl sulfoxide (DMSO 100 μl) was added a solution of NaH in DMSO (100 μl), and the solution was sonicated for 1 h. To this solution, CH_3I (150 μl) was added. After sonication for another 1 h, the reaction mixture was diluted with water and extracted with $CHCl_3$. After evaporation of $CHCl_3$, 90% HCOOH (2 ml) was added to the residue and the solution was kept at 100 °C for 1 h. HCOOH was evaporated and the residue was treated with 2 M TFA (1 ml) at 100 °C for 2 h. TFA was evaporated and the residue was dissolved in water (2 ml) and then treated with $NaBH_4$ (25 mg). After standing at room temp. for 2 h, the mixture was acidified by Dowex 50W-X1 (H^+ form) and concentrated to dryness. The residue was acetylated with Ac_2O–C_5H_5N (1 : 1, 1 ml) for 12 h at room temp. The reagent was removed by evaporation and the resulting mixture of methylated alditol acetates was subjected to gas chromatography–mass spectrum (GC-MS) analysis.[11] GC-MS was taken on a JEOL JMS-SX 102 spectrometer; on a Neutral Bond-1 column (0.25 mm i.d. × 25 m, Gasukuro Kogyo Inc.), injection temp. 200 °C, column temp. 150—250 °C (2 °C/min) and carrier gas He at 1 ml/min.

Isolation of Flavonol Glycosides The epicarps (100 g) were extracted with hot EtOH. After evaporation of the solvent, the extract (10 g) was chromatographed on a column of Diaion HP-20 with H_2O, MeOH and Me_2CO. The MeOH eluate was subjected to chromatography on RP-8 with a solvent (e) to give three fractions. Fraction 1 was subjected to HPLC with solvent (f) to produce two compounds (A and B). Compound A (22 mg): A yellow powder, $[\alpha]_D^{20}$ +54° (c=0.1, MeOH). TLC Rf 0.36 (solvent g). HPLC t_R 95 min (solvent f). Mg–HCl test: positive. Acid hydrolysis yielded L-rhamnose and D-glucose in addition to isorhamnetin. FAB-MS m/z: 625 $[M+H]^+$, 479 $[M+H-Rha]^+$, 317 $[M+H-Rha-Glc]^+$. Compound B (15 mg): A yellow powder, $[\alpha]_D^{20}$ +67° (c=0.07, MeOH). TLC Rf 0.39 (solvent g). HPLC t_R 81 min (solvent f). Mg–HCl test: positive. Acid hydrolysis gave L-rhamnose, D-galactose and isorhamnetin. FAB-MS m/z: 625 $[M+H]^+$, 479 $[M+H-Rha]^+$, 317 $[M+H-Rha-Gal]^+$.

Acid Hydrolysis of Compounds 1—8, A and B A sample (5 mg) was heated with 2 N HCl in H_2O–dioxane (1 : 1) in a sealed micro-tube at 80 °C for 3 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The aqueous layer was subjected to TLC on silica gel with the solvent (b) (detection: triphenyltetrazolium chloride reagent). Rf values were 0.17, 0.32 and 0.25 for D-glucose, L-rhamnose and D-xylose, respectively. The aqueous layer was concentrated, trimethylsilylated and subjected to gas liquid chromatography (GLC) analysis with a Shimadzu GC-8A apparatus equipped with a dual flame ionization detector; carrier gas, He 3.1 ml/min, Neutra Bond-1 column, column temp. 150 °C and injection temperature 220 °C. t_R of sugars were 6.1 min for L-rhamnose, 8.3 min for D-xylose and 22.1, 38.1 min for D-glucose. The identification of the aglycones (diosgenin and isorhamnetin) isolated from the $CHCl_3$ layer was based on ^{13}C-NMR analysis and comparison with reported data.[4,9]

Measurement of Antidiabetic Activity[12] For measurement of antidiabetic activity, male mice (Std.: dd Y strain, 20—25 g) were used in groups of five. Experimental diabetes was induced by i.p. injection of streptozotocin (200 mg/kg body weight) and blood samples were taken from the orbital sinus with micro-hematocrit tubes periodically. The glucose was determined using Dextrostix strips and a glucometer (Sankyo Co.).

Measurements of Hexokinase Activity and 2-Deoxyglucose Uptake The hexokinase activity of mouse liver cytosol was measured spectrometrically on the basis of an increase in absorbance at 340 nm due to the formation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) from $NADP^+$ in accordance with the formation of glucose-6-phosphate. The formation of 1 μmol of glucose-6-phosphate per minute was designated as 1 unit (U).

2-Deoxyglucose uptake by Ehrlich ascites tumor cells was measured by adding 20 μl of cell suspension (about 10^6 cells) to 180 μl of Krebs Ringer phosphate buffer containing 0.25 mM [^{3}H] 2-deoxyglucose, which was incubated for 2.5 minutes at 37 °C. After incubations, samples were filtered through Whatman glass filters (GF/B).

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