Hypoglycemic Polysaccharides from the Tuberous Root of *Liriope spicata*

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Previous studies showed that the water extract and crude polysaccharides from the tuberous root of *Liriope spicata* var. *prolifera* showed high hypoglycemic and hypolipidemic activities. In the present study, two new water-soluble polysaccharides, named LSP1 and LSP2, were isolated from the active crude polysaccharides by DEAE-cellulose 52 and AB-8 macroporous resin chromatography and tested for their hypoglycemic effects. On the basis of the chemical and physical data obtained, LSP1 and LSP2 are two fructans with the molecular weights 3.20 and 4.29 kDa, respectively, which both have a backbone structure of β-(1→2)-fructosyl residues that branches at O-6 with fructosyl residues and terminates with a glucosyl residue and a fructosyl residue. Preliminary activity tests revealed that both LSP1 and LSP2 caused a significant decrease of the fasting blood glucose (FBG) and a significant improvement on glucose tolerance in type 2 diabetic mice. These results suggest that LSP1 and LSP2 show high potential to be explored as natural oral hypoglycemic agents.

Polysaccharides have been found as common polymers in many families of plants, bacteria, and fungi and received considerable attention in recent years due to their antitumor and antidiabetic activities.[1−5] As a special kind of polysaccharide, the fructans comprise (2→1)-linked and/or (2→6)-linked α-D-fructofuranosyl units. Although there are many reports on the structural analysis of fructans, reports on their bioactivity are still infrequent.

Liriope spicata var. *prolifera* (Liliaceae) is indigenous to Hubei Province, China. The tuberous root, recorded as Radix Liriopes in the Pharmacopoeia of the People's Republic of China, is frequently used in prescriptions of traditional Chinese medicine as an antidiabetic agent. Because of the high availability and safety, it is regarded as both food and medicine by the Chinese Ministry of Public Health. In recent years, several chemical studies on the tuberous root of *L. spicata* have been reported, such as the structural analysis of steroidal glycosides[6,7] and the quantitative analysis of crude polysaccharides.[8] Our previous studies have shown that the water extract, containing crude polysaccharides from the tuberous root, showed hypoglycemic and hypolipidemic activities. Upon purification, the polysaccharides exhibited greater hypoglycemic effects than the original water extract.[9] In order to reveal the nature of the active polysaccharides, we report here the isolation and chemical characterization of two new polysaccharides from the active crude polysaccharides of *L. spicata* and their hypoglycemic effects on type 2 diabetic mice. These results suggest that both LSP1 and LSP2 could be responsible for the antidiabetic function of *L. spicata* and might be valuable lead compounds for the discovery of new antidiabetic agents.

The crude polysaccharides from the tuberous root of *L. spicata* were obtained by extraction, deproteination, dialysis, and precipitation. The polysaccharides were purified through DEAE-cellulose 52 and AB-8 macroporous resin columns, leading to the isolation of two polysaccharide fractions, LSP1 and LSP2.

LSP1 and LSP2 are two white powders with $[\alpha]^{20}_{D}$ −43.6 and −55.8 (*c* 0.1, H_2O), respectively. The total sugar content was estimated as 99% by the phenol-sulfuric acid method. The high-performance gel permeation chromatography (HPGPC) profiles showed a single and symmetric sharp peak, respectively, indicating that both were homogeneous polysaccharides. Through correlation with the calibration curve of dextran standards, their weight-average molecular weights were approximately 3.20 and 4.29 kDa, respectively. As determined by the *m*-hydroxydiphenyl colorimetric method,[10] LSP1 and LSP2 were both free of uronic acid. It had a negative response to the Lowry test[11] and no absorption at 280 or 260 nm in the UV spectrum, indicating the absence of protein and nucleic acid.

The strong absorption in the range 1200−1000 cm^{-1} that appeared in the IR spectra suggested that the main monosaccharides in both LSP1 and LSP2 had a furanose ring. Broad bands at 3402, 2935, and 1644 cm^{-1} in LSP1 and at 3401, 2935, and 1649 cm^{-1} in LSP2 were due to the hydroxy stretching vibration, the C−H stretching vibration, and the bound water, respectively.[12] Characteristic bands at 930 and 818 cm^{-1} for the skeleton vibration of fructofuranose suggested that both LSP1 and LSP2 were fructans.[13] Monosaccharide composition analysis by TLC and GC after hydrolysis showed that LSP1 and LSP2 comprised fructose and glucose approximately in the ratio of 19:1 and 25:1, respectively.

Periodate oxidation of LSP1 and LSP2 resulted in 1.06 and 1.03 mol of periodate consumed, respectively, and almost no formic acid produced per mole of sugar residues, indicating C-2 hydroxy groups of all the fructosyl residues in both LSP1 and LSP2 were replaced. After Smith degradation, only glycerol was found by GC-MS, indicating that the C-3 and C-4 hydroxy groups of the fructose residues were free. Therefore, both LSP1 and LSP2 mainly comprise (2→1)-linked, (2→6)-linked, and/or (1→6)-linked glycosidic bonds.

To further confirm the linkage of sugars, LSP1 and LSP2 were methylated and reductively cleaved, and then the partially methylated alditol acetates were analyzed by GC-MS to show seven peaks in both instances. The fragmentation patterns of these peaks were identified and the molar ratios were estimated (Table 1). Corresponding with the molecular weight, it can be inferred that both LSP1 and LSP2 have a backbone structure with six repeating units; each repeating unit in LSP1 comprises (1, 2→)-linked, (1, 2, 6→)-linked, and (2→)-linked fructosyl residues in the ratio of 1:1:1 and in LSP2 in the ratio of 2:1:1 and a glucosyl residue and a fructosyl residue linked with the two terminals of the backbone of both LSP1 and LSP2, respectively. This pattern of linkage is in accordance with the results of periodate oxidation and Smith degradation.

On the basis of the data available,[14,15] the resonances in the region δ 105−107 in the ^{13}C NMR spectra were attributed to C-2 of four β-D-fructofuranose residues (A, B, C, and D, respectively) in LSP1 and five β-D-fructofuranose residues (A, B, C, D, and E,

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Table 1. GC-MS Data of Alditol Acetate Derivatives from the Methylated Products of LSP1 and LSP2

methylated sugars[a]	mass fragments (*m/z*) (relative abundance, %)	type of linkage	molar ratio[b]	
			LSP1	LSP2
2,3,4,6-Me4-Gluc	43,71,87,101,129,145,161,205 (100,22,27,99,59,50,55,18)	Glc(1→	1.12	0.61
1,3,4,6-Me4-Mann	45,87,117,129,161,205 (19,32,12,100,30,11)	Fru(2→	7.14	7.02
1,3,4,6-Me4-Sorb	45,71,87,117,129,161 (16,9,30,19,100,20)			
3,4,6-Me3-Mann	43,71,87,101,129,161,189 (100,17,45,21,98,24,13)	→1)Fru(2→	6.33	12.18
3,4,6-Me3-Sorb	43,87,101,129,161,189,233 (100,48,19,98,24,11,6)			
3,4,-Me2-Mann	45,71,87,129,161,189,233 (12,9,25,100,36,30,5)	→1,2)Fru(6→	6.00	6.00
3,4,-Me2-Sorb	43,71,87,129,189 (100,11,47,98,30)			

[a] 2,3,4,6-Me4-Gluc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol, etc. [b] A fructosyl residue was reduced and derivatized into a mannitol or sorbitol acetate; thus, each type of fructosyl residue produced two peaks, and the sum of the two represented a certain type of fructosyl residue.

Table 2. ^{1}H and ^{13}C NMR Chemical Shifts of Polysaccharide LSP1 in D_2O (ppm relative to the signal for DSS)

	1		**2**		**3**		**4**		**5**		**6**	
sugar residue	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
A →2)-*β*-D-Fru-(1→	3.75[a], 3.65[b]	64.4	nd	106.7	4.17	78.5	4.08	76.8	3.92	83.7	3.83[a,b]	64.8
B →1,6)-*β*-D-Fru-(2→	3.73[a], nd[b,c]	63.7	nd	106.0	4.21	78.9	4.09	76.7	3.94	82.9	3.92[a], 3.86[b]	65.8
C →2)-*β*-D-Fru-(1→	3.64[a,b]	63.1	nd	106.2	4.22	78.6	4.08	76.9	3.91	83.6	3.81[a,b]	64.7
D *β*-D-Fru-(2→	3.64[a,b]	63.1	nd	106.9	4.20	nd	4.06	77.0	nd	nd	3.80[a,b]	nd
Glc α-D-Glc-(1→	5.42	95.0	3.54	73.8	nd	75.0	3.47	71.8	3.82	74.1	3.78[a,b]	62.7

[a] Interchangeable. [b] Interchangeable. [c] nd = not detected, or overlapped.

Table 3. ^{1}H and ^{13}C NMR Chemical Shifts of Polysaccharide LSP2 in D_2O (ppm relative to the signal for DSS)

	1		**2**		**3**		**4**		**5**		**6**	
sugar residue	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
A →2)-*β*-D-Fru-(1→	3.75[a], 3.68[b]	63.4	nd[c]	106.4	4.23	78.1	4.08	76.4	3.93	83.7	3.83[a,b]	64.8
B →1,6)-*β*-D-Fru-(2→	3.83[a], 3.73[b]	65.8	nd	105.9	4.24	78.8	4.09	76.7	3.94	82.6	3.92[a], 3.87[b]	66.0
C →2)-*β*-D-Fru-(1→	3.74[a], 3.64[b]	63.4	nd	106.6	4.17	78.3	4.09	76.5	3.93	83.4	3.83[a,b]	64.7
D *β*-D-Fru-(2→	3.67[a,b]	63.1	nd	106.2	4.22	78.6	4.10	76.6	3.94	83.6	3.81[a,b]	64.7
E *β*-D-Fru-(2→	nd	nd	nd	106.7	4.19	77.3	4.05	76.3	3.90	83.6	nd	nd
Glc α-D-Glc-(1→	5.43	94.8	3.54	73.7	nd	75.1	3.47	71.7	3.82	74.2	3.78[a,b]	62.6

[a] Interchangeable. [b] Interchangeable. [c] nd = not detected, or overlapped.

respectively) in LSP2, respectively. The anomeric carbon (C-1) signal of a terminal α-D-glucopyranose residue (Glc) in LSP1 and LSP2 resonated at *δ* 95.0 and 94.8, respectively. Both ^{1}H NMR spectra had an anomeric proton (H-1) signal at *δ* 5.42 and 5.43, respectively, which also explained the α-D-configuration of the glucosyl residue. Tables 2 and 3 summarize the ^{1}H and ^{13}C NMR data of LSP1 and LSP2, respectively, which were confirmed by COSY, TOCSY, NOESY, HSQC, and HMBC spectra.

In the TOCSY spectra, five groups of correlated signals (A, B, C, D, and Glc, respectively) in LSP1 and six groups (A, B, C, D, E, and Glc, respectively) in LSP2 were observed and confirmed by COSY. The signals of residue D in LSP1 and residue E in LSP2 were weaker, indicating that residue D in LSP1 and residue E in LSP2 might both be a terminal residue. From the HSQC spectrum, according to the assignment of hydrogen signals, all the carbon signals were confirmed except for C-2 of the fructosyl residues. In the HMBC spectrum of LSP1 (Figure 1A), several groups of correlated signals were observed, such as C-2/H-3 and H-5 in residue A, C-2/H-3 and H-5 in residue B, C-2/H-1 and H-5 in residue C, and C-2/H-3 and H-1 in residue D, which confirmed the signals of C-2 of the four fructosyl residues in LSP1. In the HMBC spectrum of LSP2 (Figure 2A), key correlated signals (C-2/H-3 in residue A, C-2/H-1 in residue B, C-2/H-3 in residue C, C-2/H-4 and H-5 in residue D, and C-2/H-3 and H-4 in residue E) were observed, which confirmed the C-2 signals of the five fructosyl residues in LSP2.

The monosaccharide sequence within the repeating unit was established by NOESY and HMBC experiments. For both LSP1 and LSP2, all groups of correlated signals in the NOESY spectra could be found in the TOCSY spectra, indicating that each glycosidic bond was formed from a C-2 hydroxy group of a fructosyl residue and a hydroxy group of another sugar residue. In the HMBC spectrum (Figure 1A), key correlations were observed including C-2 in residue B/H-1 in residue A, C-2 in residue A/H-1 in residue B, and C-2 in residue C/H-6 in residue B. These results determined the monosaccharide sequence within the repeating unit of LSP1 as shown in Figure 1B. In the HMBC spectrum (Figure 2A), key correlations were observed including C-2 in residue B/H-1 in residue A, C-2 in residue C/H-1 in residue B, C-2 in residue A/H-1 in residue C, and C-2 in residue D/H-6 in residue B. These results determined the monosaccharide sequence within the repeating unit of LSP2 as shown in Figure 2B. In both HMBC spectra (Figures 1A and 2A), the C-2 in residue A correlated to H-1 in residue Glc, indicating that the glucosyl residue is a terminal group, linked with residue A at the nonreducing ends of both LSP1 and LSP2. Due to signal overlap, there were no signals to confirm the linkage mode of residue D in LSP1 and residue E in LSP2. According to the results of methylation analysis and Smith

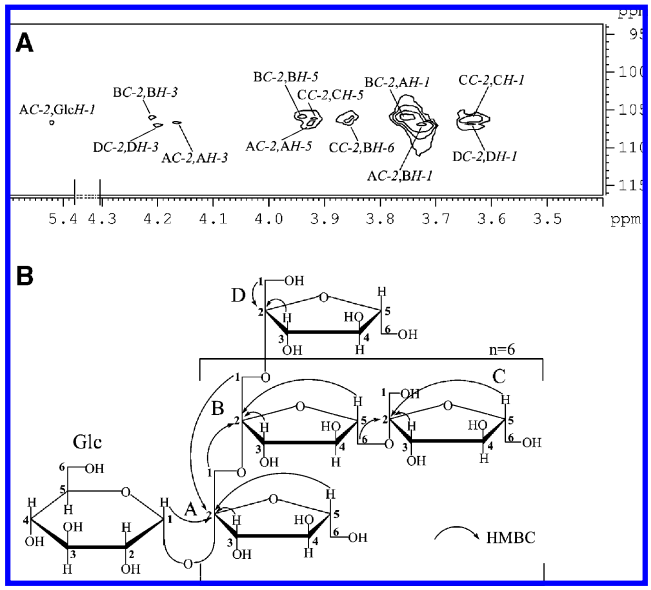

Figure 1. (A) Part of the HMBC spectrum of LSP1. Arabic numerals refer to atoms in sugar residues denoted by capital letters as shown in Table 2. (B) Proposed structure of LSP1 with key HMBC correlations shown, where n indicates the number of repeating units.

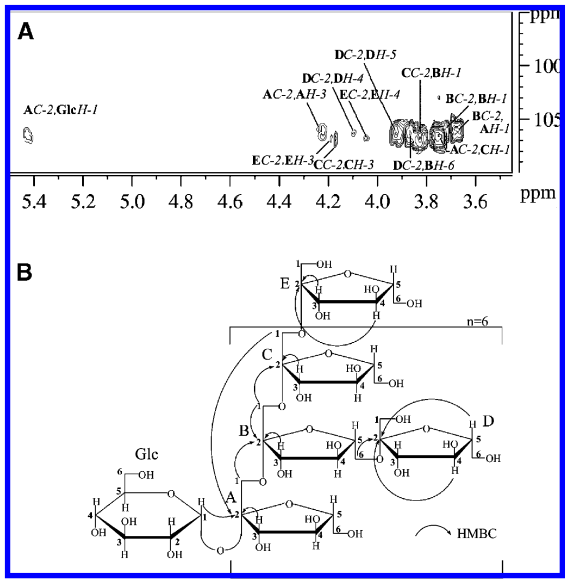

Figure 2. (A) Part of the HMBC spectrum of LSP2. Arabic numerals refer to atoms in sugar residues denoted by capital letters as shown in Table 3. (B) Proposed structure of LSP2 with key HMBC correlations shown, where n indicates the number of the repeating unit.

degradation, residue D is a terminal residue linked with residue B at the other end of LSP1; residue E is also a terminal residue linked with residue C at the other end of LSP2. According to the molecular weight, both LSP1 and LSP2 consist of six repeating units, respectively.

There are many plant fructans with different structures.[16] One of the simplest fructans is linear inulin, which consists of β-(1→2)-linked fructose residues. Another is the levan type found in grasses (Poaceae), which is a linear β-(2→6)-linked fructose polymer,[17] and mixed fructan types, called gramminans, may also be present and consist of β-(2→6)-linked fructose residues with β-(1→2) branches.[18] However, LSP1 and LSP2 are two new fructose polymers, which both have a (1→2)-linked fructosyl backbone with β-(6→2) branches.

The biological properties of LSP1 and LSP2 were elucidated. Table 4 shows the fasting blood glucose (FBG) levels of mice at baseline and each week during four weeks of oral administration. The FBG level of diabetic control was significantly higher compared with normal control ($P < 0.01$). LSP1 and LSP2 at both doses significantly reduced FBG after administration ($P < 0.01$), and the FBG levels of LSP1 and LSP2 groups were almost the same as that of the normal control when the mice were administered for four weeks. There were no significant differences between the hypoglycemic activities of the two doses for both LSP1 and LSP2.

In Figure 3, the effects of LSP1 and LSP2 on oral glucose tolerance in type 2 diabetic mice are presented. When the mice were orally administered with glucose, the rates of increase in the blood glucose level (BGL) were the same for LSP1, LSP2, rosiglitazone, and normal control groups during the first 60 min. After that the BGL of these groups recovered more rapidly compared to diabetic control ($P < 0.01$). These data suggest that LSP1 and LSP2 improve the glucose tolerance of type 2 diabetic mice.

There are many polysaccharides reported to have antidiabetic activity, such as polysaccharides from *Auricularia auricular-judae,*[2] American ginseng berry,[3] *Cordyceps militaris* and *C. sinensis*,[5] and so on. However, there are no reports about the antidiabetic activity of micromolecular fructans.

On the basis of the results mentioned above, it can be concluded that LSP1 and LSP2, obtained from tuberous root of *L. spicata*, are two new fructans having the structures shown in Figures 1B and 2B, respectively, where n indicates the number of repeating units. Preliminary activity tests revealed that both LSP1 and LSP2 showed high hypoglycemic activities and high potential to be explored as natural oral hypoglycemic agents.

In order to further corroborate the above structure, MALDI-TOF-MS analyses and single-crystal X-ray studies of LSP1 and LSP2 are currently underway. Further pharmacological evaluation is required in order to determine the mechanism of action.

Experimental Section

General Experimental Procedures. Optical rotations were measured at 20 °C with a Jasco P-1010 polarimeter. UV absorption spectra were recorded with a Varian Cary 100 UV−vis spectrophotometer. IR spectra were recorded on a Vertex 70 FT-IR spectrophotometer. Total carbohydrate content was determined by the phenol-sulfuric acid method.[19] Uronic acid and protein contents were determined by the *m*-hydroxydiphenyl colorimetric and the Lowry methods,[10,11] respectively. The hydrolysates were analyzed using TLC on a silica gel plate with a solvent system of *n*-butanol, EtOAc, 2-propanol, and H_2O [7:4:7:2 (v/v)]. The plate was visualized with *o*-phthalic acid reagent. The hydrolysates were determined by GC (Huli 9790) equipped with a DB-5 capillary column (30 m × 25 mm) as trimethylsilylated glycosides (per-*O*-TMS-glycosides), obtained by trimethylsilylation using TRI-SIL reagent for 10 h at 60 °C.[20] Identification of per-*O*-TMS-glycosides of the sugars was carried out from the retention time relative to standard sugars. GC-MS analyses were performed on a GC-MS (Aglient 6890N) instrument equipped with a DB-5 capillary column. Homogeneity and molecular weight were determined by HPGPC, performed on an HP-1100 instrument equipped with a G4000PW_{XL} column (7.8 × 300 mm) and a model 300S ELSD detector. The column was calibrated with dextran standards (MW 1, 3, 10, 40, and 70 kDa, Sigma). Then 20 μL of 1% LSP1 or LSP2 solution was injected, with water as the mobile phase at a flow rate of 0.6 μL/min. For solutions of LSP1 or LSP2 (100 mg, each) in D_2O (0.7 μL), all NMR spectra were recorded at 40 °C with a Varian Unity Inova-600 spectrometer.

Plant Material. The tuberous root of *L. spicata* var. *prolifera* was collected from its standardized planting base in Xiangfan, Hubei Province, China, in April 2006. Plant identification was done by Prof. Jiachun Chen (Faculty of Pharmaceutical Sciences, Huazhong University of Science and Technology, Wuhan, China). A voucher specimen (no. 20060405) has been kept in the herbarium of Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, Tongji School of Pharmaceutical Sciences, Huazhong University of Science and Technology.

Table 4. Effects of LSP1 and LSP2 on FBG in Type 2 Diabetic Mice (mean ± SD)[a]

	blood glucose levels (mmol/L)				
	pretreatment (week)	post-treatment (week)			
group	0	1	2	3	4
normal control	3.96 ± 0.36	4.05 ± 0.35**	4.16 ± 0.42**	4.30 ± 0.41**	4.30 ± 0.32**
Ddiabetic control	14.97 ± 1.56	14.89 ± 1.37	14.86 ± 1.58	14.89 ± 1.42	15.03 ± 1.47
rosiglitazone (2 mg/kg)	15.11 ± 1.54	9.58 ± 1.62**	5.54 ± 1.34**	5.04 ± 1.21**	4.94 ± 0.61**
LSP1 (200 mg/kg)	15.11 ± 0.55	9.35 ± 0.84**	7.25 ± 1.36**	5.44 ± 1.48**	4.83 ± 1.21**
LSP1 (100 mg/kg)	14.81 ± 0.59	9.71 ± 1.51**	7.96 ± 1.59**	5.70 ± 1.30**	5.13 ± 1.11**
LSP2 (200 mg/kg)	15.01 ± 0.58	8.65 ± 1.53**	6.16 ± 1.44**	5.14 ± 1.28**	4.78 ± 1.18**
LSP2 (100 mg/kg)	14.76 ± 0.50	10.04 ± 1.71**	7.05 ± 1.54**	4.70 ± 1.20**	4.63 ± 1.21**

[a] $^{**}P < 0.01$ as compared with diabetic control.

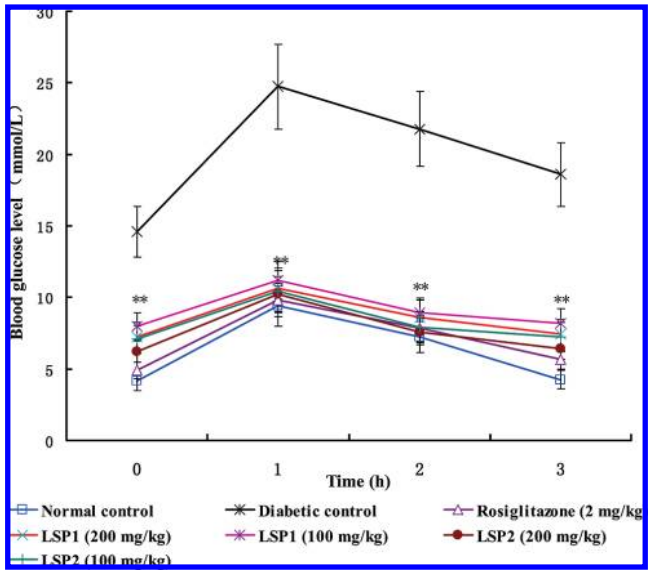

Figure 3. Effects of LSP1 and LSP2 on oral glucose tolerance in type 2 diabetic mice (mean ± SD). $^{*}P < 0.05$ as compared with diabetic control. $^{**}P < 0.01$ as compared with diabetic control.

Isolation and Purification of LSP1 and LSP2. Powdered tuberous root (100 g) was boiled in distilled H_2O (3 × 0.5 h, 1:4, 1:4, 1:2, w/v). Each extract was then filtered and combined. The H_2O extract was deproteinated by an enzymolysis method with papain.[9] The deproteinated extract was dialyzed using a regenerated cellulose membrane tube (MW cutoff 1000) against tap H_2O for 2 days and distilled H_2O for another day. The nondialysate was precipitated with 80% EtOH (v/v) to give 22.4 g of precipitate (crude polysaccharides). The crude polysaccharides were applied to a diethylaminoethyl cellulose 52 (DEAE-cellulose 52) column (2.5 × 38 cm), eluted with H_2O, followed by 0.1 M NaCl, 0.3 M NaCl, 0.5 M NaCl, and 0.1 M NaOH, respectively. The fraction eluted with H_2O was further purified on an AB-8 macroporous resin column (2.5 × 70 cm) eluted with H_2O followed by 30% EtOH. Two polysaccharide fractions were collected and lyophilized to get two white purified polysaccharides, named LSP1 (4.8 g) and LSP2 (12.3 g), respectively. LSP1 and LSP2 were analyzed as a single peak by HPGPC, respectively. Both LSP1 and LSP2 were used in the subsequent studies.

Chemical and Physical Analyses of LSP1 and LSP2. LSP1 was a white powder; IR (KBr) ν_{max} 3401, 2934, 1642, 1461, 1423, 1132, 1025, 928, 819, 597 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; COSY, TOCSY, NOESY, HSQC, and HMBC experiments were also performed; partial HMBC spectrum see Figure 1A. In the TOCSY spectrum, five groups of correlated signals were observed: H-3/H-1a and H-1b, H-3/H-4, H-3/H-5, H-3/H-6 in residue A; H-3/H-1a, H-4/H-1a, H-4/H-3, H-4/H-5, H-4/H-6a and H-6b in residue B; H-3/H-1, H-3/H-4, H-3/H-5, H-3/H-6 in residue C; H-1/H-3, H-1/H-4, H-1/H-6 in residue D; H-2/H-5, H-2/H-6, H-1/H-2, H-5/H-4 in residue Glc.

LSP2 was a white powder; IR (KBr) ν_{max} 3401, 2935, 1649, 1459, 1425, 1133, 1024, 930, 818, 597 cm^{-1}; ^1H and ^{13}C NMR data, Table 3; COSY, TOCSY, NOESY, HSQC, and HMBC experiments were also performed: partial HMBC spectrum see Figure 2A. In the TOCSY spectrum, six groups of correlated signals were observed: H-3/H-1a and H-1b, H-3/H-4, H-3/H-5, H-3/H-6a and H-6b in residue A; H-3/H-1a and H-1b, H-3/H-4, H-3/H-5, H-5/H-6a and H-6b in residue B; H-3/H-1a and H-1b, H-3/H-4, H-3/H-5, H-3/H-6a and H-6b in residue C; H-3/H-1a and H-1b, H-3/H-4, H-3/H-6a and H-6b, H-6ab/H-5 in residue D; H-3/H-4 (very weak), H-3/H-5 (very weak) in residue E; H-1/H-2, H-1/H-5, H-1/H-6a and H-6b, H-5/H-4 in residue Glc.

In order to determine the composition of monosaccharides, LSP1 and LSP2 (10.0 mg each) were completely hydrolyzed with 0.1 M TFA (2 mL) at 70 °C for 2 h. After the TFA was removed under vacuum, the hydrolysates were co-analyzed by TLC and GC methods.

To confirm the linkage of sugars by periodate oxidation and Smith degradation, LSP1 and LSP2 (20.0 mg) were separately dissolved in 0.015 M $NaIO_4$ (20 mL). The solutions were kept in the dark at 4 °C, and 0.1 mL aliquots were withdrawn each day, diluted to 25 mL with distilled H_2O, and read in a spectrophometer at 223 nm.[21,22] Complete oxidation, identified by a stable absorbance, was reached in 7 days. Consumption of $NaIO_4$ was measured by a spectrophotometric method.[23] The solution of the periodate product (2 mL) was sampled to calculate the yield of HCO_2H by titration with 0.01 M NaOH. The rest was extensively dialyzed against distilled H_2O, and the nondialysate was reduced by $NaBH_4$ (30 mg, 10 h), neutralized with 0.1 M HOAc, dialyzed against distilled H_2O again, and hydrolyzed with 0.1 M TFA at 70 °C for 2 h. The hydrolyzsates were finally converted into alditol acetates and analyzed by GC-MS.[24]

For further proof of the linkage of sugars by methylation, LSP1 and LSP2 (10.0 mg each) were methylated six times by the Needs and Selvendran method.[25] Complete methylation was confirmed by the lack of a hydroxy absorption in the IR spectrum. The permethylated product was depolymerized with 90% HCO_2H at 70 °C for 2 h and further hydrolyzed with 0.1 M TFA at 70 °C for 2 h. The partially methylated residues were reduced and acetylated.[22] The resulting products were analyzed by GC-MS. Partially methylated alditol acetates were identified by their fragment ions in EIMS,[26] and the molar ratios were estimated from the peak areas and response factors.[27,28]

Assay of Hypoglycemic Effect on Type 2 Diabetic Mice. Male BABL/c mice (No. SCXK (Hubei) 2003-0005) weighing 17−20 g were procured from The Center for Disease Prevention and Control in Hubei Province, China. Type 2 diabetes was induced by prescribed methods.[9] The diabetic mice were randomly divided into four groups of 10 mice each. Group 1, which served as the diabetic control, was orally administered 10 mL/kg of body weight/day (BW/D) of physiological NaCl solution (vehicle); group 2 was orally administered the standard oral hypoglycemic agent rosiglitazone (2 mg/kg BW/D) in the same vehicle; groups 3 and 4 received LSP1 in the same vehicle (200 and 100 mg/kg BW/D, respectively); and groups 5 and 6 received LSP2 in the same vehicle (200 and 100 mg/kg BW/D, respectively). Ten normal mice, which served as the normal control, were orally administered 10 mL/kg BW/D of vehicle. The mice of groups 1−6 were fed with diets high in fat and sugar during the study,[9,29] while the mice of the normal control group were fed with common pellet diets.

In all groups blood samples for the FBG level analysis were obtained from the tail vein of the overnight fasted mice at baseline and after 1, 2, 3, and 4 weeks of oral administration, and estimated using a one-touch glucometer (JPS-7, Yicheng, Beijing). The data were tested for statistical differences using the one-way ANOVA followed by the Dunnett post hoc test.

When orally administered for 14 days, mice in all groups were orally given glucose (50 mmol/kg) after 60 min of drug administration. Blood samples were obtained at baseline and 1, 2, and 3 h after glucose loading, and estimated using a one-touch glucometer.

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