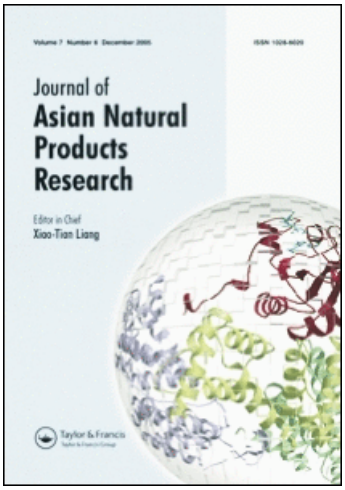


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# Structure Elucidation of Glycan of Glycoconjugate LbGp3 Isolated from the Fruit of *Lycium barbarum* L

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# STRUCTURE ELUCIDATION OF GLYCAN OF GLYCOCONJUGATE LbGp3 ISOLATED FROM THE FRUIT OF *LYCIUM BARBARUM* L.

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The structure of the repeat unit of the glycan of glycoconjugate LbGp3 with pronounced immunoactivity, isolated from the fruit of *Lycium barbarum* L. was elucidated based on methylation analysis, partial acid hydrolysis and $^{1}H$, $^{13}C$ NMR spectroscopy of the original glycan and products of its partial hydrolysis.

*Keywords:* *Lycium barbarum* L.; Glycoconjugate; Structure; Immunoactivity

## INTRODUCTION

An immunoactive glycoconjugate named LbGp3 was isolated from the fruit of *Lycium barbarum* L. [1]. Its carbohydrate content was up to 93.6%. Component analysis showed that it was composed of Ara and Gal in a molar ratio of 1 : 1, and 18 amino acids. Its MW was 92.5 kd as determined by size exclusive chromatography (SEC) with standard glycans. The linkage between the glycan and the core protein backbone may be O-linkage. As is well known, the glycan moiety of glycoconjugate possesses important biological function in regulating the activity of protein and giving signal of cell recognition [2]. So it is important to elucidate the glycan structure and to study its structure–function relationship.

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## RESULTS AND DISCUSSION

### Release of Glycan from LbGp3

Glycan was isolated on Sephadex G-100 column after it was released by pronase E. The elution pattern was obtained as shown in Fig. 1. The fraction containing sugar was collected and named LbGp3-OL. The result of its elemental analysis was C 43.30%, H 6.42%, and N 0%.

### Component Analysis of LbGp3-OL

Figure 2 showed that LbGp3-OL was composed of Ara and Gal in a molar ratio of 1 : 1 measured by the GC method.

### Methylation Analysis of LbGp3-OL

LbGp3-OL was completely methylated, the IR spectrum showed absence of hydroxyl groups, then hydrolyzed with HCOOH (88%) and 0.125 M $H_2SO_4$, consecutively, and prepared as alditol acetate as before. Results of the GC-MS analysis are shown in Fig. 3 and Table I.

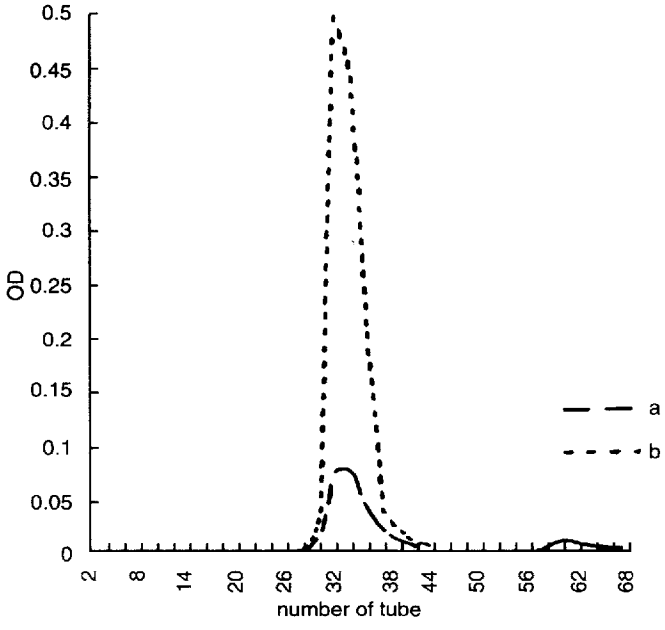



FIGURE 1 Elution pattern of glycan from LbGp3 on Sephadex G-100 Column (1.5 × 100 cm): eluent, 0.10 M NaCl; flow rate, 0.5 ml/min; (a) $\lambda_{280\,nm}$, (b) $\lambda_{490\,nm}$.

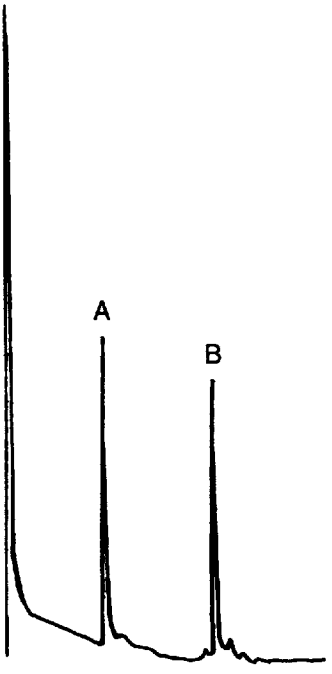



FIGURE 2 GC measurement of alditol acetate derivatives of LbGp3-OL on 3% OV-225 capillary column (0.3 mm × 25 m). Temperature: 180°C (5 min) 2°C/min 200°C (30 min). A: Ara, B: Gal.



Peak 5 and peak 8 were non-glycan peaks. Results of the GC-MS analysis showed that they represented o-phthalic acid which was a plasticity agent.

The peak area ratio of methylated fragments was as molar ratio, but molar response factors of partially methylated alditol acetates are calculated by the "effective carbon response", originally based on the predicted ionization potential of organic constituents in an FID [3]. As shown in Table I, a large amount of the branching unit in LbGp3-OL was -3,-4)Gal(1-, the non-reducing end was Ara(1- only. Peaks 1, 2 were furanoside, and peaks 4, 6, 7, 9 were pyranoside, but peak 3 was not ascertained.

## Partial Acid Hydrolysis

It is known that furanose is easily hydrolyzed in dilute $H_2SO_4$ with a rate of nearly 2 orders of magnitude higher than pyranose [4]. When LbGp3-OL was first hydrolyzed in 20 mM $H_2SO_4$ at 80°C for 12 h, paper chromatography showed that only Ara was liberated.

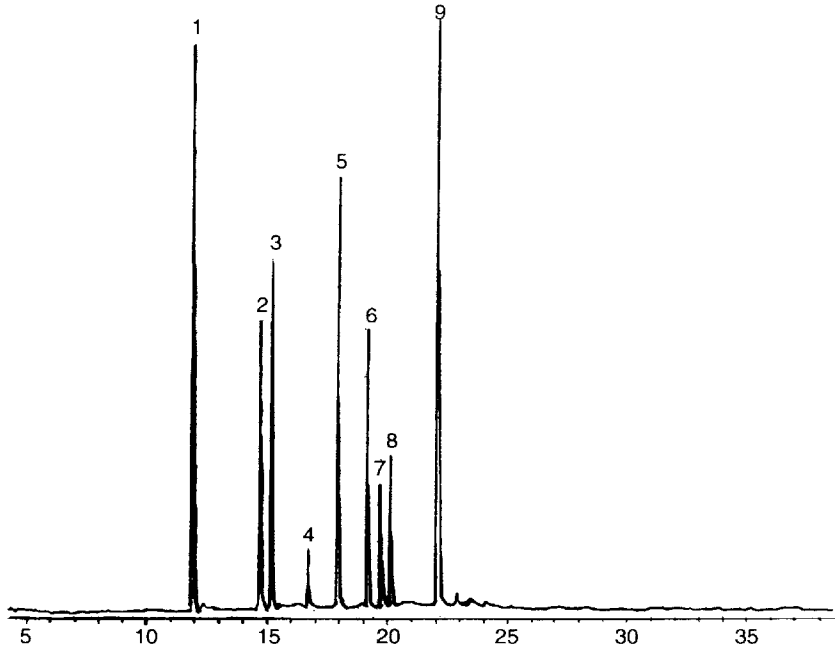



FIGURE 3 GC measurement of the partially methylated alditol acetates of LbGp3-OL on OV-17 capillary column (30 m × 0.2 mm). Temperature: 150°C (3 min) 5°C/min 260°C (30 min).

TABLE I Partially methylated alditol acetates of LbGp3-OL

| *GC peak* | *Fragment* | *Configure* | *Relative molar ratio* |
|---|---|---|---|
| 1 | 2,3,5-Me3-Ara | Ara(1 → | 5 |
| 2 | 2,5-Me2-Ara | → 3)Ara(1 → | 2 |
| 3 | 2,3,-Me2-Ara | → 4 or 5)Ara(1 → | 2 |
| 4 | 2,3,4,6-Me4-Gal | Gal(1 → | trace |
| 6 | 2,4,6-Me3-Gal | → 3)Gal(1 → | 2 |
| 7 | 2,3,6-Me3-Gal | → 4)Gal(1 → | 1 |
| 9 | 2,6-Me2-Gal | → 3, → 4)Gal(1 → | 5 |

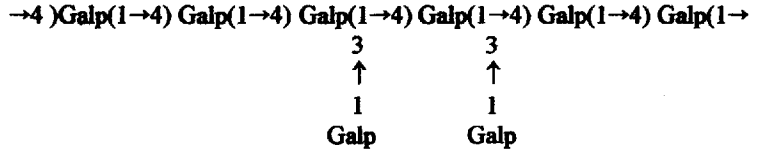
In the hydrolysate, named LbGp3-OL′ the molar ratio of Ara to Gal was 1.0 : 13. Results of methylation analysis of LbGp3-OL and LbGp3-OL′ by GC-MS were compared, as shown in Table II.

As shown in Table II, the increase of three -4)Gal(1- in LbGp3-OL′ was equal to the decrease of -3,-4)Gal(1- branching unit in LbGp3-OL, which meant that the main chain of LbGp3-OL′ was composed of -4)Gal(1-. The two new terminal Gal(1- in LbGp3-OL′ was equal to the decrease of -3)Gal(1- in LbGp3-OL and the number of -3,-4)Gal(1-, the branching unit, was equal to Gal(1- in LbGp3-OL′. So there were two Gal(1- chains linked to two branching -3,-4)Gal(1- in the main chain of LbGp3-OL′. Therefore,

TABLE II Methylation analysis of LbGp3-OL and LbGp3-OL′

| *Configuration* | *Relative molar ratio* | |
|---|---|---|
| | *LbGp3-OL* | *LbGp3-OL′* |
| Ara(1 → | 5 | trace |
| → 3)Ara(1 → | 2 | trace |
| → 4 or 5)Ara(1 → | 2 | trace |
| Gal(1 → | trace | 2 |
| → 3)Gal(1 → | 2 | trace |
| → 4)Gal(1 → | 1 | 4 |
| → 3, → 4)Gal(1 → | 5 | 2 |

we suggest that the structure of LbGp3-OL′ is probably as follows:




## Analysis of NMR

To determine the anomeric configuration, methylglycosides were selected as model compounds as shown in Table III [5,6].

$^{1}H$ and $^{13}C$ 300 MHz spectra of LbGp3-OL and LbGp3-OL′ are shown in Figs. 4 and 5.

As shown in Figs. 4 and 5, $^{1}H$ 5.319 ppm, $^{13}C$ 109–110 ppm in LbGp3-OL were not found in LbGp3-OL′, so Ara(1- was $\alpha$-furanose. $^{1}H$ 5.32 ppm and 5.17–5.15 ppm in LbGp3-OL disappeared in LbGp3-OL′ and ratio of integral area between $^{1}H$ 5.32 ppm and 5.17–5.15 ppm was about 1.12 : 1, therefore, -3)Ara(1- and -5)Ara(1- were all $\beta$ furanose. From Fig. 5 and Table III, we can deduce that all Gal in glycan were $\beta$-pyranose. Hence, we suggest that the structure of LbGp3-OL may be as follows:

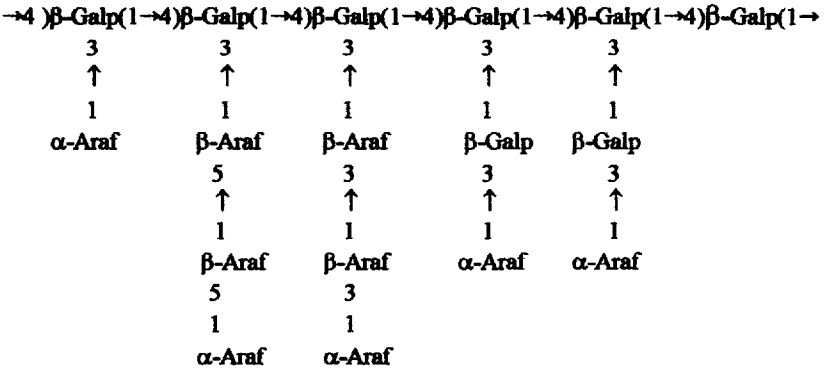


TABLE III Anomeric $^{1}H$ and $^{13}C$ NMR chemical shifts of some methylglycosides

| | $^{1}H$ (ppm) | | $^{13}C$ (ppm) | |
|---|---|---|---|---|
| | *p* | *f* | *p* | *f* |
| α-D-Ara | 4.16 | 5.28 | 105.1 | 109.2 |
| β-D-Ara | 4.72 | 5.12 | 101.0 | 103.2 |
| α-D-Gal | 4.73 | 99.2 | 103.5 | |
| β-D-Gal | 4.20 | 104.5 | 109.6 | |

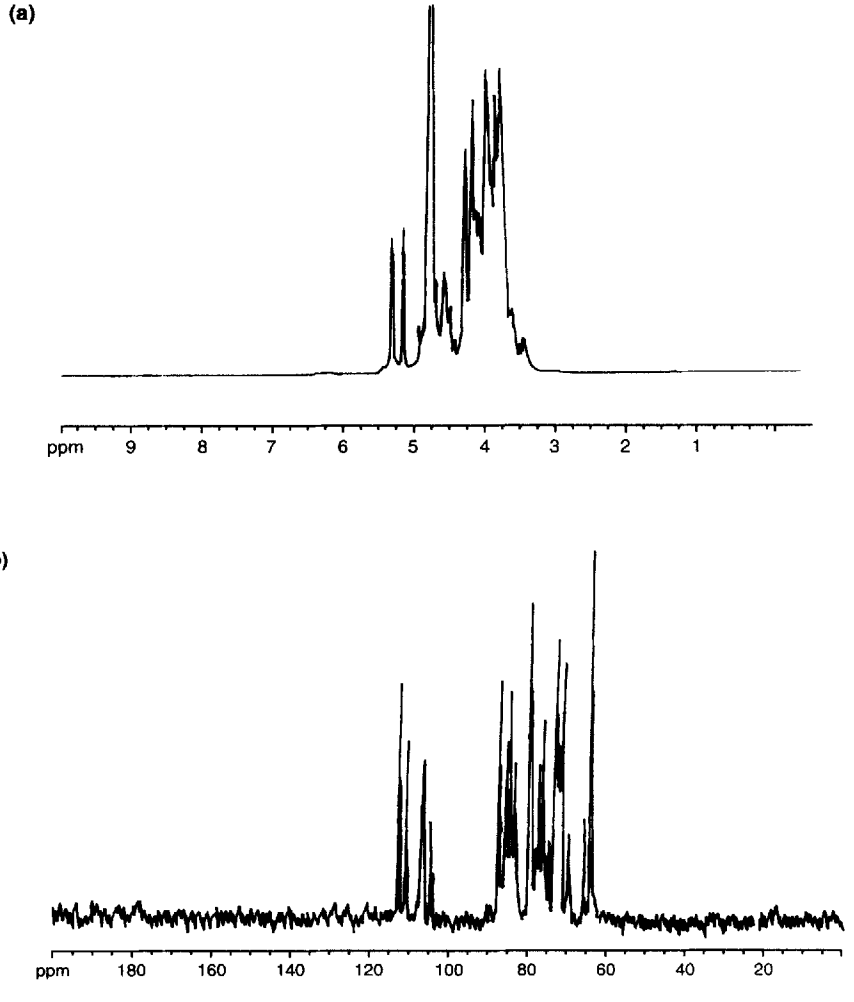



FIGURE 4 (a) $^{1}H$ 300 MHz spectra of LbGp3-OL. (b) $^{13}C$ 300 MHz spectra of LbGp3-OL.

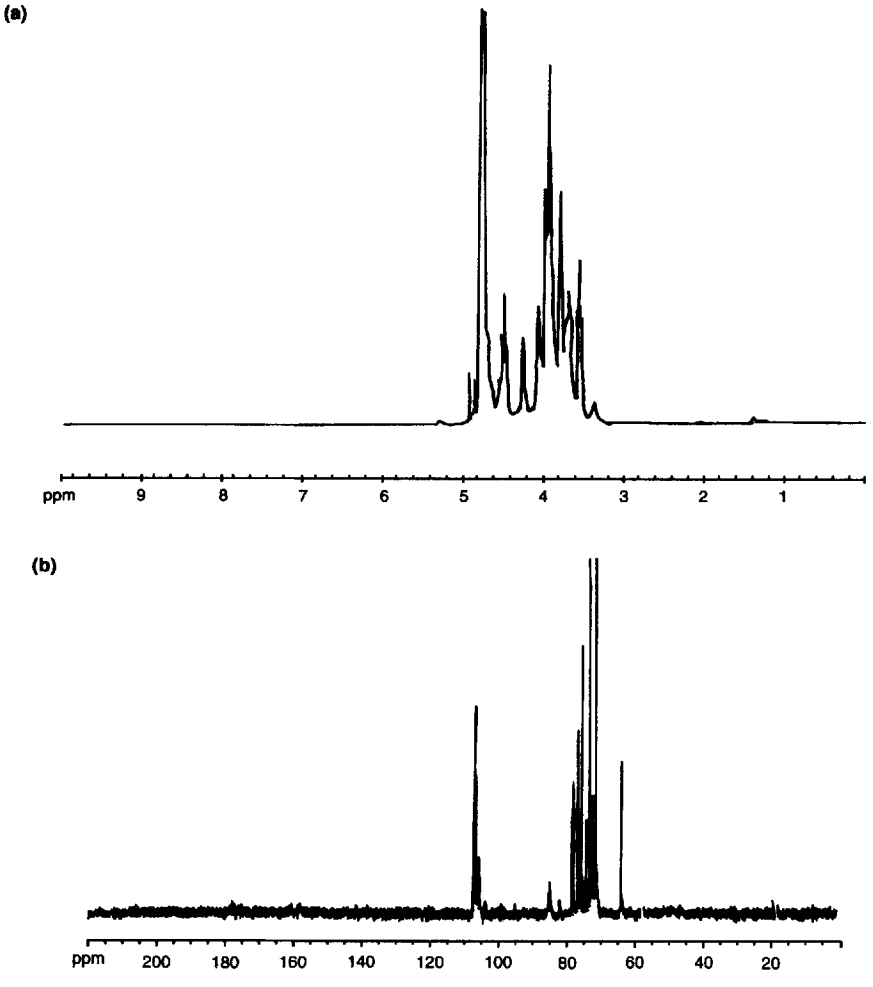



FIGURE 5 (a) $^{1}H$ 300 MHz spectra of LbGp3-OL′. (b) $^{13}C$ 300 MHz spectra of LbGp3-OL′.

This kind of structure was found commonly in plant arabinogalactan-proteins, but their glycan possessing immunoactivity was newly found.

## EXPERIMENTAL SECTION

### General Experimental Procedures

Sephadex G-25 and Sephadex G-100 were purchased from Pharmacia. Heavy water (99.8%) was from the Beijing Chemical Plant. All the other

chemical agents were of AR grade. The Spectrophotometer 722 for colorimetric analysis was the product of Shanghai Third Analytical Instrument Factory, GC analysis was carried out with a Varian VISTA402. GC-MS spectra was obtained on a Shimadzu QP 5000, and NMR spectra at 300 MHz on a Bruker-MX-300.

### Plant Material

*Lycium barbarum* L. was the product of Ning Xia Huizu Autonomous Region, People's Republic of China.

### Release of Glycan from LbGp3

LbGp3 was prepared according to Ref. [1]. Fifty mg LbGp3 was dissolved in 2 ml reaction buffer (100 mM Tris-HCl, pH 8.0, 1 mM $CaCl_2$). Pronase E (0.5 mg) was added into the reaction system and incubated for 72 h at 37°C, with further amounts of 0.5 mg pronase E added for every 24 h. Then, the product was isolated on Sephadex G-100 column, eluted with 0.1 M NaCl at a flow rate of 0.5 ml/min. Eluted fractions were monitored by UV absorption at 280 nm, and by phenol–$H_2SO_4$ colorimetry at 490 nm.The fractions containing sugar were collected, then loaded on Sephadex G-25 chromatography to desalt, and then freeze dried.



### Component Analysis of Sugar

The sample was hydrolyzed in 1.0 M $H_2SO_4$ at 100°C for 4 h, neutralized with barium carbonate, reduced to alditol by $NaBH_4$ with trace ammonia solution (25%), and acetylated with acetic anhydride/pyridine (V : V = 1 : 1) at room temperature overnight [7]. The alditol acetate derivative after hydrolysis was analyzed using GC with 3% OV-225 capillary column (0.3 mm × 25 m).

### Methylation Analysis

The sample was methylated with DMSO/SMSM/$CH_3I$ containing $Me_4U$ [8], then methylated again with NaOH (solid)/$CH_3I$/DMSO [9], hydrolyzed in HCOOH (88%) at 100°C for 3 h, and 0.125 M $H_2SO_4$ at 100°C for 16 h, then transferred into alditol acetates as before [10]. GC measurement of the partially methylated alditol acetates was carried out on OV-17 capillary column (0.2 mm × 30 m).

## Partial Acid Hydrolysis

The sample was hydrolyzed with 20 mM $H_2SO_4$ at 80°C for 12 h. The process of hydrolysis was monitored by paper chromatography. The hydrolysate was then dialyzed against water and freeze dried.

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