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Note

Structural identification and cytotoxic activity of a polysaccharide from the fruits of *Lagenaria siceraria* (Lau)

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ABSTRACT

A water-soluble polysaccharide, isolated from fruiting bodies of *Lagenaria siceraria,* is composed of methyl-α-D-galacturonate, 3-*O*-acetyl methyl-α-D-galacturonate, and β-D-galactose in a ratio of nearly 1:1:1. Compositional analysis, methylation analysis, periodate oxidation, and NMR studies (^{1}H, ^{13}C, 2D-COSY, TOCSY, NOESY, HMQC, and HMBC) revealed the presence of the following repeating unit in the polysaccharide:

OAc
↓
3
→4)-α-D-Gal*p*A6Me-(1→2)-α-D-Gal*p*A6Me-(1→4)-β-D-Gal*p*-(1→

This polysaccharide showed cytotoxic activity in vitro against human breast adenocarcinoma cell line (MCF-7).

Bottle gourd is an excellent fruit in nature having all the essential constituents required for normal and sound health. It cures pain, ulcers, fever, asthma, and other bronchial disorders.[1] The fruit extract of *Lagenaria siceraria* possesses cardioprotective[2] as well as anti-inflammatory[3] effects. It is also used as a purgative and a diuretic.[4,5] The antihepatotoxic activity[6] of different fractions of the ethanolic extract of fruit was evaluated and showed significant activity in a dose of 250 mg/kg. Modern phytochemical screening methods showed the presence of triterpenoid, cucurbitacins B, D, G, H[7–9] and flavone C-glycosides[10] in fruits. The seeds of *L. siceraria* are used in the treatment of headache and pain[1,11] and a novel ribosome inactivating protein, lagenin isolated from it reported to show antitumor and anti-HIV activities.[12]

Detailed structural works on polysaccharide isolated from the stem of *L. siceraria* were carried out and reported[13] by our group in *Carbohydrate Research*. Here, in this case, a different polysaccharide from the fruits (pepos) of *L. siceraria* was isolated from hot water extract followed by acetic acid treatment and gel filtration. We are reporting herein the detailed structural studies and cytotoxic activity of this polysaccharide on breast adenocarcinoma cell line (MCF-7).

The molecular weight of the polysaccharide was estimated as ~78,000 Da from a calibration curve prepared with standard dextrans.[14] The pure polysaccharide has $[\alpha]_D^{25}$ +11.6 (*c* 0.68, water). Paper chromatographic analysis[15] of the hydrolyzed product showed the presence of galacturonic acid and galactose only. The GLC analysis of the alditol acetates of the sugars showed the presence of galactose and carboxyl-reduced polysaccharide on hydrolysis followed by GLC examination of the corresponding alditol acetates which also showed the presence of galactose. The absolute configurations of the sugars were determined by the method of Gerwig et al.[16] taking intact and carboxyl-reduced polysaccharide. The polysaccharide was methylated by the Ciucanu and Kerek method[17] and then hydrolyzed. The alditol acetates of methylated product were analyzed by GLC–MS analysis and showed the presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-galactitol only. This result indicates that either (1→4)-galactopyranosyl or (1→5)-galactofuranosyl moiety may be present in the polysaccharide. The carboxyl-reduced polysaccharide[18] was methylated, and alditol acetates of methylated sugars were identified by GLC–MS analysis, which showed the presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-galactitol and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-galactitol in a molar ratio of nearly 2:1. This result indicates that (1→4)-linked galactopyranose or (1→5)-galactofuranose, (1→4) and (1→2)-linked galacturonic acid may be present in the polysaccharide. Then, a periodate oxidation experiment was carried out with this polysaccharide. The periodate-oxidized, reduced material was

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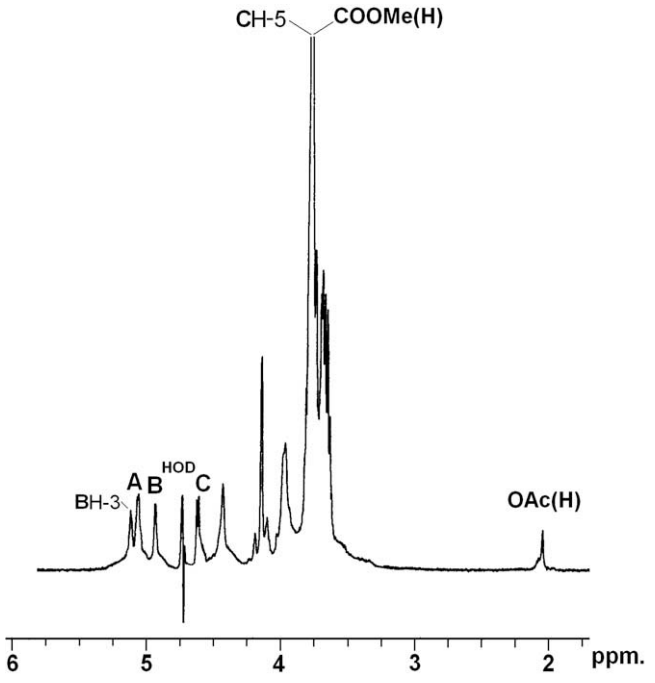

Figure 1. ^{1}H NMR spectrum (500 MHz, D_2O, 27 °C) of polysaccharide, isolated from the fruits of *Lagenaria siceraria.*

hydrolyzed and the paper chromatographic studies showed the presence of galacturonic acid only. Thus, the periodate oxidation study only confirms the presence of a (1→4)-linked galactopyranosyl or a (1→5)-galactofuranosyl residue, but the linkage analysis of galacturonic acid remains unconfirmed due to its survival under the same conditions. Hence, its linkage information was confirmed through NMR experiments.

The ^{1}H NMR (500 Hz) spectrum of the polysaccharide (Fig. 1) showed three anomeric proton signals at δ 5.06, δ 4.94, and δ 4.62 ppm in a ratio of nearly 1:1:1. The sugar residues were assigned as **A, B**, and **C** according to their decreasing anomeric chemical shifts (Table 1). In the ^{13}C NMR (125 MHz) spectrum (Fig. 2, Table 1), three anomeric carbon signals appeared at δ 104.7, δ 100.7, and δ 99.8 ppm in a ratio of nearly 1:1:1. Further δ 53.3 ppm and 20.5 ppm were assigned for carbomethoxy carbon and acetyl carbon, respectively. All the ^{1}H and ^{13}C signals were assigned using DQF-COSY, TOCSY, HMQC, and HMBC (Fig. 3) NMR experiments.

Residue **A** has an anomeric chemical shift at δ 5.06 and $J_{H\text{-}1,\ H\text{-}2}$ ~2.8 Hz and $J_{H\text{-}1,C\text{-}1}$ ~170 Hz, indicating an α-linked residue. The spin system of this residue, which consisted of only five protons with a relatively high chemical shift of the H-5 signal (δ 4.43) and weak coupling between H-3, H-4, and H-5, indicated that residue **A** is a D-galacturonosyl moiety. The C-1 signal of residue **A** at δ 99.8 was confirmed by the appearance of cross-peak **A**C-1, **B**H-2 in the HMBC experiment (Fig. 3, Table 3). The C-4 peak of residue **A** at δ 79.4 showed a down field shift compared to that of standard methyl glycosides[19] due to the effect of glycosylation. The presence of a carboxy methyl group in residue **A** is confirmed by the appearance of intra-residual coupling between ester carbonyl carbon (δ 171.0) and carboxy methyl proton (δ 3.78) in HMBC experiment (Fig. 3, Table 3). These results indicate that residue **A** is (1→4)-α-D-Gal*p*A6Me.

Residue **B** has an anomeric proton chemical shift at 4.94 (unresolved) and $J_{H\text{-}1,C\text{-}1}$ ~171 Hz indicating that it is an α-linked residue. This residue showed only five proton signals with two relatively high chemical shifts of H-5 (δ 4.43) and H-3 (δ 5.12) observed. The high chemical shift of H-3 (δ 5.12) is due to the presence of an acetyl group at the C-3 of this residue. The appearance of intra-residual coupling between acetyl carbonyl carbon (δ 171.0) and H-3 (δ 5.12) in the HMBC experiment (Fig. 3, Table 3) indicates that the acetyl group is attached at the C-3 of residue **B**. The anomeric carbon chemical shift of moiety **B** at δ 100.7 was confirmed by the presence of cross-peak **B**C-1, **C**H-4 in the HMBC experiment (Fig. 3, Table 3). The down field shift of C-2 (δ 74.9) compared to that of standard methyl glycosides[19] was due to the effect of glycosylation. The appearance of intra-residual coupling between carbonyl carbon (δ 171.0) and carboxymethyl proton (δ 3.78) in the HMBC experiment clearly indicates that the carboxyl group of galacturonic acid is present as a methyl ester. These indicate that residue **B** is (1→2)-3-*O*-Ac-α-D-Gal*p*A6Me.

Residue **C** has an anomeric proton chemical shift at 4.62 ppm. A large coupling constant $J_{H\text{-}1,H\text{-}2}$ value (~7.8 Hz) and $J_{H\text{-}1,C\text{-}1}$ value (~160 Hz) indicates that it is a β-linked residue. The $J_{H\text{-}2,H\text{-}3}$ value (~9.0 Hz) and the $J_{H\text{-}3,H\text{-}4}$ value (~3.5 Hz) for residue **C** indicate that it is a β-D-galactosyl residue. The C-1 signal of residue C at 104.7 ppm was confirmed by the presence of cross-peak **C**C-1, **A**H-4 in the HMBC experiment (Fig. 3, Table 3). The down field shift of C-4 (δ 78.7) with respect to standard methyl glycosides[19] indicates that residue **C** is present as a (1→4)-β-D-galactopyranosyl residue and not as a (1→5)-β-D-galactofuranosyl moiety whose anomeric carbon signal appears nearly at δ 109.6 ppm. Hence, the presence of a galactose in the polysaccharide in the furanose form does not arise.

The sequence of glycosyl residues of the polysaccharide was determined from NOESY (Fig. 4, Table 2) as well as from ROESY experiments (Fig. not shown) followed by confirmation with an

Table 1
^{1}H NMR[a] and ^{13}C NMR[b] chemical shifts of the polysaccharide isolated from the fruits of *Lagenaria siceraria* recorded in D_2O at 27 °C

Sugar residue	H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6a/6b/C-6	COOMe	3-*O*-COMe
→4)-α-D-Gal*p*A6Me-(1→	5.06	3.82	3.97	4.11	4.43		3.78	
A	99.8	68.9	68.1	79.4	72.2	171.0	53.3	
→2)-3-*O*-Ac-α-D-Gal*p*A6Me-(1→	4.94	3.70	5.12	3.97	4.43		3.78	2.04[c]
B	100.7	74.9	72.2	68.9	73.7	171.0	53.3	171.0[d], 20.5[e]
→4)-β-D-Gal*p*-(1→	4.62	3.66	3.68	4.14	3.78	3.74, 3.64		
C	104.7	72.2	68.9	78.7	70.9	61.1		

[a] The values of chemical shifts were recorded keeping HOD signal fixed at δ 4.73 ppm.
[b] The values of chemical shifts were recorded with reference to acetone as internal standard and fixed at δ 31.05 ppm at 27 °C.
[c] The values of the acetyl methyl proton.
[d] The values of the acetyl carbonyl carbon.
[e] The values of the acetyl methyl carbon.