Carbohydrate Polymers 86 (2011) 1252–1259

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structural characterization of a heteropolysaccharide isolated from the rhizomes of *Curcuma zedoaria* (Sati)

Chanchal K. Nandan[a], Ramsankar Sarkar[a], Sunil K. Bhanja[a], Soumitra Mondal[b], Syed S. Islam[a,*]

[a] Department of Chemistry and Chemical Technology, Vidyasagar University, Midnapore 721102, West Bengal, India
[b] Department of Chemistry, Panskura Banamali College, Panskura, Purba Medinipur, West Bengal, India

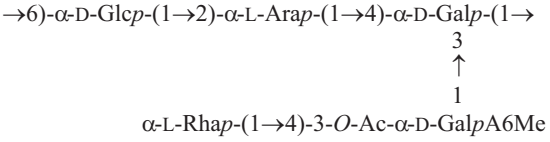
ARTICLE INFO

Article history:
Received 27 September 2010
Received in revised form 7 June 2011
Accepted 9 June 2011
Available online 16 June 2011

Keywords:
Curcuma zedoaria
Polysaccharide
Structure
NMR spectroscopy

ABSTRACT

A water-soluble polysaccharide, having an apparent molecular weight of 1.88×10^2 kDa, isolated from the aqueous extract of the rhizomes of *Curcuma zedoaria*, was found to consist of D-glucose, D-galactose, L-arabinose, D-methyl galacturonate, L-rhamnose with a molar ratio of nearly 1:1:1:1:1. On the basis of acid hydrolysis, methylation analysis, periodate oxidation, NMR experiments (^{1}H, ^{13}C, TOCSY, DQF-COSY, NOESY, ROESY, HMQC, and HMBC), the repeating unit of the polysaccharide was established as



1. Introduction

Curcuma zedoaria, an important member of the genus *Curcuma*, belongs to the family Zingiberaceae. In many Asian countries such as China, Korea and Japan it is used as traditional medicine for the improvement of blood circulation, menstrual flow, abdominal cramps and rheumatic pain. Rhizomes of *C. zedoaria* contain several specific sesquiterpenes that have anti-cancer and antioxidant properties (Maeda, Sunagane, & Kubota, 1984; Ruby, Kuttan, Babu, Rajasekharan, & Kuttan, 1995). These are also effective against flatulent colic, amenorrhea, and hepatocirrhosis (Limtrakul, Lipigorngoson, Namwong, Apisariyakul, & Dunn, 1997) and blood aggregation (Srivastava, Bordia, & Verma, 1995). Kim et al. found that some partially purified polysaccharides isolated from *C. zedoaria* showed strong dose dependent antitumor activity (Kim et al., 2000) and macrophage activation (Kim et al., 2001). Keeping in mind the usefulness of *C. zedoaria*, polysaccharide was isolated and its structural studies were carried out, since no work relating to the structural studies on the polysaccharide has been reported. In our present investigation two polysaccharides, water-soluble and water-insoluble, were isolated from hot water extract of rhizomes of *C. zedoaria* and structural characterization of the water-soluble part is only reported herein.

2. Materials and methods

2.1. Isolation, fractionation, and purification of the crude polysaccharide

The extraction and fractionation were performed according to the method previously reported (Chandra, Ghosh, Ojha, & Islam, 2009; Maiti et al., 2008). The steps are shown in the following diagram.

* Corresponding author. Tel.: +91 03222 276558x437/9932629971; fax: +91 03222 275329.
E-mail address: sirajul_1999@yahoo.com (S.S. Islam).

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doi:10.1016/j.carbpol.2011.06.021

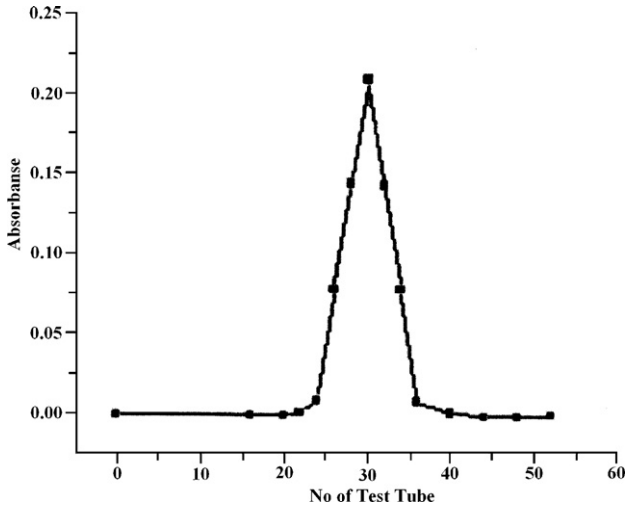
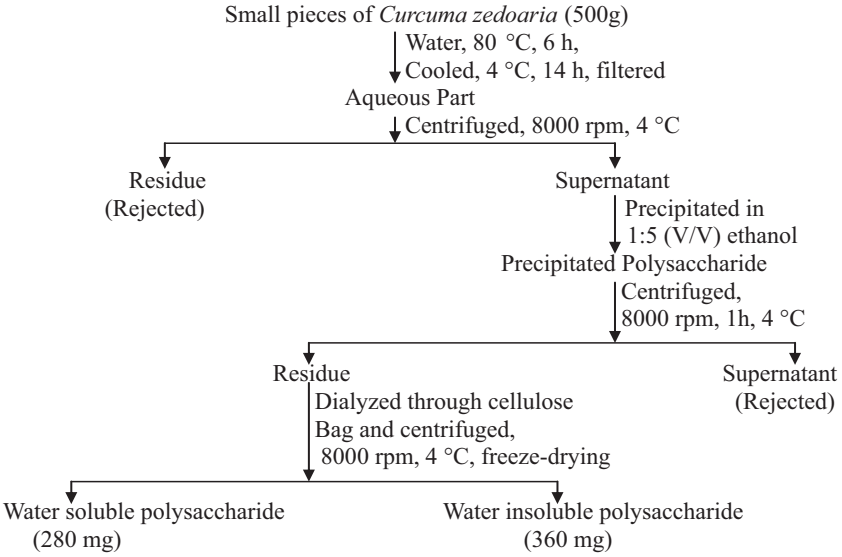


Fig. 1. Gel permeation chromatogram of the polysaccharide, isolated from rhizomes of *Curcuma zedoaria* using Sepharose 6B column.



The crude water-soluble polysaccharide (30 mg) was purified by gel permeation chromatography on column (90 cm × 2.1 cm) of Sepharose 6B using water as eluent with a flow rate of 0.4 mL min^{-1} using Redifrac fraction collector. Ninety-five test tubes (2 mL each) were collected, and monitored spectrophotometrically at 490 nm with phenol–sulfuric acid reagent (York, Darvill, McNeil, Stevenson, & Albersheim, 1985) using Shimadzu UV–vis spectrophotometer, model-1601. One homogeneous fraction of a symmetrical peak (Fig. 1) was observed in gel permeation chromatography and test tubes 24–34 were collected and freeze-dried, yield: 12 mg. The purification process was carried out in several lots.

2.2. Monosaccharide analysis

2.2.1. Alditol acetate analysis

The polysaccharide sample (4.0 mg) was hydrolyzed with 2 M CF_3COOH (2 mL) in a round-bottom flask at 100 °C for 18 h in a boiling water bath. The excess acid was completely removed by co-distillation with water. The hydrolyzed product was then reduced with $NaBH_4$ (9 mg), followed by acidification with dilute CH_3COOH, and then co-distilled with pure CH_3OH to remove excess boric acid. The reduced sugars (alditol) were acetylated with 1:1 pyridine–Ac_2O in a boiling water bath for 1 h to give the alditol acetates (Lindhall, 1970), which were analyzed by GLC and GLC–MS using Hewlett-Packard 5970A automatic GLC–MS system, fitted with an HP-5 capillary column (25 m × 25 mm). The program was isothermal at 150 °C; hold time 2 min, with a temperature gradient of 4 °C min^{-1} up to a final temperature of 200 °C. Quantization was carried out from the peak area, using response factors from standard monosaccharides.

2.2.2. Preparation of carboxy methyl reduced polysaccharide

The polysaccharide (1.5 mg) was dissolved in 1 M imidazole–hydrochloric acid buffer, pH 7.0 (200 μL/mg) and cooled on ice. $NaBH_4$ (40 mg) was then added, and the reaction mixture was maintained on ice for at least 1 h. Excess $NaBH_4$ was decomposed by adding HOAc (100 μL/40 mg $NaBH_4$) slowly to the cooled sample. An equal volume of redistilled water was added and the reduced polysaccharide was precipitated by adding 3–4 vols. 95% (v/v) EtOH (2 mL). The sample was reprecipitated two more times with 95% (v/v) EtOH and freeze dried. The carboxy methyl reduced polysaccharide was hydrolyzed with 2 M CF_3COOH for 18 h at 100 °C, and after the usual treatment, the sugars were analyzed by GLC and GLC–MS on a Hewlett-Packard Model 5730.

2.3. Methylation analysis

The polysaccharide was methylated using the procedure described by Ciucanu and Kerek (1984) and the methylated product was isolated by making partition between $CHCl_3$ and water (5:2, v/v). The organic layer containing product was washed with water for several times and dried. The methylated polysaccharide was hydrolyzed with 90% HCOOH (1 mL) at 100 °C for 1 h, and excess HCOOH was evaporated by co-distillation with distilled water. The hydrolyzed product was then reduced with $NaBH_4$ and acetylated with pyridine and Ac_2O. The alditol acetates of the methylated sugars were analyzed by GLC and GLC–MS (using HP-5 fused silica capillary column).

Another portion of the methylated product (2.0 mg) was dissolved in dry THF (2 mL) and refluxed with $LiAlH_4$ (40 mg) (Abdel-Akher & Smith, 1950) for 5 h and kept overnight at room temperature. The excess of the reductant was decomposed by drop wise addition of EtOAc and aqueous THF. The inorganic materials were filtered off. The filtrate was evaporated to dryness giving the carboxy-reduced permethylated product. The product was hydrolyzed with HCOOH as before, and the alditol acetates of the

reduced, methylated sugars were prepared in the usual way and analyzed by GLC and GLC–MS.

2.4. Periodate oxidation

The PS (5 mg) was oxidized by 2 mL 0.1 M $NaIO_4$ (sodium meta periodate) in dark for 48 h at room temperature. Excess of $NaIO_4$ was destroyed by addition of ethylene glycol, and dialyzed against distilled water for 3–4 h. The dialyzed product was reduced with $NaBH_4$ for overnight and neutralized with AcOH. The resulting material was obtained by co-distillation with CH_3OH (Goldstein, Hay, Lewis, & Smith, 1965; Hay, Lewis, & Smith, 1965). The residue was subjected to both hydrolysis and methylation by the same process described earlier and the product was analyzed for GLC.

2.5. Paper chromatographic studies

Paper partition chromatographic studies were performed on Whatmann nos. 1 and 3 mm sheets. Solvent systems used were: (X) BuOH–HOAc–H_2O (v/v/v, 4:1:5, upper phase) and (Y) EtOAc–pyridine–H_2O (v/v/v, 8:2:1). The spray reagent used was alkaline silver nitrate solution (Hoffman, Lindberg, & Svensson, 1972).

2.6. Absolute configuration of monosaccharides

The method used was based on Gerwig, Kamarling, and Vliegenthart (1978). The polysaccharide (1.0 mg) was hydrolyzed with CF_3COOH, and then the acid was removed. A solution of 250 μL of 0.625 M HCl in R-(+)-2-butanol was added and heated at 80 °C for 16 h. Then the reactants were evaporated and TMS-derivatives were prepared with bis(trimethylsilyl) trifluroacetamide (BSTFA). The products were analyzed by GLC using a capillary column SPB-1 (30 m × 0.26 mm), a temperature program (3 °C min^{-1}) from 150 to 210 °C. The 2,3,4,6-tetra-*O*-TMS-(+)-2-butylglycosides obtained were identified by comparison with those prepared from the D and L enantiomers of different monosaccharides.

2.7. Optical rotation

Optical rotation was measured on a Jasco Polarimeter model P-1020 at 25.5 °C.

2.8. Colorimetric estimations

Colorimetric estimations were carried out on a Shimadzu UV–vis spectrophotometer, model 1601.

2.9. Determination of molecular weight

The molecular weight of the polysaccharide was determined by gel-chromatographic technique. Standard dextrans (Hara, Kiho, Tanaka, & Ukai, 1982) T-40, T-60, T-70, T-200 and T-250 were passed through a sepharose-6B column, and then the elution volumes were plotted against the logarithms of their respective molecular weights. The elution volume of PS was then plotted in the same graph, and apparent molecular weight of polysaccharide was determined.

2.10. GLC and GLC–MS experiments

All gas liquid chromatography experiments were performed on a Hewlett-Packard Model 5730A. A gas chromatograph having a flame ionization detector and glass columns (1.8 m × 6 mm) packed with 3% ECNSS-M (A) on Gas Chrom Q (100–120 mesh) and 1% OV-225 (B) on Gas Chrom Q (100–120 mesh). All GLC analyses were performed at 170 °C. All the GLC–MS experiments were carried out using HP-5 fused silica capillary column. The program was isothermal at 150 °C; hold time 2 min, with a temperature gradient of 4 °C min^{-1} up to a final temperature of 200 °C.

2.11. NMR studies

The ^1H and ^{13}C NMR experiments were carried out at 500 MHz and 125 MHz, respectively, using Bruker Avance DPX-500 spectrometer respectively using a 5 mm broad-band probe. For NMR studies, PS-1 was dried in vacuum over P_2O_5 for several days, and then exchanged with deuterium (Dueñas Chaso et al., 1997) by lyophilizing with D_2O for three times. The deuterium-exchanged polysaccharide (4 mg) was dissolved in 0.7 mL D_2O (99.96% atom ^2H, Aldrich). The ^1H and ^{13}C NMR spectra (both ^1H coupled and decoupled) were recorded at 27 °C. Acetone was used as an internal standard (δ 31.05) for ^{13}C spectrum. The ^1H NMR spectrum was recorded fixing HOD signal at δ 4.73 at 27 °C using the WEFT pulse sequence (Hård, Zadelhoff, Moonen, Kamarling, & Vliegenthart, 1992). 2D (DQF-COSY) NMR experiment was performed using standard Bruker software. The TOCSY experiment was recorded at mixing time of 150 ms, and complete assignment required several TOCSY experiments having mixing times ranging from 60 to 300 ms. The NOESY and ROESY mixing delay was 300 ms. The delay time in the HMBC experiment was 80 ms.

3. Results and discussion

3.1. Isolation, purification and chemical analysis of the polysaccharide

500 g of fresh, thoroughly washed rhizomes of *C. zedoaria* was boiled with distilled water in a beaker for 6 h and then kept overnight at 4 °C. It was then centrifuged and the supernatant was precipitated in ethanol. The precipitated material was dissolved in minimum volume of distilled water and dialyzed using cellulose tubing membrane, retaining molecular weight >12,400 for 18 h to remove the low molecular weight materials. In cellulose tubing membrane the material was divided into two parts, water soluble (260 mg) and water insoluble (380 mg). The structural characterization of the water soluble polysaccharide (PS) was only carried out and reported herein. The water soluble PS was fractionated through S-6B column yielding only one homogeneous peak (Fig. 1).

The apparent molecular weight (Hara et al., 1982) of the polysaccharide was estimated from a calibration curve prepared with standard dextrans as 1.88×10^2 kDa and showed specific rotation of $[\alpha]_D^{25.5}$ + 134.8 (*c* 0.54, water). The polysaccharide (4.0 mg) on acid hydrolysis with 2 M CF_3CO_2H (2 mL) followed by paper chromatographic analysis (Hoffman et al., 1972) showed the presence of rhamnose, glucose, galactose, galacturonic acid and arabinose. GLC analysis of alditol acetates of the sugars showed the presence of L-rhamnose, D-glucose, D-galactose and L-arabinose in a molar ratio of 1:1:1:1, but carboxyl-reduced (Maness, Ryan, & Mort, 1990) PS on hydrolysis followed by GLC examination showed the presence of above sugars now in a molar ratio of 1:1:2:1. This result confirmed the presence of D-galacturonic acid in the polysaccharide. The absolute configuration of the polysaccharide was determined by the method of Gerwig et al. (1978) and confirmed by NMR-experiment.

The polysaccharide was methylated using Ciucanu and Kerek method (1984) followed by hydrolysis and alditol acetate preparation. The alditol acetates of the methylated polysaccharide were analyzed and identified by GLC–MS experiment, which revealed the presence of 2,3,4-Me_3-Rha, 2,3,4-Me_3-Glc, 2,6-Me_2-Gal and 3,4-Me_2-Ara in a molar ratio of nearly 1:1:1:1. These result indicated the presence of terminal rhamnopyranosyl, (1 → 6)-linked glu-

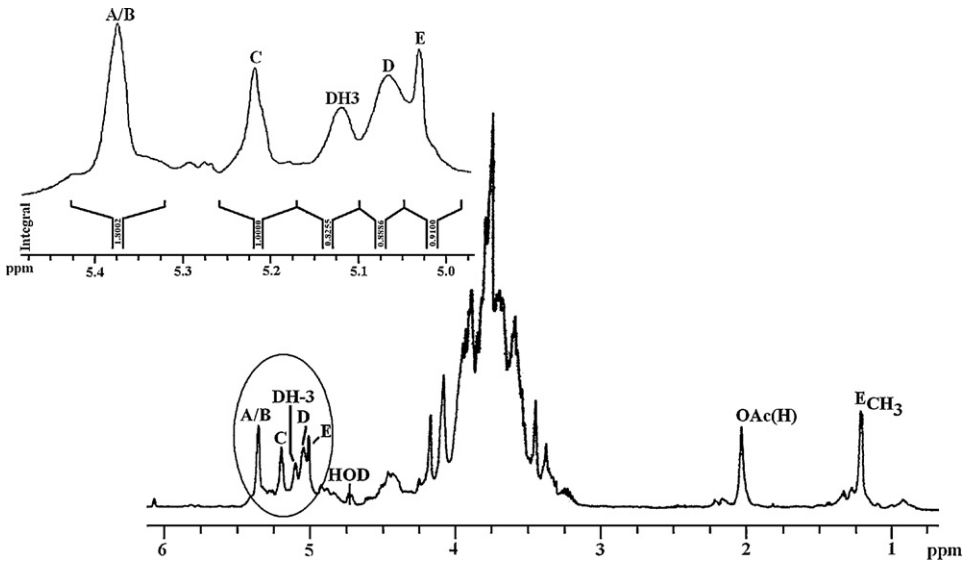


Fig. 2. ^{1}H NMR spectrum (500 MHz, D_2O, 27 °C) of the polysaccharide, isolated from rhizomes of *Curcuma zedoaria*. The inset figure shows extended form of the anomeric region with respective integral values.

copyranosyl, (1 → 3,4)-linked galactopyranosyl and (1 → 2)-linked arabinopyranosyl moieties. The alditol acetates of the methylated, carboxyl reduced (Abdel-Akher & Smith, 1950) PS showed the above peaks in a molar ratio of 1:1:2:1 which indicated the presence of (1 → 3,4)-linked Gal*p*A. But the carboxyl reduced methylated PS showed the presence of 2,3,6-Me_3-Gal along with the above peaks in a molar ratio of 1:1:1:1:1 indicating that Gal*p*A was present as (1 → 4) linked and the 3-position of galacturonic acid was linked with OAc group, which was confirmed by NMR experiment. The periodate oxidation (Goldstein et al., 1965; Hay et al., 1965) was carried out with the polysaccharide. The periodate-oxidized, $NaBH_4$-reduced material obtained from the polysaccharide, upon hydrolysis with TFA followed by GLC analysis showed the presence of D-galactose only. A part of periodate-oxidized PS on hydrolysis showed the presence of galacturonic acid in addition to the above sugar in the paper chromatographic examination (Hoffman et al., 1972). The GLC–MS analysis of the alditol acetates of the periodate-oxidized, carboxyl-reduced (Abdel-Akher & Smith, 1950) methylated polysaccharide showed the presence of 2,6-Me_2-Gal and 2,3,6-Me_3-Gal. These result confirmed that galactopyranosyl and galacturonic acid were present as (1 → 3,4)-linked and (1 → 4)-linked moieties, respectively.

3.2. NMR and structural analysis of the polysaccharide

The ^{1}H NMR spectrum (500 MHz, Fig. 2 and Table 1) of this polysaccharide at 27 °C showed five signals in the anomeric region 5.38, 5.22, 5.12, 5.07 and 5.04 ppm. The integral values of the signals at δ 5.22, 5.12, 5.07 and 5.04 were nearly same, but the signal at δ 5.38 was almost double than that of others. These observations indicated that all the signals corresponded to one proton except the signal at δ 5.38, which corresponded to two protons. In the ^{13}C NMR spectrum (125 MHz, Fig. 3 and Table 1) at 27 °C, four anomeric signals appeared at δ 103.5, 100.6, 100.1 and 100.0 ppm. From the H-1/C-1 correlation in HMQC experiment the signal at δ 103.5 was correlated to one proton at δ 5.04 whereas the signals at δ 100.0 and 100.1 corresponded to the signal at δ 5.38 and the signal at δ 100.6 corresponded to two protons at δ 5.22 and 5.07 indicating that the proton signal at δ 5.38 and the carbon signal at δ 100.6 were overlapping signals of two anomeric protons and two anomeric carbons respectively. All the ^{1}H and ^{13}C signals were assigned using DQF-COSY, TOCSY, HMQC, and HMBC NMR experiments. The resonance at δ 5.12 was correlated to δ 72.1 (C-3 signal of **D**) indicating that it was not for anomeric proton, but for H-3 of residue **D** whose C-3 position was attached by OAc group for which H-3 proton has been shifted downfield towards anomeric region. The five residues were designated as **A**–**E**, according to their decreasing anomeric chemical shifts in ^{1}H NMR spectrum.

Residue **A** had an anomeric proton signal at δ 5.38 with small coupling constants $^{3}J_{H\text{-}1,H\text{-}2} \sim 3.2$ Hz and $^{1}J_{H\text{-}1,C\text{-}1} \sim 170$ Hz indicating that it was an α-linked moiety. Large coupling constants, $^{3}J_{H\text{-}2,H\text{-}3}$ (~10 Hz) and $^{3}J_{H\text{-}3,H\text{-}4}$ (~10 Hz) for **A** indicated that it was a D-glucosyl moiety. The anomeric carbon chemical shift of residue **A** at δ 100.0 was confirmed from an H-1/C-1 correlation in HMQC experiment. The downfield shifts of C-6 (δ 68.9) carbon signal with respect to the standard values of methyl glycosides (Agrawal, 1992; Rinaudo & Vincendon, 1982) indicated that residue **A** was linked at this position. This observation was also supported by the GC–MS data for this linkage. Thus considering the results of methylation analysis and NMR experiment, it may be concluded that **A** was a (1 → 6)-linked D-glucose. The carbon chemical shifts of residue **A** were observed at 72.1, 74.5, 70.7 and 72.5 ppm corresponding to C-2, C-3, C-4 and C-5 respectively.

B, at δ 5.38, was assigned as α-linked residue as indicated from their coupling constant value $J_{H\text{-}1,H\text{-}2} \sim 3.4$ Hz and $J_{H\text{-}1,C\text{-}1} \sim 170$ Hz. A large $J_{H\text{-}2,H\text{-}3}$ (~9.5 Hz) and relatively small coupling constant $J_{H\text{-}3,H\text{-}4}$ (~4.0 Hz) for residue **B** indicated that it was an α-D-galactosyl residue. The anomeric carbon chemical shift of residue **B** at δ 100.1 was confirmed from an H-1/C-1 correlation in HMQC experiment. The downfield shifts of C-3 (78.9 ppm) and C-4 (81.7 ppm) signals with respect to the standard value of methyl glycosides (Agrawal, 1992; Rinaudo & Vincendon, 1982) indicated that **B** residue was (1 → 3,4)-linked D-galactose moiety present in the polysaccharide. The carbon signals at δ 72.1, 70.7 and 60.9 corresponded to C-2, C-5 and C-6 respectively.

Residue **C** was assigned to L-Ara*p* as it showed two H-5 signals at δ 3.72 and δ 3.98. The anomeric proton chemical shift for the moiety **C** at δ 5.22 had coupling constant values of $J_{H\text{-}1,H\text{-}2} \sim 7.9$ Hz and $J_{H\text{-}1,C\text{-}1} \sim 161$ Hz indicated that L-arabinopyranosyl residue was α-linked. The anomeric carbon chemical shift of residue **C** at δ 100.6 was confirmed from an H-1/C-1 correlation in HMQC experiment. The C-2 signal of residue **C** at δ 78.5 showed a downfield shift due to the effect of glycosylation indicating that it was (1 → 2)-linked L-Ara*p*.

Table 1
The ^{1}H and ^{13}C NMR chemical shifts of this polysaccharide isolated from *Curcuma zedoaria*[a,b] (δ, ppm) 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-5a, H-5b/C-5	H-6a, H-6b/C-6	COOMe	4-OAc
A	5.38	3.61	3.96	3.57	3.83	3.62, 3.98		
→6)-α-D-Glc*p*-(1→	100.0	72.1	74.5	70.7	72.5	68.9		
B	5.38	3.59	3.93	4.2	3.99	3.73, 3.83		
→3,4)-α-D-Gal*p*-(1→	100.1	72.1	78.9	81.7	70.7	60.9		
C	5.22	3.92	4.1	3.7	3.72, 3.98			
→2)-α-L-Ara*p*-(1→	100.6	78.5	68.9	68.4	68.9			
D	5.07	3.88	5.12	3.67	4.43		3.8[c]	2.05[f], 20.0[g]
→4)-α-D-Gal*p*A6Me-(1→ 3 ↑ OAc	100.6	70.7	72.1	77.0	71.6	171.2[e]	53.3[d]	171.2[h]
E	5.04	4.13	3.7	3.4	4.0	1.24		
α-L-Rha*p*-(1→	103.5	68.9	68.4	70.7	69.8	16.9		

[a] Values of the ^{13}C chemical shifts were recorded with reference to using acetone as internal standard and fixed at 31.05 ppm at 27 °C.
[b] Values of the ^{1}H chemical shifts recorded and assigned with respect to the HOD signal fixed at 4.74 ppm at 27 °C.
[c] Proton value of the ester group.
[d] ^{13}C chemical shift value of the methyl carbon in ester group.
[e] ^{13}C chemical shifts value of the carbonyl group of the carboxyl group.
[f] Methyl proton value of OAc group.
[g] Methyl carbon value of OAc group.
[h] Carbonyl carbon value of OAc group.

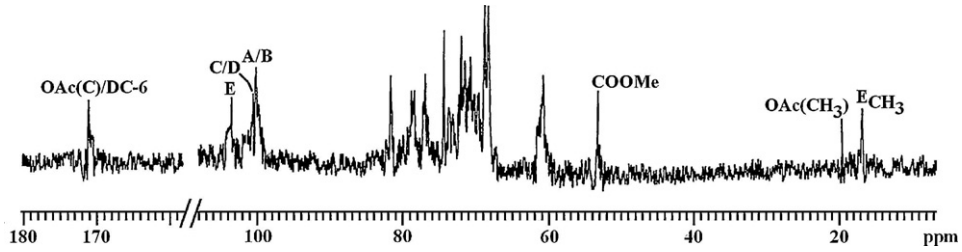


Fig. 3. ^{13}C NMR spectrum (125 MHz, D_2O, 27 °C) of the polysaccharide, isolated from rhizomes of *C. zedoaria*.

The residue **D** had an anomeric proton chemical shift at δ 5.07 ppm and this spin system consisted of only five protons with a low chemical shift of the H-5 signal (δ 4.43) and weak coupling between H-3, H-4, and H-4, H-5 indicating that it was a D-Gal*p*A moiety. The characteristic $J_{H\text{-}1,H\text{-}2}$ coupling constant value of 3.1 Hz and $J_{H\text{-}1,C\text{-}1} \sim 170$ Hz, showed that the residue **D** was α-D-Gal*p*A. The large downfield shift of the H-3 proton of residue **D** at 5.12 ppm strongly suggested that *O*-3 of the Gal*p* was the site of the acetylation, which was confirmed by the appearance of a cross-peak between H-3 proton (δ 5.12) of residue **D** and acetyl carbonyl carbon (δ 171.2) [**D** H-3, OAc(C)] in the HMBC experiment (Fig. 5 and Table 3). The downfield shift of C-4 (77.0 ppm) signal indicated that residue **D** was (1 → 4)-linked 3-*O*-acetyl-α-D-Gal*p*A. The carbon signals at 72.1, 70.7, 71.6 and 171.2 ppm of residue **D** corresponded to C-2, C-3, C-5 and C-6 respectively. The galacturonic acid moiety was present as methyl ester. The carbonyl group of methyl ester was confirmed by the appearance of intra-residual coupling between the ester carbonyl carbon (δ 171.2) and the carboxymethyl proton (δ 3.8) in the HMBC experiment (Fig. 5 and Table 3). Thus from NOESY as well as HMBC experiments it may be concluded that residue **D** was the methyl ester of a 1,4-linked 3-*O*-acetyl-α-D-Gal*p*A.

Residue **E** had an anomeric proton chemical shift at 5.04 ppm. Residue **E** was determined as L-Rha*p* due to signals for an exocyclic CH_3 group at δ_H 1.24 ppm and δ_C 16.9 ppm and the weak coupling between H-1, H-2, and H-2, H-3. $J_{H\text{-}1,H\text{-}2} \sim 1.9$ Hz, $J_{H\text{-}1,C\text{-}1} \sim 170$ Hz, indicated that it was an α-linked residue. It was also supported by the appearance of the C-5 signal of rhamnose at 69.8 ppm, instead of 73.4 ppm, which was the characteristic signal for α-rhamnopyranosyl residue. The anomeric carbon chemical shift of residue **E** at δ 100.6 was confirmed from an H-1/C-1 correlation in HMQC experiment. The carbon signals of residue **E** were observed at 68.9, 68.4, 70.7, 69.8, and 16.9 corresponding to C-2, C-3, C-4, C-5, and C-6 respectively. Thus, considering the result of methylation analysis and NMR experiments, it may be concluded that residue **E** was a terminal α-L-rhamnopyranosyl moiety.

The sequences of glycosyl residues in PS was determined on the basis of NOESY experiment (Fig. 4 and Table 2), followed by confirmation with HMBC experiment (Fig. 5 and Table 3). Residue **A** had inter-residue NOE contacts from H-1 to H-2 of residue **C**, residue **B** had NOE contacts from H-1 to H-6a and H-6b of **A**, residue **C** had NOE contacts from H-1 to H-4 of **B**, residue **D** had NOE contacts from H-1 to H-3 of **B** and similarly residue **E** had NOE contacts from H-1 to H-4 of **D**. Hence, the following sequences were established as

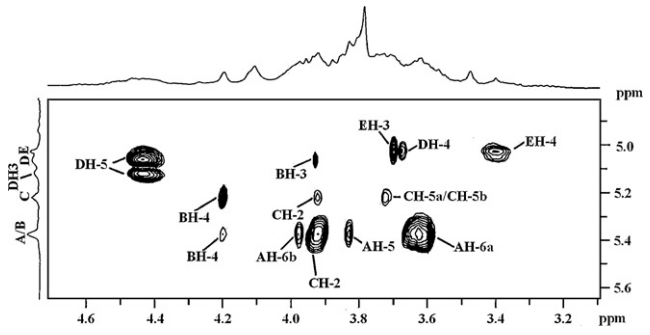


Fig. 4. The NOESY spectra of the polysaccharide, isolated from rhizomes of *C. zedoaria*. The NOESY mixing time was 300 ms.

Table 2
NOE effect of polysaccharide isolated from *Curcuma zedoaria*, observed in the NOESY spectrum recorded in D_2O at 27 °C.

Anomeric protons glycosyl residue	δ_H	NOE contact protons		
		δ_H	Residue	Atoms
→6)-α-D-Glc*p*-(1→	5.38	3.92	**C**	H-2
A		3.83	**A**	H-5
→3,4)-α-D-Gal*p*-(1→	5.38	3.62	**A**	H-6a
B		3.98	**A**	H-6b
		4.2	**B**	H-4
→2)-α-L-Ara*p*-(1→	5.22	4.2	**B**	H-4
C		3.92	**C**	H-2
		3.72	**C**	H-5a/H-5b
→4)-α-D-Gal*p*A6Me-(1→ 3 ↑ OAc	5.07	3.93	**B**	H-3
		5.12	**D**	H-3
D		4.43	**D**	H-5
α-L-Rha*p*-(1→	5.04	3.67	**D**	H-4
E		3.7	**E**	H-3
		3.4	**E**	H-4

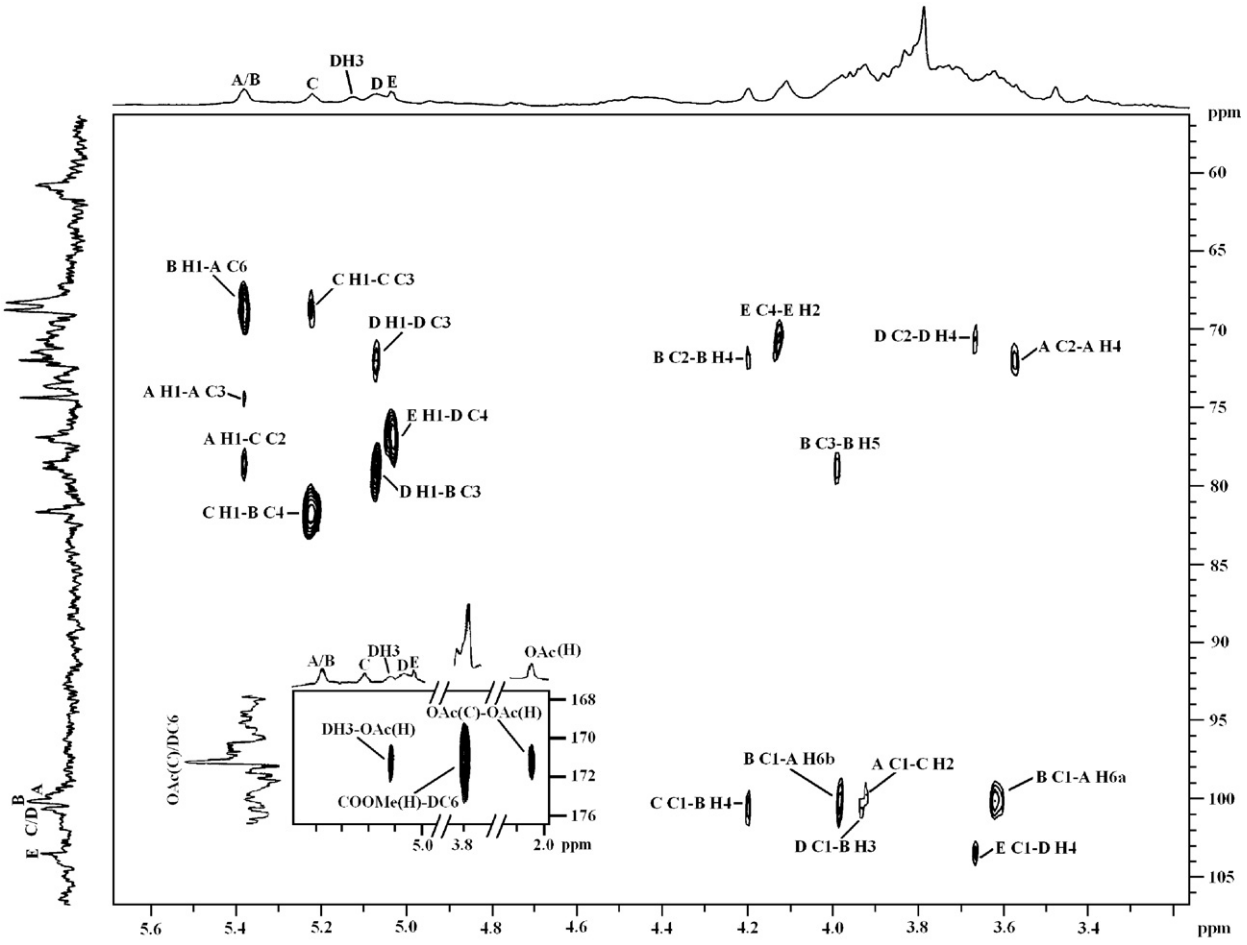


Fig. 5. HMBC spectrum of the polysaccharide isolated from *C. zedoaria*. The inset figure shows the coupling between acetyl carbon and its corresponding proton. The delay time in the HMBC experiment was 80 ms.

Table 3
The significant $^{3}J_{H,C}$ connectivities observed in an HMBC spectrum for the anomeric protons/carbons of the polysaccharide isolated from *Curcuma zedoaria*.

Residue	Sugar linkage	Observed connectivites	
		Inter	Intra
A	→6)-α-D-Glc*p*-(1→	**A**H1/**C**C2	**A**H-1/**A**C-3
		AC1/**C**H2	**A**C-2/**A**H-4
B	→3,4)-α-D-Gal*p*-(1→	**B**H1/**A**C6	**B**C-2/**B**H4
		BC1/**A**H6a, **B**C1/**A**H6b	**B**C-3/**B**H-5
C	→2)-α-L-Ara*p*-(1→	**C**H1/**B**C4	**C**H-1/**C**C-3
		CC1/**B**H4	
D	→4)-α-D-Gal*p*A6Me-(1→ 3 ↑ OAc	**D**H1/**B**C3	**D**H-1/**D**C-3
		DC1/**B**H3	**D**C-2/**D**H-4
E	α-L-Rha*p*-(1→	**E**H1/**D**C4	**E**C-4/**E**H-2
		EC1/**D**H4	
D	→4)-α-D-Gal*p*A6Me-(1→ 3 ↑ OAc		**D**COOMe(H)/**D**C-6
			DOAc(H)/**D**OAc(CO)
			DOAc(CO)/**D**H-3

A **C**
→6)-α-D-Glc*p*-(1→2)- α-L-Ara*p*-(1→

B **A**
→4)-α-D-Gal*p*-(1→6)- α-D-Glc*p*-(1→
3
↑

C **B**
→2)α-L-Ara*p*-(1→4)-α-D-Gal*p*-(1→
3
↑

D **B**
→4)-α-D-Gal*p*A6Me-(1→3)-α-D-Gal*p*-(1→
3 4
↑ ↑
OAc

E **D**
α-L-Rha*p*-(1→4)-α-D-Gal*p*A6Me-(1→
3
↑
OAc

The sequences of these linkages were further confirmed by the HMBC experiment (Fig. 5 and Table 3). Cross-peaks were found between H-1 (δ 5.38) of residue **A** and C-2 (δ 78.5) of residue **C** (**A** H-1, **C** C-2); and between C-1 (δ 100.0) of residue **A** and H-2 (δ 3.92) of residue **C** (**A** C-1, **C** H-2). The cross-peaks between H-1 (δ 5.38) of residue **B** and C-6 (δ 68.9) of residue **A** (**B** H-1, **A** C-6); and between C-1 (δ 100.1) of residue **B** and H-6a (δ 3.62) and H-6b (δ 3.98) of residue **A** (**B** C-1, **A** H-6a; **B** C-1, **A** H-6b) were observed. The cross-peaks were also found between H-1 (δ 5.22) of residue **C** and C-4 (δ 81.7) of residue **B** (**C** H-1, **B** C-4); and between C-1 (δ 100.6) of residue **C** and H-4 (δ 4.2) of residue **B** (**C** C-1, **B** H-4). Again cross-peak were observed between H-1 (δ 5.07) of residue **D** and C-3 (δ 78.9) of residue **B** (**D** H-1, **B** C-3); and between C-1 (δ 100.6) of residue **D** and H-3 (δ 3.93) of residue **B** (**D** C-1, **B** H-3). The 3-position of residue **D** attached with acetyl group was confirmed by the presence of cross-peak **D** H-3, **D** OAc (carbonyl carbon). Finally cross-peak between H-1 (δ 5.04) of residue **E** and C-4 (δ 77.0) of residue **D** (**E** H-1, **D** C-4); and between C-1 (δ 103.5) of residue **E** and H-4 (δ 3.67) of residue **D** (**E** C-1, **D** H-4) were observed.

4. Conclusion

A water soluble polysaccharide was isolated from the rhizomes of *C. zedoaria* and purified by gel-filtration chromatography. On the basis of chemical analysis and NMR studies the structure of the pentasaccharide repeating unit in the polysaccharide was established as

A **C** **B**
→6)- α-D-Glc*p*-(1→2)- α-L-Ara*p*-(1→4)-α-D-Gal*p*-(1→
3
↑
1
α-L-Rha*p*-(1→4)-3-*O*-Ac-α-D-Gal*p*A6Me
E **D**

Acknowledgements

Dr. S. Roy, Director and Dr. A.K. Sen (Jr.), IICB, Kolkata, are gratefully acknowledged for providing instrumental facilities. The authors do not fail to acknowledge the services of Mr. Barun Majumder of Bose Institute, Kolkata, for preparing NMR spectra. We are also indebted to DST, Govt. of India, for sanctioning a project (Ref No: SR/S1/OC-52/2006 dated 19/02/2007). One of the authors (C.K.N.) is grateful to UGC, New Delhi for providing Junior Research Fellowship.

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