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ISOLATION AND STRUCTURE ELUCIDATION OF NOVEL OLIGOSACCHARIDE FROM GOAT MILK

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ABSTRACT

Milk is a primary dynamic biological fluid responsible for proper growth and development of mammalian neonates. Besides the other regular constituents it has oligosaccharides in it which are responsible for anti-tumor, anti-cancer, anti-pathogenic, anti-inflammatory, anti-coagulant, anti-complementary, antigenic, hypoglycemic, immunological, immunostimulant and prebiotic activities. Goat milk oligosaccharides have anti-inflammatory properties and are involved in the repairing process after a dextran sodium sulphate-induced colitis.

In the present studies, we have selected the goat milk for its oligosaccharide contents for which it was processed by modified Kobata and Ginsburg method followed by the HPLC and CC techniques. The structure of the isolated oligosaccharide was elucidated by chemical transformation, chemical degradation, ^{1}H, ^{13}C, 2D-NMR (COSY, TOCSY and HSQC) and mass spectrometry as under.

Gal-β(1→3)GlcNAc-β(1→6)Gal-β(1→4)Glc
|
GlcNAc-β(1→4)

KEYWORDS:- Milk Oligosaccharides, pentasaccharide, melose, 2D-NMR, biological activity.

1. INTRODUCTION

Milk is a gift of nature to mankind that contains proteins, fatty acids, minerals, vitamins and carbohydrates especially lactose and large number of oligosaccharides. Oligosaccharides in milk exert various bioactivities and modulate the immune system.[1] Oligosaccharides play a critical role in preventing inflammatory processes, reducing diabetes, obesity and cardiovascular risks, modulating the gut flora and affecting different gastrointestinal activities.[2-4] Goat milk oligosaccharides have anti-inflammatory effects in rats with trinitrobenzenesulfonic (T) acid-induced colitis and may be useful in the management of inflammatory bowel disease.[5] Goat milk oligosaccharides play an important role in intestinal protection and repair after damage caused by DSS (dextran sodium sulphate)-induced colitis and their implication in human intestinal inflammation.[6,7] Goat milk possesses anti-hypertensive and immunomodulatory properties.[8]

In the present study we have described the structure elucidation of novel oligosaccharide isolated from goat milk by the modified method of Kobata and Ginsburg followed by chemical degradation, chemical transformation and various spectroscopic techniques like ^{1}H, ^{13}C, COSY, TOCSY, HSQC and Mass spectrometry.

2. EXPERIMENTAL

2.1. General procedure

Optical rotations were measured with a PERKIN-ELMER 241 automatic polarimeter in 1cm tube. ^{1}H and ^{13}C NMR spectra of oligosaccharides were recorded in D_2O and the spectra of acetylated oligosaccharides were recorded in $CDCl_3$ at 25^0C on a Bruker AM 300 FT NMR spectrometer. The electrospray mass spectra were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. The samples (dissolved in suitable solvents such as methanol/acetonitrile/water) were introduced into the ESI source through a syringe pump at the rate 5µl per min. The ESI capillary was set at 3.5 KV and the cone voltage was 40 V. The spectra were collected in 6s scans and the print outs are averaged spectra of 6-8 scans. The C, H and N analysis were recorded on CARLO-ELBA 1108 an elemental analyzer. The sugars were visualized on TLC with 30% aqueous H_2SO_4 reagent and on Paper Chromatography with acetyl acetone and p-dimethyl amino benzaldehyde reagents. The absorbent for TLC was silica gel G (SRL) and CC silica gel (SRL, 60-120 mesh). PC was performed on Whatman No.1 filter paper using solvent system ethylacetate-pyridine (2:1) saturated with H_2O. Sephadex G-25 (PHARMACIA) was used in gel permeation chromatography. Freeze

drying of the compound was done with the help of CT 60e (HETO) lyophilized and centrifuged by a cooling centrifuged Remi instruments C-23 JJRCI 763. To check the homogeneity of the compounds reverse phase HPLC system was used equipped with Perkin Elmer 250 solvent delivering system, 235 diod array detector and G.P. 100 printer plotter. Authentic samples of glucosamine, galactosamine, galactose and glucose were purchased from Aldrich Chemicals.

2.2. Isolation of goat milk oligosaccharides by the modified Kobata & Ginsburg method

12 litres goat milk was collected from a domestic goat and equal amount of ethanol was added to it. It was processed by the modified method of Kobata and Ginsburg[9,10] and centrifuged for 15 min at 5000 rpm at 4^0C. The solidified lipid layer was removed by filtration through glass wool column in cold. Ethanol was added to clear filtrate to a final concentration of 68% and the resulting solution was left overnight at 0^0C. The white precipitate formed, mainly of lactose and protein was removed by centrifugation and washed twice with 68% ethanol at 0^0C. The supernatant and washings were combined and filtered through a micro filter and lyophilized affording crude oligosaccharide mixture (326 gm).

The lyophilized material responded positively to Morgan-Elson test[11] and thiobarbituric-acid assay[12] suggesting the presence of N-acetyl sugars and sialic acid in oligosaccharide mixture. This lyophilized material (mixture of oligosaccharides) was further purified by fractionating it on Sephadex G-25 chromatography using glass triple distilled water as eluant at a flow rate of 3 ml/min. Each fraction was analyzed by phenol sulphuric acid reagent[13] for the presence of neutral sugar (219 gm).

2.3. Acetylation of oligosaccharide mixture

For acetylation, 12 gm of pooled fractions which gave positive phenol-sulphuric acid test[14] were acetylated with pyridine (12 ml) and acetic anhydride (12 ml) at 60°C and the solution was stirred overnight. The mixture was evaporated under reduced pressure and the viscous residue was taken in $CHCl_3$ (500 ml) and washed in sequence with 2N-HCl (1 x 25 ml), ice cold 2N-$NaHCO_3$ (2 x 25 ml) and finally with H_2O (2 x 25 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness yielding the acetylated mixture (16.7 gm).

2.4. Purification of compounds on silica gel chromatography

Separation or purification of acetylated oligosaccharides (16.7 gm) was carried over silica gel (500 gm) using varying proportion of Hex:$CHCl_3$, $CHCl_3$ and $CHCl_3$:MeOH as eluents, collecting fractions of 300 ml each. So ten fractions namely I (4.28 gm), II (736 mg), III (3.29 gm), IV (468 mg), V (380 mg) ,VI (2.007 gm), VII (1.06 gm), VIII (767 mg), IX (319 mg) and X (137 mg) respectively obtained. All these fractions were checked on TLC and those showing similar spots were taken together for further investigations. Substance A (98 mg) was obtained from fraction II.

2.5. Deacetylation of compound melose acetate

Melose acetate (98 mg) obtained from column chromatography of acetylated oligosaccharide mixture were dissolved individually in acetone (3 ml) and NH_3 (3 ml) was added and left overnight in a stoppered hydrolysis flask. After 24 h ammonia was removed under reduced pressure and the compound was washed with (3 x 5 ml) $CHCl_3$ (to remove acetamide) and the water layer was finally freeze dried giving the deacetylated oligosaccharide i.e. Melose (87mg).

2.6. Methyl glycosidation/Acid Hydrolysis of melose

Melose was ref1uxed with absolute MeOH (2 ml) at 70°C for 18 h in the presence of cation exchange !R-l20 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To a solution of methyl glycoside of Melose in 1,4-dioxane (1 ml), 0.1 N H_2SO_4 (1 ml) was added and the solution was warmed for 30 minutes at 50^0C. The hydrolysis was complete after 22 h. The hydrolysate was neutralized with freshly prepared $BaCO_3$ filtered and concentrated under reduced pressure to afford α-and β-methyl glucosides along with the Glc, Gal and GlcNAc. Their identification was confirmed by comparison with authentic samples (TLC, PC).

2.7. Kiliani hydrolysis of melose

Melose was dissolved in 2 ml of Kiliani mixture (AcOH-H_2O-HCI, 7:11:2)[14] separately and heated at 100^0C for 1 h followed by evaporation under reduced pressure. It was dissolved in 2 ml of H_2O and extracted twice with 3 ml $CHCl_3$. The aqueous residual solution was made neutral by addition of 1-2 drops of 2N NaOH to it and was evaporated under reduced pressure to afford glucose, galactose and GlcNAc on comparison with authentic samples of glucose, galactose and GlcNAc.

2.8. Description of compound

Compound Melose obtained from fraction of chromatography-II. On deacetylation of 100 mg of substance Melose acetate with NH_3/acetone it afford substance B (81 mg) as a viscous mass, $[\alpha]_D$ $+52.8^0$ (c,$4H_2O$).

Elemental analysis: Cald. %C 44.83, %H 6.37, %N 3.08 and found %C 44.79, %H 6.29, %N 3.06. The molecular formula of compound Melose was $C_{34}H_{58}N_2O_{26.}$ For experimental analysis, this compound was dried over P_2O_5 at 100^0C and 0.1 mm pressure for 8 hr. The presence of sugar units in compound Melose have been confirmed by NMR and Mass spectrometry.

^{1}HNMR values of Melose in D_2O

2.00[s, 3H, $NHCOCH_3$, β-GlcNAc (S-3)], 3.28[t, 1H, J=7.0 Hz, β-Glc(S-1), H-2],3.93[, β-Gal (S-2), H-4],4.06[t, 1H, β-GlcNAc(S-3), H-3],4.47[d,2H, J=7.5 Hz, β-Gal (S-2 and S-4), H-1] ,4.53 [d, 1H, J=7.8 Hz, β-GlcNAc (S-3), H-1],4.56[d, 1H, J=9.0 Hz, β-GlcNAc (S-5), H-1],,4.67[d , 1H ,J=8.7 Hz, β-Glc (S-1), H-1], 5.23 [d,1H, J=3.8 Hz, α-Glc (S-1),H-1].

^{13}C NMR values of Melose in D_2O

21.8 [s,$NHCOCH_3$]., 24.9 [s,$NHCOCH_3$], 91.8[α-Glc (S-1),C-1], 96.5 [β-Glc (S-1),C-1], 102.3 [β-GlcNAc (S-3), C-1], 102.8[β-Gal (S-2 and S-4),& β-GlcNAc (S-5), C-1], 173.6 [s,$NHCOCH_3$].

^{1}H NMR values of Acetylated Melose in $CDCl_3$

4.48[d, 1H, J=8.4 Hz, β-GlcNAc (S-3), H-1], 4.49 [d,2H, J=8.3 Hz, β-Gal (S-2 and S-4), H-1], 4.51[d, 1H, J=9.0 Hz, β-GlcNAc (S-5), H-1], 5.68 [d, 1H ,J=8.7 Hz, β-Glc (S-1), H-1], 6.25[d,1H, J=3.6 Hz, α-Glc (S-1),H-1].

^{13}C NMR values of Acetylated Melose in $CDCl_3$

89.1[α-Glc (S-1),C-1], 91.7[β-Glc (S-1),C-1], 101.1[β-GlcNAc (S-3& S-5), C-1]., 101.3 [β-Gal (S-2 & S-4), C-1].

ES Mass of Melose

933$[M+Na]^+$, 892, 861, 848, 844, 837, 819, 819, 789, 771, 758, 748, 730, 699, 688, 681, 659, 652, 621, 621, 611, 593, 590, 579, 576, 558, 545, 527, 496, 479, 465, 462, 406, 402, 384, 375, 357, 342, 342, 325, 293, 275, 266, 257, 239, 218, 200, 190, 180.

3. RESULT AND DISCUSSION

The structure of novel Melose was elucidated with the help of NMR spectroscopy (^{1}H, ^{13}C, and 2D-NMR), mass spectrometry, chemical degradation and chemical transformation.

Melose, $C_{34}H_{58}N_2O_{26}$ $[\alpha]_D$ +52.8 gave positive Phenol- sulphuric acid test,[13] Feigl test,[15] Morgon-Elson test[11] showed the presence of normal and amino sugar(s) in the compound. The HSQC spectrum of acetylated Melose acetate in $CDCl_3$ showed the presence of five cross peaks of six anomeric protons and carbons in the respective region at δ 6.25 x 89.1 (1C), δ 5.69 x 91.7 (1C), δ 4.48 x 101.1 (1C), δ 4.51 x 101.1 (1C) and δ 4.49 x 101.3 (2C) suggesting the presence of six anomeric protons and carbons in it. Further ^{1}H NMR spectrum of Melose acetate at 300 MHz in $CDCl_3$ showed five signals for six anomeric proton at δ 6.25 (1H), δ 5.69 (1H), δ 4.48 (1H), δ 4.51 (1H) and δ 4.49 (2H) indicating that the Melose acetate may be pentasaccharide in its reducing form. It was further supported by appearance of five signals for the six anomeric carbons at δ 89.1 (1C), δ 91.7 (1C), δ 101.1 (1C), δ 101.1 (1C) and δ 101.3 (2C) in the ^{13}C NMR spectrum of acetylated Melose in $CDCl_3$. The ^{1}H NMR spectrum of Melose at 300 MHz in D_2O exhibited five doublets for six anomeric proton signals at δ 5.23 (1H), 4.67 (1H), 4.56 (1H), 4.53 (1H) and 4.47 (2H) indicating that the compound may be a pentasaccharide in its reducing form giving signals for α and β anomers of glucose at its reducing end. Methyl glycosidation of Melose by $MeOH/H^+$ followed by its acid hydrolysis led to isolation of α and β- methyl glucosides, which suggested the presence of glucose at the reducing end of the oligosaccharide. It was also confirmed by the presence of two anomeric proton signals at δ 5.23 and δ 4.67 for α and β-Glc. The pentasaccharide nature of Melose was further confirmed by the presence of four doublets for six anomeric carbon at δ 91.8 (1C), δ 96.5 (1C), δ 102.3 (1C) and δ 102.8 (3C) in ^{13}C NMR of Melose in D_2O. The five monosaccharides present in Melose have been designated as S_1, S_2, S_3, S_4, and S_5 for convenience starting from reducing end. To confirm the monosaccharide constituents in Melose, it was hydrolysed under strong acidic conditions. In Kiliani hydrolysis under strong acid condition, it gave three monosaccharides i.e. glucose, galactose and N-acetyl-glucosamine, confirming that the pentasaccharide consists of three types of monosaccharide units. The ^{1}HNMR of Melose acetate contains two anomeric proton signals at δ6.25 and δ5.69 showed the presence of α and β-glucose (S_1) present at the reducing end. The anomeric proton present at δ5.69 contain three cross peaks at δ 5.69x5.02, δ5.69x5.23 and δ5.69x3.69 in the TOCSY spectrum of Melose acetate, out of which the cross peak present at δ3.69 was

assigned for glycosidic linkage which was later identified for H-4 of the reducing glucose by the COSY spectrum of Melose acetate confirming that H-4 of reducing glucose was available for glycosidic linkage by the next monosaccharide. Further another anomeric proton doublet which was present at δ4.61 was due to presence of galactose which was linked to H-4 of reducing glucose, showing presence of Lactosyl moiety at the reducing end in Melose. Since the presence of glucose was also confirmed at its reducing end of Melose by methyl glycosidation/acid hydrolysis was also supported by ^{1}H NMR of Melose which contains two anomeric proton signals for α- and β-Glc at δ 5.23 (J=3.8 Hz) and at δ 4.67 (J=8.7 Hz). It also contains another anomeric proton doublet at δ 4.47 (J=7.5 Hz) which was due to presence of β-Gal (S_2) moiety in the Melose. The large coupling constant of anomeric proton signal of Gal (S_2) δ 4.47 (J=7.5 Hz) confirmed the β glycosidic linkage between S_2 and S_1. Presence of reducing glucose along with β-Gal moiety present in Melose suggested the presence of a lactosyl moiety i.e. Gal-β-(1→4) Glc in Melose which was further confirmed by β-Glc (S_1) H-2 signal (a structure reporter group) which appeared as a triplet at δ 3.29 confirmed the presence of lactosyl moiety in Melose. Further the anomeric proton of β-galactose present at δ4.48 showed three cross peaks at 4.48x5.15 and 4.48x3.73 in the TOCSY spectrum of Melose acetate, out of which a cross peak present at δ3.73 showed availability for glycosidic linkage which was later identified for H-6 of the β-galactose by the COSY spectrum of Melose acetate confirming that H-6 of β-galactose (S_2) was available for glycosidic linkage by the next monosaccharide. The presence of another anomeric proton signal present at δ4.51 in the ^{1}HNMR of Melose acetate was due to presence of GlcNAc showed that the GlcNAc was present as the third monosaccharide unit linked to Gal S_2 at H-6. Further the presence of another anomeric proton doublet at δ 4.53 (J=7.8Hz) along with signal of amide methyl group at δ 2.00 ^{1}HNMR of Melose in D_2O, was due to the presence of β-GlcNAc (S_3) moiety. The large coupling constant of anomeric proton signal of GlcNAc (S_3) δ4.51 (J=7.5 Hz) confirmed the β glycosidic linkage between S_3 and S_2. The H-4 proton resonance of β Gal (S_2), which appeared at δ 3.93 implies that the β-GlcNAc (S_3) may be 1→6 linked to β-Gal (S_2) (SRG). This was confirmed on the basis of presence of β-Gal (S_2), H-6 at δ3.84 and C-6 at δ73.8 and chemical shift analogies of β-GlcNAc (1→6) Gal-β as given by Dua et.al (S_3). Further the anomeric proton of β-GlcNAc showed three cross peaks at δ 4.51x4.12, δ 4.51x3.62 and δ 4.51x3.80 in TOCSY spectrum of Melose acetate. Further the COSY spectrum of Melose acetate confirmed that signal at chemical shift at δ4.12 was

due to H-2 methene proton of GlcNAc (S_3), while the signal at δ3.80 and δ3.62 was due to H-3 and H-4 protons of S_3 respectively, out of which the positions of H-3 and H-4 protons of β-GlcNAc (S_3) at δ 3.80 and δ 3.62 respectively which imply that H-3 and H-4 of β-GlcNAc (S_3) were involved in the glycosydation with the next monosaccharide unit. The presence of another anomeric proton signal present at δ4.49 was due to the presence galactose in the spectrum of Melose acetate. Since H-4 showed a signal at δ3.62 confirmed that Gal S_4 was glycosydically linked to H-3 of S_3. The large coupling constant of anomeric proton signal of Gal (S_4) δ4.51 (J=7.5 Hz) confirmed the β-glycosidic linkage between S_4 and S_3. Further the presence of another anomeric proton signal appeared at δ 4.47 (J=7.5 Hz) which was due to presence of a β-GlcNAc moiety (S_5) confirmed that S_5(GlcNAc) was linked to H-4 of β-GlcNAc(S_3) in spectrum of Melose in D_2O. The large coupling constant of anomeric proton signal of GlcNAc (S_5) δ4.51 (J=7.5 Hz) confirmed the β glycosidic linkage between S_5 and S_3. The linkage between S_3 and S_4 was established on the basis of presence of downfield shifted H-3 proton of GlcNAc (S_3) at δ 4.06 as triplet. It was further confirmed by anomeric proton chemical shift value of this β-Gal moiety (S_4), which was identical with the anomeric proton chemical shift value of β-Gal (S_2) of lactosyl moiety, which is a structure reporter group for S_4 and S_3 linkage. Another anomeric signals appeared at δ 4.56 (J=9.0 Hz) along with signal of methyl group at δ 2.00 was due to presence of β-GlcNAc moiety (S_5) which was linked to H-4 of β-GlcNAc (S_3).

Table 1: ^{1}H and ^{13}C NMR values in D_2O.

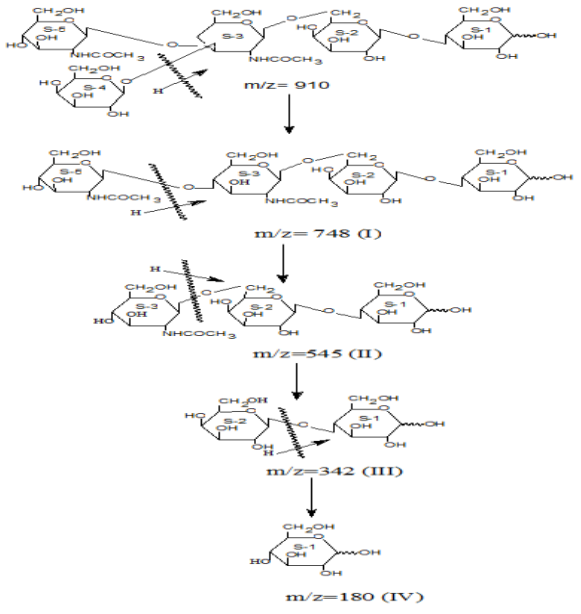
	^{1}H NMR	^{13}C NMR	Coupling Constt.(J)
α-Glc	5.23	91.8	3.8
β-Glc	4.67	96.5	8.7
β-Gal	4.47	102.8	7.5
β-GlcNAc	4.53	102.3	7.8
β-Gal	4.47	102.8	7.5
β-GlcNAc	4.56	102.8	9.0

The ^{13}C NMR values of anomeric carbons and ring carbons of Melose are given in table 2. The various values of ring carbons are in accordance with ^{13}C value of their respective monosaccharides, which also supports the derived structure.

Table 2: ^{13}C NMR values of Melose.

	C-1	C-2	C-3	C-4	C-5	C-6	CO	CH_3
α-Glc	91.8	73.6	71.65	78.01	70.9	60.44		
β-Glc	96.5	74.9	75.2	77.59	74.9	62.0		
β-Gal	102.8	70.9	73.5	70.7	70.8	73.8		
β-GlcNAc	102.3	60.9	78.5	78.01	74.9	62.0	173.6	21.8
β-Gal	102.8	71.0	72.4	70.9	76.18	60.9		
β-GlcNAc	102.8	61.9	74.9	72.4	75.29	62.0	173.6	24.9

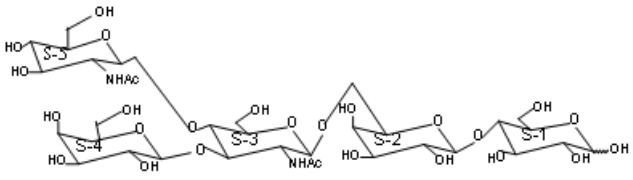
The pentasaccharide nature of Melose was further confirmed by the spectral studies of acetylated product of compound B. These studies are made on the basis of HOMOCOSY, TOCSY and HSQC connectivities. The glycosidic linkages were assigned by the cross peaks for glycosidically linked carbons with their protons in HSQC spectrum of Melose acetate. The values of these cross peaks are as- β-Glc (S_1) H-4 and C-4 at δ3.79x 75.94 shows (1→4) linkage, β-Gal (S_2 & S_4) H-6 and C-6 at δδ 3.82 x 73.67 shows (1→6) linkage, β-GlcNAc(S_3) H-3 and C-3 atδ 3.88 x 75.94 shows (1→3) linkage and also it's H-4 and C-4 at δ 3.82 x 75.81 shows (1→4) linkage respectively. The ES-MASS spectrum of Melose not only confirmed the derived structure but also confirmed the sequences of the monosaccharides in Melose. The highest mass ion peak were recorded m/z 933 which was due to $[M+Na]^+$. It confirmed that the molecular weight of compound was 910. Further the mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water. The fragmentation pathway confirmed the sequence of monosaccharide units in the pentasaccharide (scheme 1).



Scheme 1: ES-Mass Fragmentation of Compound Melose.

The pentasaccharide on fragmentation gave a mass ion peak at m/z 748(I), corresponding to tetrasaccharide unit, which was due to loss of S-4 sugar unit i.e. Gal (S-4) sugar unit linked to the S-3 of pentasaccharide. It was supported by its respective fragment at m/z 180, that confirmed the presence of Gal (S-4) at non reducing end. The tetrasaccharide on fragmentation gave a mass ion peak at m/z 545(II), which was due to loss S-5 sugar unit i.e. GlcNAc (S-5) sugar unit linked to the S-3 of tetrasaccharide unit. It was supported by m/z 527 (545-H_2O). The trisaccharide on fragmentation gave mass ion peak at m/z 342(III), which was due to loss of S-3 sugar unit i.e. GlcNAc (S-3) sugar unit linked to the S-2 of trisaccharide unit. This disaccharide on further fragmentation gave a mass ion peak at m/z 180(IV), which was due to loss of S-2 sugar unit i.e. Gal (S-2) sugar unit linked to the S-1 of disaccharide. The other mass fragments obtained at m/z 892(910-H_2O), m/z 844(892-CH_2OH), m/z 771(844-CH_2OH), m/z 848(910-2CH_2OH), m/z 789(848-CH_2CO-OH), m/z 758(789- CH_2OH), m/z 861(910-CH_2OH-H_2O), m/z 819(861-CH_2CO), m/z 771(819-CH_2OH-OH), m/z837(910-CH_2OH-CH_2CO) and m/z 819(837-H_2O). The pentasaccharide m/z 910 on fragmentation gave tetrasaccharide m/z748 (M-S_4), which was further confirmed by its other fragment ions at m/z 730(748-H_2O), m/z 699(730-CH_2OH), m/z 681(699-H_2O), m/z 621(681-CH_2OHCHO), m/z 590(621-CH_2OH), m/z 688(748-CH_2OHCHO), m/z 652(688-2H_2O), m/z 621(652-CH_2OH), m/z 579(621-CH_2CO), m/z 659(748-$NHCOCH_3$-CH_2OH), m/z 611(659-CH_2OH-OH), m/z 593(611-H_2O), m/z 576(593-OH) and m/z 558(576-H_2O). The tetrasaccharide m/z 748 on fragmentation gave trisaccharide m/z 527 due to (M-S_5- H_2O), which was further confirmed by its other fragment ions at m/z 465(527-2CH_2OH), m/z 406(465- CH_2CO-OH), m/z 375(406- CH_2OH), m/z 357(375- H_2O), m/z 496(545- CH_2OH- H_2O), m/z 479(496-CH_2OH- H_2O), m/z 462(479-OH), m/z 402(462-CH_2OHCHO), m/z 384(402- H_2O) and m/z 342(384- CH_2CO). The trisaccharide m/z 545 on fragmentation gave disaccharide m/z 342, which was further confirmed by its other fragment ions at m/z 293(342- CH_2OH- H_2O), m/z 275(293- H_2O), m/z 257(275- H_2O), m/z 239(257-H_2O), m/z 190(239- CH_2OH- H_2O), m/z 325(342-OH),m/z 266(325- CH_2CO- OH), m/z 218(266- CH_2CO- OH) and m/z 200(218- H_2O).The disaccharide m/z 342 on fragmentation gave monosaccharide m/z 180. Based on the results obtained from chemical degradation and chemical transformation, mass spectrometry and ^{1}H, ^{13}C, HOMOCOSY, TOCSY, HSQC NMR, the structure of the isolated pentasaccharide is deduced as.

Gal-β(1→3)GlcNAcβ(1→6)Gal-β(1→4)Glc
4
|
1GlcNAc β



Melose

4. CONCLUSION

From the above informations, we conclude that the structure of isolated goat milk oligosaccharide, Melose. This oligosaccharide was reported for the first time from any natural source or any milk and elucidated with the help of spectroscopic technique like ^{1}H, ^{13}C, 2D-NMR (COSY, TOCSY and HSQC) spectroscopy and mass spectrometry.

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