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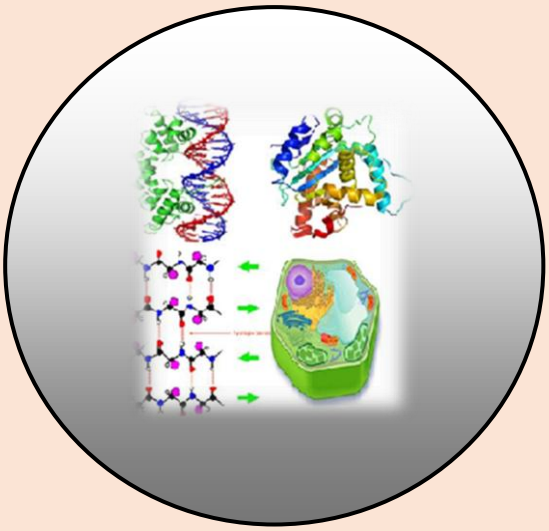

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Isolation and Structure Elucidation of Novel Oligosaccharide Harose from Mare Milk

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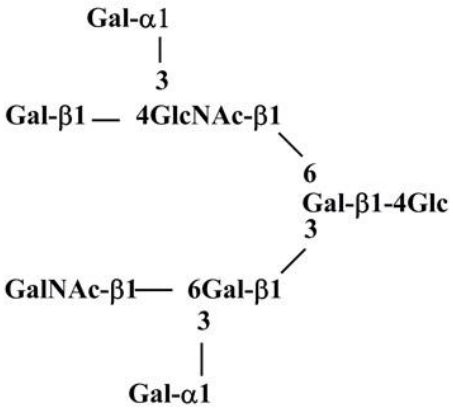
ABSTRACT

Milk Oligosaccharides have been proposed to play an important role in new born defense, blocking bacterial adhesion to the intestinal mucosa and preventing infections. Oligosaccharides are relevant components of milk, which have been quite well studied for their prebiotic effect and their capacity in stimulating the immune system. Since we have chosen mare milk for its oligosaccharide contents because it has antioxidant, lipid lowering, and mineral absorption regulating activities etc. The mare milk was collected in bulk and processed by the modified method of Kobata and Ginsburg involving deproteination, centrifugation and lyophilzation followed by gel filtration, affording crude oligosaccharide mixture which on acetylation and silica gel column chromatography afforded a purified novel compound Harose. The structure of isolated oligosaccharide was elucidated by chemical transformation, chemical degradation, NMR (^{1}H, ^{13}C and 2D COSY, TOCSY and HSQC) and mass spectrometry as under.



HAROSE

Keywords: Mare milk, oligosaccharide and Harose.

INTRODUCTION

Oligosaccharides are a heterogeneous group of carbohydrates comprising the third most abundant constituents in milk. It have established themselves as an effective class of organic biomolecules impacting various physiological and pathological processes such as molecular recognition, signal transaction, differentiation and developmental events and exhibit varied biological activities such as antitumor (Schwonzen et al., 1992), immuno-stimulant (Abe et al., 1983), anti-cancer (Fang et al., 1985), anti-complementary, anti-coagulant, anti-inflamatory (Srivastava and Kulshrestra 1989), hypoglycemic, anti-viral, antithrombotic (Witczak and Nieforth, 1977), and immuno-logical activities (Ehresmann et al,1979 and Ovodov et al,1983). They also have inhibitory effect on certain virulence-related abilities of monocytes, lymphocytes and neutrophils adhesion of endothelial cells. Oligosaccharides isolated from milk of different origin like Human, Bovine, Goat, Buffalo, Donkey, Horse, Sheep etc. have shown structural homology with the carbohydrates carried by glycoproteins and glycolipids on cell surface. Bovine's milk oligosaccharides reduce the adhesion of enterotoxic *E. coli* strain of the calf (Johansson et al, 2005). Goat milk oligosaccharides have important role in intestinal protection and repair after a change caused by DSS (dextron sodium sulphate) induced colitis and their implication in human intenstinal inflammation (Villosladaa et al., 2006). Donkey milk oligosaccharides have ability to stimulate non-specific and specific immunological resistance (Deepak et al., 1998). The oligosaccharide isolated from elephant milk contained a high ratio of sialyl oligosaccharides which may be significant with respect to formation of brain components such as gangliosides of suckling claves (Ostho et al., 2007). Mare's milk oligosaccharides include numerous biologically activities such as antioxidant and lipid lowering activities (Srivastava et al., 2012). It has an excellent medicinal importance usually in the treatment of the metabolic gastrointestinal and liver problems and for recovering after surgery and severe illness. Because of its importance in the treatment of inflammatory disorder (especially inflammation of intestine), in liver disorder in cholesterol and skin disorders, we confined our studies on isolation of oligosaccharide from mare milk. In the present study, we have elucidated the structure of a novel milk oligosaccharide namely Harose with the help of chemical degradation, chemical transformation, spectroscopic techniques like (^{1}H NMR, ^{13}C NMR and 2D NMR).

MATERIAL AND METHODS

GENERAL PROCEDURE

General procedure was same as described in our previous communication (Maurya et al., 2017).

Isolation of Mare milk oligosaccharide by the modified method of Kobata and Ginsburg-

Isolation of Mare milk oligosaccharides was done by the modified method of Kobata and Ginsburg method, which was described in our previous communication (Maurya et al., 2017), except the isolation, was done from 10 litre of mare milk and the yield of oligosaccharide mixture was 315 gm.

Acetylation of Mare milk oligosaccharide mixture

Dry oligosaccharides of pooled fractions (11.5gm) which gave positive phenol-sulphuric acid test (Dubois et al., 1956) were acetylated with pyridine (12ml) and acetic anhydride (12ml) at 60° C for 24 hr. The mixture was evaporated under reduced pressure and viscous residue was taken in $CHCl_3$ and washed in sequence with 2 N HCl, ice cold 2N $NaHCO_3$and finally with H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness yielding the acetylated olgosaccharide mixture (12.3g).

Column chromatography of acetylated oligosaccharide mixture

Acetylated Mare's milk oligosaccharides mixture (12.3g) gave ten spots a,b,c,d,e,f,g,h,i,j on TLC which on repeated colomn chromatography by various proportion of $CHCl_3$ and $CHCl_3$:MeOH resulted into isolation of compound a Harose (46mg) in pure form.

Deacetylation of Compound (a) Harose Acetate by NH_3/ Acetone

Compound a (30 mg) was dissolved in acetone (5 ml) and 5 ml of NH_3 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 3 ml) $CHCl_3$ and the water layer was finally freeze dried giving the deacetylated oligosaccharide A Harose (25 mg).

Methylation/ acid hydrolysis of compound A

Compound A Harose (6 mg) was refluxed with absolute MeOH (2 ml) at 70^0C for 18 h in the presence of cation exchange IR-120 (H^+) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To a solution of methylglycoside of I 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^oC.The hydrolysis was completed after 26 h. The hydrolysate were neutralized with freshly prepared $BaCO_3$, filtered and concentrated under reduced pressure to afford α and β-methylglucosides along with the Gal, GalNAc and GlcNAc. Their identification was confirmed by the comparison with authentic samples (TLC, PC).

Kiliani Hydrolysis of Compound A Harose

Compound A (9 mg) was dissolved in 4 ml Kiliani mixture (AcOH-H_2O-HCl, 7:11:2) 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 GalNAc, GlcNAc, Gal and Glc and these are identified by comparison with authentic sample (TLC, PC).

Description of isolated compound Harose

Compound A (146 mg) obtained from fraction 25-37 of column chromatography-9, on deacetylation with NH_3/ acetone it afforded compound A (85 mg) as a viscous mass, $[\alpha]_D$ +75.16 ^{0} (c, 0.27, H_2O). For elemental analysis, this compound was dried over P_2O_5 at 100°C and 0.1 mm pressure for 8 hr.

$C_{52}H_{88}N_2O_{41}$	% C	% H	% N
Calcd.	44.69	6.30	2.00
Found	44.66	6.31	2.00

It gave positive Phenol-sulphuric acid test, Feigl test, Morgon-Elson test.

δ in D_2O (ppm) : ^{1}H NMR

δ 2.24 [s, 6H, β-GalNAc(S_5) &β-GlcNAc (S_6), NHCO<u>CH_3</u>], 3.29 (t, 1H, J= 8.4 Hz, β-Glc (S_1) H-2), 4.47 [d, 3 H, J=7.5 Hz, β-Gal (S_2,S_3& S_8) H-1], 4.57 (d, 1H, J=7.2 Hz, β-GalNAc (S_5) H-1), 4.66 (d, 1H, J= 7.8 Hz, β-Glc (S_1) H-1), 4.81 (d, 1H, J= 7.8 Hz, β-GlcNAc (S_6) H-1), δ 5.22 (d, 2H, J=3.6 Hz, α-Gal (S_4& S_7), δ 5.25 (d, 1H J=3.0 Hz, α-Glc (S_1) H-1).

δ in D_20 (ppm) : ^{13}C NMR

24.1 [β-GlcNAc (S_6)- NHCO<u>CH_3</u>], δ27.9 [β-GalNAc (S_5)-NHCO<u>CH_3</u>,], δ 88.5 α Glc (S_1) C1, δ 94.7 βGlc (S_1) C-1, δ 98.6 α Gal (S_4& S_7) C-1, δ 99.7 βGlcNAc (S_6) C-1, δ103.8 β-GalNAc (S_5) C-1, δ105.8 β-Gal(S_2, S_3 & S_8)C-1, 180.2 β-GlcNAc (S_6) NH<u>CO</u>CH_3, 184.3 β-GalNAc(S_5) NH<u>CO</u>CH_3.

ES-MS:

1458 $[M+Na+K]^+$ and m/z 1396 $[M]^+$ and other fragments at m/z 1338, 1302, 1290, 1247, 1234, 1217, 1203, 1178, 1125, 1072, 1049, 1031, 1010, 998, 933, 910, 869, 839, 780, 748, 723, 708, 707, 666, 659, 642, 602, 600, 547, 504, 497, 455, 406, 342, 319, 291, 277, 260, 242, 223, 205, 187, 180, 174, 170, 112, 60, 43.

RESULT AND DISCUSSION

COMPOUND A (HAROSE)

Compound A, $[\alpha]_D$ +75.16 ^{0} (c, 0.27, H_2O), $C_{52}H_{88}O_{41}$ N_2 gave positive Phenol-sulphuric acid test, Fiegl test and Morgon-Elson test indicating the presence of normal and amino sugar(s) in the moiety. The ^{1}H NMR spectrum of A at 300 MHz exhibited nine signals in the anomeric proton region as doublets at δ5.25 (1H), δ5.22 (2H), 4.81 (1H),4.66 (1H), 4.57 (1H) and 4.44 (3H) for nine protons leading to the

presence of nine anomeric protons in compound. It was further supported by the appearance of nine signals for nine anomeric carbons at δ 88.5 (1C), δ 94.7 (1C), δ 98.6 (2C), δ 99.7 (1C), δ 103.8 (1C) and 105.8 (3C) in the ^{13}C NMR spectrum of Harose. These data led to the suggestion that A may be an Octasaccharide in its reducing form. The Electrospray mass spectrum of compound Harose showed the highest mass ion peak at m/z 1458 $[M+Na+K]^+$ and 1396 $[M]^+$, which was indication of the composition C_{52} H_{88} O_{41} N_2 with the molecular ion expected at m/z 1396 for a Octasaccharide. The nine-monosaccharide units present in compound I have been designated as S_1, S_2, S_3, S_4, S_5, S_6, S_7 and S_8 for convenience starting from the reducing end. The Kiliani acid hydrolysis of compound Harose gave four spots on the paper chromatography, which were identified as Glc, Gal, GlcNAc and GalNAc by co-chromatography with authentic samples suggesting that this Octasaccharide is comprised from these four monosaccharides in varied numbers. Methylglycosidation of 'A' by $MeOH/H^+$ followed by acid hydrolysis led to the isolation of α and β-methyl glucoside, which suggested the presence of glucose at the reducing end in the oligosaccharide.

The reducing and free nature of glucose was further supported by the presence of two anomeric proton signals as doublets and their coupling constants, for α and β Glc at 5.25 ppm(1H, J=3.0 Hz) and δ 4.66 ppm(1H, (J=7.8 Hz) respectively. Beside, α and β Glucose (S_1), another anomeric proton doublet present at δ 4.47 ppm (3H) J=7.5 Hz for Gal (S_2) residue suggested the presence of a lactosyl moiety i.e. Gal β (1→4) Glc in compound Harose. This was further confirmed by β Glc (S_1) H-2 signal appeared as a triplet at δ 3.296, J=8.4 Hz, these ^1H NMR of data of lactosyl moiety was compared with structural reporter group.

Further in the ^1H NMR spectrum of Harose, third anomeric proton appeared as a doublet at δ 4.47 (3H, J=7.5 Hz) which was due to the presence of another β Gal (S_3) moiety in it. In the ^1H NMR of acetylated Harose, signal present at δ 3.67 ppm was assigned for H-3 of Gal (S_2) which was confirmed by COSY and TOCSY experiments, this chemical shift value was implies that β Gal (S_3) glycosidically linked to the Gal (S_2) by [1→3] linkage which was compared with reported values of earlier isolated compound of horse milk. Further, in ^1H NMR spectrum, the fourth anomeric proton of Harose which appeared as a doublet at δ 5.22 ppm (J=3.6 Hz) showed the presence of α Gal (S_4) in Harose, which was complimented by signal which appeared at δ 3.66 ppm assigned for H-3 of S-3 showing the glycosidation of α Gal (S_4) with C-3 of β Gal (S_3). This linkage was compared by acetylated ^1H-^1H COSY and TOCSY spectrum of Harose, in which H-3 proton of Gal (S_3) was present at up field region δ 3.77 ppm. Further, the fifth anomeric proton present as a doublet at δ 4.574 ppm (1H, J=7.2 Hz) along with singlet signal of NHAc of three protons at δ 2.248 ppm was due to the presence of GalNAc (S_5) moiety, the linkage between (S_5) and (S_3) was assigned as [1→6] by the signal of H-6 of (S_3) which appeared at δ 3.74 ppm in the ^1H NMR of acetylated Harose. This data led to the assignment the β GalNAc (S_5) was glycosidically linked to C-6 of β Gal (S_3). This linkage was confirmed with acetylated ^1H NMR, COSY and TOCSY spectrum data of compound Harose.

Table. ^1H AND ^{13}C NMR Values of Compound A.

	S-1(α)	S-1 (β)	S-2	S-3	S-4	S-5	S-6	S-7	S-8
^1H	5.25	4.66	4.47	4.47	5.22	4.57	4.81	5.22	4.47
^{13}C	88.1	94.7	105.8	105.8	98.6	103.8	99.7	98.6	105.8

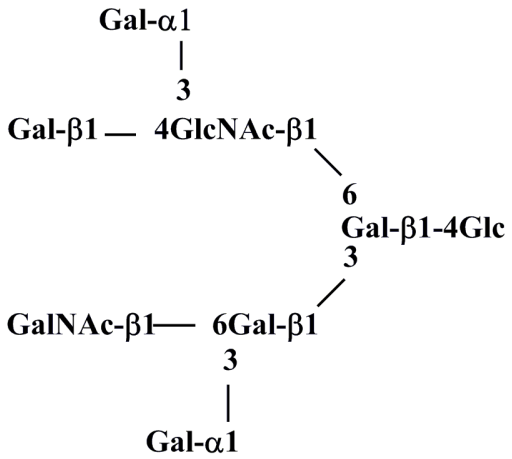
Further, the ^1H NMR spectrum showed another anomeric proton signal appeared as a doublet at δ 4.81ppm (1H, J=7.8) along with singlet NHAc of three protons at δ 2.00 ppm showed the presence of GlcNAc (S_6) which was present in Harose. The linkage of GlcNAc (S_6) showed the structure resemblance with earlier isolated compound of horse milk which showed a signal H-6 of Gal (S_2) at δ 3.614 ppm assigned to H-6 of β-Gal (S_2). This assignment was further confirmed by ^1H-^1H COSY and TOCSY spectrum of acetylated Harose, which showing that the C-6 of β Gal (S_2) was involved in inter residual glycosidation GlcNAc (S_6) [1→6] β-Gal (S_2).

Further, the seventh anomeric proton which appear as a doublet at δ 5.22 ppm (J=3.6 Hz) of α -Gal (S_7) that was glycosidically linked to the C-3 of GlcNAc (S_6) because H-3 proton of (S_6) was present at δ 3.864 ppm in ^{1}H NMR of Harose, which was confirmed by acetylated ^{1}H-^{1}H COSY and TOCSY spectra of acetylated compound of Harose, In which H-3 proton of (S_6) appear at up field position δ 3.700 ppm indicates that C-3 of GlcNAc (S_6) was involved in inter residual glycosidation with S_7 sugar. In the ^{1}H NMR of Harose another anomeric proton signal present as a doublet at δ 4.47 (3H, J=7.5 Hz) showed another β-Gal (S_8) which was present in next to GlcNAc (S_6). The ^{1}H NMR spectrum of acetylated Harose showed a signal at δ 3.86 ppm which assigned for H-4 of GlcNAc (S_6) which was confirmed by COSY and TOCSY NMR experiments of acetylated Harose, this implies that β-Gal (S_8) glycosidically linked to the C-4 of GlcNAc (S_6) and this linkage Gal (S_8) [1→4] β-GlcNAc (S_6) was confirmed by ^{1}H-^{1}H COSY and TOCSY spectrum of acetylated compound of Harose.

Table. ^{13}C NMR Values of Compound 'A' HAROSE.

	C-1	C-2	C-3	C-4	C-5	C-6	-CO	-CH_3
α-Glc(S_1)	88.5	73.0	71.5	82.2	71.4	63.0		
β-Glc(S_1)	94.7	73.0	71.4	82.3	77.2	63.9		
β-Gal(S_2)	105.8	71.5	78.2	71.4	74.0	73.0		
β-Gal(S_3)	105.8	71.4	75.4	71.4	73.8	63.9		
α-Gal (S_4)	98.6	71.5	71.4	73.6	75.4	63.0		
β-GalNAc(S_5)	103.8	62.9	73.6	74.0	77.7	62.9	184.3	27.9
β-GlcNAc(S_6)	99.7	62.9	73.6	74.0	77.7	63.0	180.2	24.1
α-Gal(S_7)	98.6	71.5	71.4	73.0	75.4	63.0		
β-Gal(S_8)	105.8	71.4	77.2	71.4	73.8	62.9		

The resembling pattern of ^{1}H NMR spectrum of Harose with reported Pentasaccharide Gal β (1-4) GlcNAc β (1-6) [Gal β (1-3)] Gal β (1-4) Glc from Horse milk (Mare), showed the presence of reported Pentasaccharide in it with substituted Gal (S_3) at C-3 and C-6 by α Gal (S_4) and β GalNAc (S_5) respectively along with substituted GlcNAc (S_6) at C-3 by β Gal (S_8), indicates that this Octasaccharide contains three more monosaccharide units which are fixed on different positions of sugar unit of earlier reported Pentasaccharide molecule (from horse) gave birth to a novel Octasaccharide Harose. The Octasaccharide nature of isolated oligosaccharide Harose was further confirmed by spectral studies of acetylated Harose.



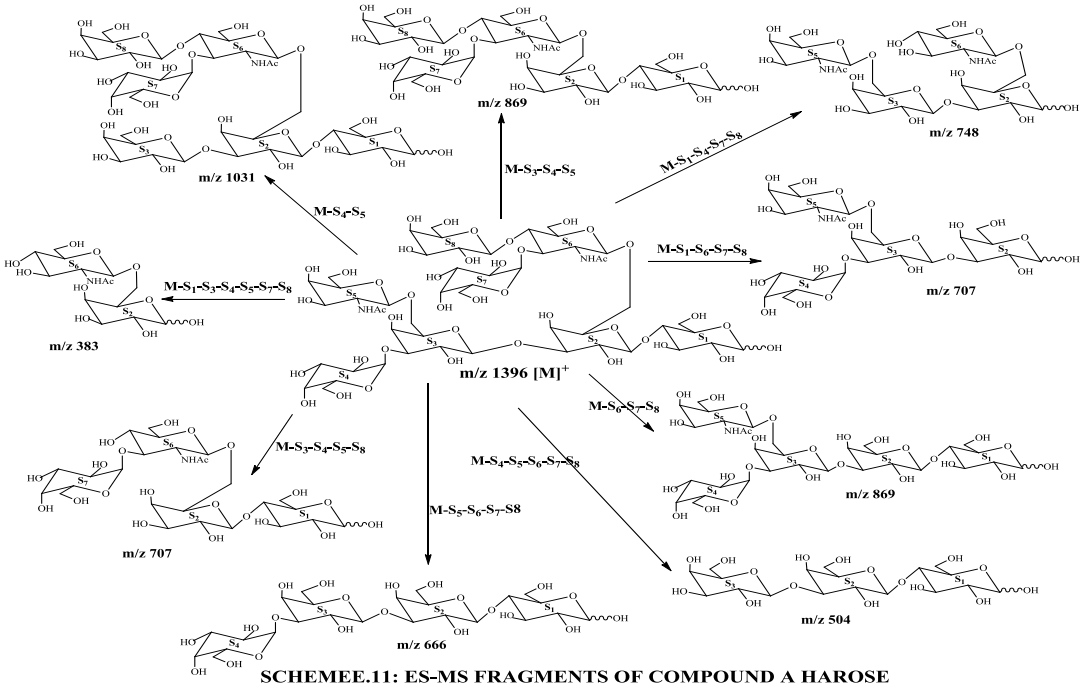
The heteronuclear single quantum-coherence (HSQC) spectrum of acetylated product of compound A confirmed anomeric assignments in ^{1}H and ^{13}C NMR spectra of A by showing the ^{1}H and ^{13}C cross peaks of α-Glc at δ6.22 x δ 90.1 and β-Glc at δ 5.6 x 92.2, and it also contains seven other anomeric cross peaks signals at 4.32 x δ 103.8, δ 4.47 x δ104.1, δ5.29 x δ92.2, 4.55 x δ104.3, δ4.67 x δ95.3, 5.36 x δ90.3 and δ 4.47 x δ101.7 for (S_2), (S_3), (S_4), (S_5), (S_6), (S_7) and (S_8) respectively along with carbons which involved in the inter residual glycosidation in acetylated compound Harose present at δ3.59 x δ 82.1for α-Glc (S_1) and at δ 3.55 x 82.5 for β-Glc (S_1), and it also contains six other glycosidic linkage crosspeaks obtained at 3.50 x δ 69.4 and δ 3.67 x δ70.6 for C-3 & C-6 of β Gal (S_2), δ3.77 x δ72.08 and 3.74 x δ74.7 for C-3 & C-6 of β Gal (S_3) and δ3.70 x δ72.6 & 3.80 x δ73.1 for C-3 & C-4 of β GlcNAc (S_6) sugar residues. Based on the pattern of chemical shift of ^{1}H, ^{13}C, HOMOCOSY, TOCSY and HSQC NMR experiments, it was interpreted that the compound Harose was a branched Octasaccharide having one reducing end Glc, three β Gal, one βGlcNAc, one βGalNAc and two α Gal moieties.

The Electrospray Mass Spectrometric data of compound Harose helped in substantiating the sequence of monosaccharide units in it. The highest mass ion peak were recorded at m/z 1458 of $[M+Na+K]^+$ and m/z 1396 $[M]^+$ confirming the molecular weight of Harose as 1396.

Further the mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water. This fragmentation path way confirmed the sequence of monosaccharides in the oligosaccharide. The ES-MS spectrum not only confirmed the derived structure but also confirmed the sequence of monosaccharide units in Harose.The Electrospray mass spectrum of compound 'A' showed the highest mass ion peak at m/z 1458 $[M+Na+K]^+$ and m/z at 1396 which was due to $[M]^+$ confirming the molecular weight of Harose as m/z 1396. Further the mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water at m/z 1234 with loss of terminal normal hexose, Gal residue (S_8), further the mass ion peak was obtained at m/z 1072 by loss of m/z 162 ofGal (S_7) Sugar residue, the mass ion peak obtained at m/z 910 by loss of (S_4)Sugar residue of m/z 162 from remaining hexasaccharide of m/z 1072, further the mass ion peak obtained at m/z 707 with lose of m/z 203 of HexNAc moiety (S_5) from remaining sugar residue m/z 910, the remaining molecular ion mass m/z 707 lose another HexNAc moiety, GlcNAc (S_6) [707-S_6] and remain m/z 504. The remaining trisaccharide of molecular mass m/z 504 further break in to the disaccharide molecule of m/z 342 with lose of m/z 162 of normal Hexose, Gal (S_3) residue. Further after fragmentation of disaccharide of m/z 342 with loss of Gal (S2) m/z 162, give a monosaccharide Glucose of molecular mass m/z 180.

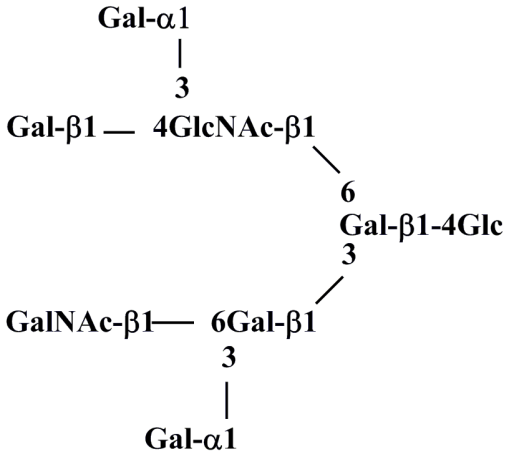
The molecular ion peak of compound Harose obtained at 1396 which fragmented in many ways and gave different molecular ion mass peak after removal of monosaccharide residues and these residues further fragmented to give mass peak. The M^+ mass 1396 fragmented to give other respective mass ion peak at m/z 1338[1396-$NHCOCH_3$], 1290[1338-CH_2OH-OH], 1302[1338-2 H_2O], 1247[1338-CH_3OH-OH-CH_2C=O], 1178 [1247-3OH-H_2O], 1125[1178-OH-2H_2O], 1049[1125-2OH-CH_2C=O]. The Electrospray mass spectrum of compound 'I' showed mass ion peaks at m/z 1234 (M-S_8) and gives other fragment at m/z 1217[1234-OH], 1203[1234-CH_2OH], 1125[1203-2H_2O-CH_2C=O], 1049[1125-H_2O-$NHCOCH_3$], 998[1049-3OH], 933[998-2OH-CH_2OH]. Further the mass ion peak are obtained at m/z 1072 [M-S_8- S_7] along with other respective fragments at 1010[1072-2CH_2OH], 933[1010-OH-CH_2C=O-H_2O] 869[933-2CH_3OH], 839[933-2OH-H_2O], 780[839-OH-CH_2C=O], 708[780-4H_2O], 666[708-CH_2C=O]. Further, with lose of Hexose S_4 moiety of mass 162 from remaining molecular ion mass m/z 1072, it gives mass ion peak at m/z 910 with its respective fragments at m/z 839[910-3H_2O-OH], 780[839-OH-CH_2C=O], 748[780-CH_3OH], 666[748-3OH-CH_2OH]. The mass ion peak obtained at m/z 707 with loss of m/z 203 of HexNAc moiety (S_5) from m/z 910 and it fragmented to 659[707-OH-CH_2OH], 547[707-2OH-CH_3OH-2H_2O-$NHCOCH_3$], 97[547-CH_3OH-H_2O], 455[497-CH_2C=O], 406[455-CH_3OH-OH] and supported the fragment m/z 707 mass ion peak.

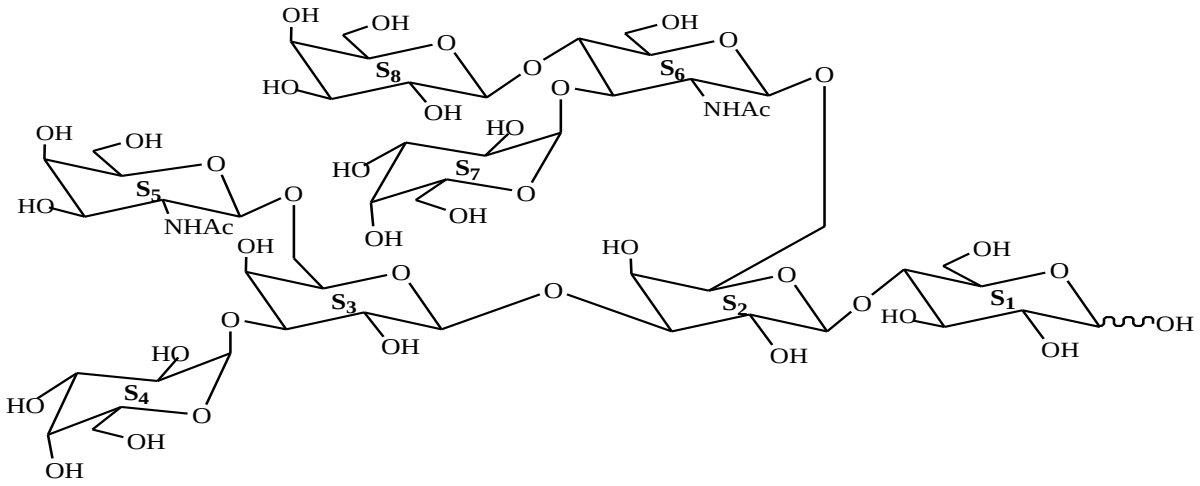
Further lose of another HexNAc moiety (S_6) from m/z 707, along with respective fragments at m/z 455[504-CH_3OH-OH], 406[455-H_2O-CH_2OH], 319[406-2H_2O-3OH], 277[319-CH_2C=O], 260[277-OH], 242[260- H_2O]. The remaining Trisaccharide m/z 504 further break in to the m/z 342 with lose of normal Hexose residue and gives fragment at 291[342-3OH], 260[291-CH_2OH], 187[260-CH_2C=O-CH_2OH], 170[187-OH], 223[291-4OH], 205[223-H_2O], 174[205-CH_2OH]. On further fragmentation of disaccharide 342 gave a monosaccharide m/z 180 with respective mass ion at m/z 112[180-4OH], 60[112-2OH-H_2O], 43[60-OH]. The different fragmentation pathway of compound 'A' shows molecular ion fragments at m/z 1031[M-S_5-S_4], 869[M-S_5-S_4-S_3], 748[M-S_8-S_7-S_4-S_1], 707[M-S_8-S_5-S_4-S_3 or M-S_8-S_7-S_6-S_1], 666[M-S_8-S_7-S_6-S_5] and 504[M-S_8-S_7-S_6-S_5-S_4] and 383[M-S_1-S_3-S_4-S_5-S_7-S_8] which confirms the Octasaccharide nature of compound Harose.



SCHEMEE.11: ES-MS FRAGMENTS OF COMPOUND A HAROSE

Based on the pattern of chemical shift of ^1H, ^{13}C, HOMOCOSY, TOCSY and HSQC NMR experiments, chemical transformation, chemical degradation / acid hydrolysis and ES-MS studies of Harose and acetylated Harose the structure of Novel compound HAROSE was confirmed as –





HAROSE

CONCLUSION

In summary, the novel milk oligosaccharide is an octasaccharide namely Harose has been isolated from mare milk and elucidated with the help of ^{1}H, ^{13}C, 2D NMR spectroscopy and mass spectrometry.

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