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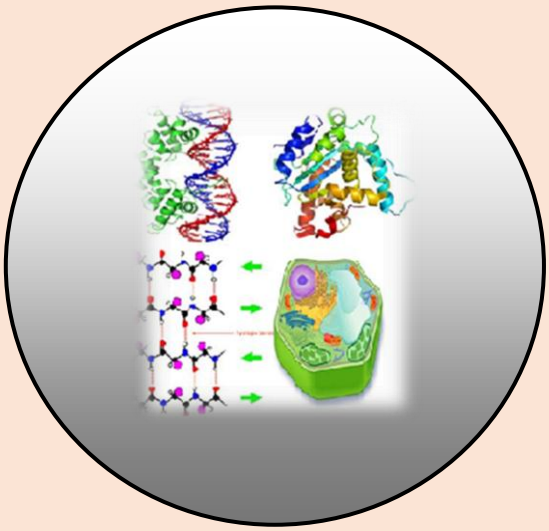

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Isolation and Structure Elucidation of a Novel Oligosaccharide Theriose from Buffalo Milk

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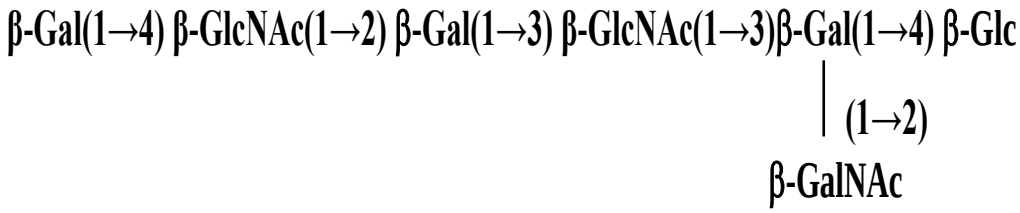
ABSTRACT

The Carbohydrate part of mammalian milk is mainly consisting of lactose and oligosaccharides. Oligosaccharides are present in milk either in free form or conjugated with protein and lipid is beneficial for new born babies as well as for adults. Among all mammals buffalo is a major milk cattle of north India. Buffalo milk is beneficial for cardiovascular health and its oligosaccharide part induces the stimulation of antibodies and it also stimulates the nonspecific immune response of the animals measured in terms of macrophage migration index. In continuation of our previous work on Buffalo milk, another novel heptasaccharide Theriose was isolated from buffalo milk and its structure was elucidated. In the present study, buffalo milk was collected and processed by modified method of Kobata and Ginsberg followed by gel filtration, HPLC, and Column Chromatography. Structure of the isolated oligosaccharide was elucidated by chemical transformation, chemical degradation, NMR (^{1}H, ^{13}C NMR) and 2D-NMR (COSY, TOCSY and HSQC) techniques. The structure of novel isolated oligosaccharide was deduced as under –



THERIOSE

Keywords: Immunostimulant, Oligosaccharide, Heptasaccharide and Theriose.

INTRODUCTION

Milk is a highly nutritious liquid formed in the mammary glands of mammals. Various mammals like -human, goat (Kumar et al., 2016), buffalo (Gangwar et al., 2017), sheep (Ranjan et al., 2015), mare (Maurya et al., 2017), cow (Gunjan et al., 2016), etc. produces milk with good concentration of bioactive oligosaccharides. The composition of milk oligosaccharides is under genetic control and shows strong individual variability between lactating mammals. The milk oligosaccharides are classified as neutral and acidic oligosaccharides. Neutral oligosaccharide contains uncharged carbohydrate residues whereas acidic oligosaccharides have negatively charged -COOH residues which are due to the presence of sialic acid (Snead T. Morrin et al., 2018). The oligosaccharide concentration and the acidic and neutral charge profile changes considerably during the lactation period. The highest concentrations are found within the first week of lactation then a subsequent decrease in the concentration of oligosaccharides take place. In the past, complex oligosaccharides were thought of as a food source of a healthy diet and their biological roles were limited to antigenic properties of various blood groups only but in present scenario, various studies shows that they have large biomedical spectrum. In the present study, we have selected buffalo milk as experimental substrate. Buffalo milk is a rich source of protein, calcium, iron, phosphorous, vitamin A and oligosaccharides. Buffalo milk is high in calcium and low in cholesterol as compare to other milk sources which is beneficial for the health of heart. Buffalo milk boasts of decent potassium content that is extremely crucial for stable blood pressure. Buffalo milk oligosaccharides have ability to stimulate non-specific immunological resistance of the host against parasitic infections (R. Saksena, et al., 1999).

In our previous works we have isolated various buffalo milk oligosaccharides such as-Orientose (Kumar et al., 2019), Bubaliose (Gangwar et al., 2017) and their structures were elucidated with the help of various spectroscopic techniques. Keeping in mind the biological activity of buffalo milk oligosaccharide and in continuation of our previous works we have isolated another oligosaccharide from Buffalo (*Bubalus bubalis*) milk and the structure of this novel oligosaccharide (Theriose) was elucidated with the help of spectroscopic techniques (^{1}H, ^{13}C, COSY, TOCSY and HSQC) along with other traditional techniques like deacetylation, hydrolysis, chemical degradation and ES-MS (mass spectrometry).

MATERIALS AND METHODS

General Procedure

Isolation of Buffalo Milk Oligosaccharide by Modified Method of Kobata and Ginsberg

Isolation of buffalo milk oligosaccharide was done by modified method of Kobata and Ginsberg (Kumar et al., 2016) which was described in our previous communication (Khan et al., 2018). The isolation was done from 10 litre of buffalo milk and the yield of oligosaccharide mixture was 450 gm.

Acetylation of Buffalo milk Oligosaccharides

10 gm of oligosaccharide mixture was obtained from sephadex chromatography of crude oligosaccharide mixture which was acetylated by adding pyridine (10 ml) and acetic anhydride (10 ml) at 60°C with constant stirring and was kept overnight. The mixture was evaporated under reduced pressure and the viscous residue was taken in $CHCl_3$ (250 ml) and washed with ice cold water.

The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness yielding the acetylated oligosaccharide mixture (12.60 gm). The acetylation converted the free sugars into their nonpolar acetyl derivatives which were resolved nicely on TLC, giving eight spots on TLC i.e. a, b, c, d, e, f, g and h.

Purification of Acetylated Oligosaccharide Mixture by column chromatography Acetylated Buffalo's milk oligosaccharides mixture (12.60g) gave eight spots a,b,c,d,e,f,g and h, on TLC which on repeated colomn chromatography by various proportion of $CHCl_3$ and $CHCl_3$:MeOH resulted into isolation of compound b (65 mg) in pure form.

Deacetylation of Compound b (Theriose acetate)

Compound'b' (65 mg) was obtained from column chromatography of acetylated oligosaccharide mixture. 25 mg of compound b was dissolved in acetone (2 ml) and 3 ml of NH_4OH was added in it and was left overnight in a stoppered hydrolysis flask. After 24 h ammonia was removed under reduced pressure and the compound was washed thrice with $CHCl_3$ (5 ml) (to remove acetamide) and the water layer was finally freeze dried giving the natural oligosaccharide B (19 mg). $[\alpha]_D$ **+** 8.44^0. For experimental analysis, this compound was dried over P_2O_5 at 100^o C and 0.1 mm pressure for 8 hrs.

$C_{48}H_{81}O_{36}N_3$	%C	%H	%N
Calculated	45.17	6.35	3.29
Found	45.16	6.34	3.29

It gave positive Phenol sulphuric acid test, Feigl test and Morgan Elson test.

Methyl glycosidation /Acid hydrolysis of compound B (Theriose)

Compound B (8 mg) was ref1uxed with absolute MeOH (2 ml) at 70°C for 18 h in the presence of cation exchange IR-l20 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. In the reaction mixture, 1,4-dioxane (1 ml), and 0.1N H_2S0_4 (1 ml) was added and the solution was warmed for 30 minutes at 50^oC. The hydrolysis was completed in 24 h. The hydrolysate was neutralized with freshly prepared $BaCO_3$ filtered and concentrated under reduced pressure to afford α-and β-methylglucosides along with the Glc, Gal, GalNAc and GlcNAc. The identification of monosaccharides were confirmed by comparison with authentic samples (TLC, PC).

Kiliani hydrolysis of compound B (Theriose)

Compound B (5 mg) was dissolved in 2 ml Kiliani mixture (AcOH-H_2O-HCl, 7:11:2) and heated at 100^oC 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 and was evaporated under reduced pressure to afford Glc, Gal, GalNAc and GlcNAc on comparison with authentic samples of Glc, Gal, GalNAc and GlcNAc.

^{1}H NMR of compound b (Theriose acetate) in $CDCl_3$

δ 5.53[d, 1H, J=4Hz, α- Glc (S_1)], δ 5.41 [d, 1H, J=8Hz, β-Glc (S_1)], δ 5.32 [d, 1H, J=8Hz, β-GlcNAc (S_3)], δ 4.75 [d, 1H, J=8Hz, β-GlcNAc (S_5)], δ 4.62 [d, 1H, J=8Hz, β-GalNAc (S_7)], δ4.53 [d,1H, J=8Hz, β-Gal(S_6)], δ4.47 [d,1H, J=8Hz, β-Gal(S_2)], δ4.39 [d,1H, J=8Hz, β-Gal(S_4)], δ3.90 [m, 1H, J=8Hz, β-Glc(S_1), H-4], δ 3.84 [m,1H, J=8Hz, β-Gal(S_2), H-2, β-GlcNAc(S_5), H-4], δ 3.75 [m,1H, J=8Hz, β-Gal(S_4), H-2], δ3.70 [m,1H, J=8Hz, β-GlcNAc(S_3), H-3], δ 3.51 [m,1H, J=8Hz, β-Gal(S_2), H-3].

^{13}C NMR of compound b (Theriose acetate) in $CDCl_3$

δ 103.5 [1C, β-Gal (S_2), C-1], δ 103.0 [1C, β-Gal (S_4), C-1], δ 101.0 [2C, β-Gal (S_6), β-GalNAc (S_7), C-1], δ 95.5 [1C, β-GlcNAc (S_5), C-1], δ 92.5 [1C, β-GlcNAc (S_3), C-1], δ 90.0 [1C, β-Glc (S_1), C-1], δ 90.0 [1C, α-Glc (S_1), C-1].

^{1}H NMR of Theriose in D_2O

δ 5.08 [d, 1H, J=4Hz, α- Glc (S_1)], δ 4.52 [d, 1H, J=8Hz, β-Glc (S_1)], δ 4.44 [d, 1H, J=8Hz, β-GlcNAc (S_3)], δ 4.39 [d, 2H, J=8Hz, β-GalNAc (S_7), β-GlcNAc (S_5)], δ4.30 [d,3H, J=8Hz, β-Gal(S_2), β-Gal(S_4) & β-Gal(S_6)], δ3.15 [t,1H, J=8Hz, β-Glc(S_1), H-2], δ 1.88 [s, 3H, β-GalNAc(S_7),NHCOC<u>H</u>$_3$], δ 1.85 [s, 3H, β-GlcNAc(S_5),NHCOC<u>H</u>$_3$] δ 1.75 [s, 3H, β-GlcNAc(S_3),NHCOC<u>H</u>$_3$].

^{13}C NMR of Theriose in D_2O

δ 104.0 [1C, β-Gal (S_4), C-1], δ 103.0 [3C, β-Gal (S_6), β-Gal (S_2) & β-GalNAc (S_7), C-1], δ 96.0 [2C, β-GlcNAc (S_3), β-GlcNAc (S_5), C-1], δ 92.0 [2C, β-Glc (S_1), α-Glc (S_1), C-1].

ES-mass

m/z 1337 $[M+Na+K]^+$, m/z 1298 $[M+Na]^+$, m/z 1275 $[M]^+$, m/z 1246, m/z 1239, m/z 1205, m/z 1113, m/z 1110, m/z 1079, m/z 1050, m/z 1033, m/z 1002, m/z 910, m/z 876, m/z 847, m/z 830, m/z 748, m/z 712, m/z 678, m/z 545, m/z 511, m/z 509, m/z 482, m/z 465, m/z 342, m/z 325, m/z 291, m/z 180.

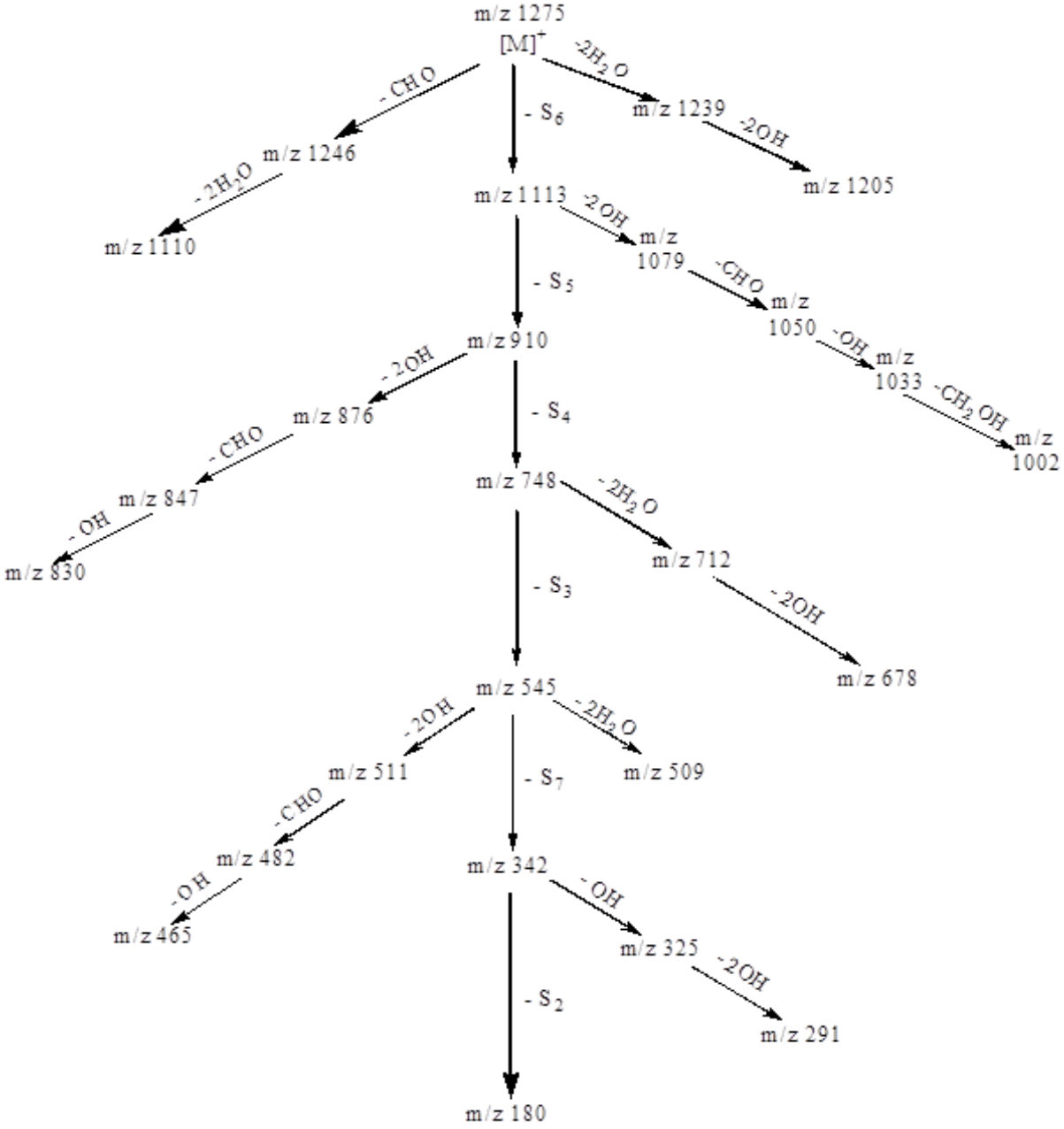
RESULT AND DISCUSSION

Compound B, $C_{48}H_{81}O_{36}N_3$, $[\alpha]_D$ $+8.44^0$, gave positive Phenol-sulphuric acid test (Dubois et al., 1956), Fiegl test (Feigl et al., 1975), and Morgon-Elson test (Gey et al., 1996) showing the presence of normal and amino sugar moieties in the compound Theriose. In the ^{1}H NMR spectrum of Theriose in D_2O at 400 MHz eight anomeric proton signals of eight anomeric protons as doublets appeared at δ 5.08 (1H), δ 4.52 (1H), δ 4.44 (1H), δ 4.39 (2H) and δ 4.30 (3H), showing it to be a heptasaccharide in its form. The heptasaccharide nature of Theriose was also supported by the presence of eight anomeric carbons at 104.0 (1C), 103.0 (3C), 96.0 (2C) and 92.0 (2C) in the ^{13}C NMR spectrum of Theriose in D_2O at 400 MHz. The HSQC spectrum of acetylated Theriose showed the presence of eight cross peaks of anomeric protons and carbons in the respective region at δ 5.53 x 90.0, δ 5.41 x 90.0, δ 5.32 x 92.5, δ 4.75 x 95.5, δ 4.62 x 101.0, δ 4.53 x 101.0, δ 4.47 x 103.0 and δ 4.39 x 103.5, suggesting the presence of eight anomeric protons and carbons in it. The presence of eight anomeric protons were further confirmed by the presence of eight anomeric doublets at δ 5.53 (1H), δ 5.41 (1H), δ 5.32 (1H), δ 4.75 (1H), δ 4.62 (1H), δ 4.53 (1H), δ 4.47 (1H), and δ 4.39 (1H) in the ^{1}H NMR spectrum of acetylated Theriose in $CDCl_3$ at 400 MHz. The heptasaccharide nature of Theriose was also confirmed by the presence eight anomeric carbons at δ 104.0 (1C), δ 103.0 (3C), δ 96.0 (2C) and δ 92.0 (2C) in ^{13}C NMR spectrum of Theriose in D_2O at 400 MHz. Further, the presence of eight anomeric carbons were also confirmed by eight anomeric carbon signals at δ 103.5 (1C), δ 103.0 (1C), δ 101.0 (2C), δ 95.5 (1C), δ 92.5 (1C) and δ 90.0 (2C) in the ^{13}C NMR spectrum of acetylated Theriose in $CDCl_3$ at 400 MHz. Since all these spectrums contained downfield shifted α and β anomeric proton and carbon suggested that compound Theriose may be a heptasaccharide in its reducing form.

The reducing nature of compound Theriose was further confirmed by methylglycosylation of compound Theriose by $MeOH/H^+$ followed by its acid hydrolysis which led to the isolation of α and β-methyl glucosides, suggesting the presence of glucose at the reducing end. For convenience the seven monosaccharides present in compound Theriose have been designated as S-1, S-2, S-3, S-4, S-5, S-6 and S-7 respectively starting from glucose (S-1) the reducing end. The monosachharide constituents in compound Theriose were confirmed by its Killiani hydrolysis (Killiani et al., 1930) under strong acidic conditions, followed by its Paper chromatography and TLC. In this hydrolysis four spots were found on TLC and PC which were found identical with authentic samples of Glucose, Galactose, GlcNAc and GalNAc by co- chromatography. Thus, confirming that the heptasaccharide contained four types of monosaccharide units i.e., Glc, Gal, GalNAc and GlcNAc in it. The chemical shifts values of anomeric protons and carbon observed in ^1H NMR and ^{13}C NMR spectrum of Theriose were also in agreement with the reported values of ^1H and ^{13}C anomeric chemical shifts of Glc, Gal, GalNAc and GlcNAc confirming the presence of these monosaccharides in the compound Theriose. Further, the presence of two anomeric proton signals at δ 5.08 (J=4.0 Hz) and δ 4.52 (J=8.0 Hz) in the ^1H NMR spectrum of Theriose in D_2O at 400 MHz were assigned for α and β anomers of glucose (S-1) confirming the presence of Glc(S-1) at the reducing end in compound Theriose. In the TOCSY spectrum of Theriose acetate the anomeric signal of β-Glc (S-1) at δ 5.41 gave a cross peak in the linkage region at δ 3.90 suggesting that one position in S-1 was available for glycosidic linkage, which was further confirmed as H-4 of β-Glc (S-1) by the COSY spectrum of Theriose acetate. This shows that β-Glc(S-1) was 1→4 linked with next monosaccharide unit. Further the presence of another anomeric proton doublet at δ 4.30 (J=8.0 Hz) in the ^1H NMR of Theriose in D_2O showed the presence of β-Gal(S-2) residue as the next monosaccharide, which was 1→4 linked with β-Glc(S-1). The 1→4 linkage between β-Gal (S-2) and β-Glc (S-1) was further confirmed by the appearance of β-Glc(S-1) H-2 signal as triplet at δ 3.15 (Structure reporter group) (Dabrowski et al., 1983) (Wengang et al., 2005) and hence the presence of lactosyl moiety was confirmed at the reducing end. The coupling constant of anomeric signal of β-Gal (S-2) with J value of 8.0 Hz confirmed the β-configuration of the β-Gal (S-2) moiety and hence β 1→4 glycosidic linkage between S-2 and S-1. Further the anomeric proton signal of β-Gal(S-2) at δ 4.47 in the ^1H NMR of Theriose acetate in $CDCl_3$ showed the two consequent complementary signals in the linkage region at δ 3.84 and δ 3.51 in the TOCSY spectrum of Theriose acetate showing that the two hydroxyl groups of β-Gal (S-2) were involved in glycosidic linkages by next monosaccharide moieties. These signals were identified for H-2 and H-3 of β-Gal(S-2) by the COSY spectrum of Theriose acetate suggesting that H-2 and H-3 of β-Gal(S-2) were available for glycosidic linkages by the next monosaccharide units. The next anomeric proton signal which appeared as doublet at δ 4.44 (J=8.0 Hz) in the ^1H NMR spectrum of Theriose in D_2O at 400 MHz along with a singlet of amide methyl ($-NHCOCH_3$) at δ 1.75 was due to the presence of β-GlcNAc(S-3) moiety. The downfield shifted H-4 proton of β-Gal (S-2) at 4.12 in the ^1H NMR spectrum of Theriose at 400 MHz in D_2O suggested that β-Gal (S-2) was glycosidically linked at C-3 position by β-GlcNAc (S-3) moiety (SRG). The 1→3 linkage between β-GlcNAc (S-3) and β-Gal (S-2) was further supported by the ^1H NMR spectrum of acetylated Theriose in which the signal for H-3 of β-Gal (S-2) appeared at δ 3.51 which was later confirmed by COSY, TOCSY and HSQC spectrum of acetylated Theriose at 400 MHz in $CDCl_3$**.**

The coupling constant of anomeric signal of (S-3) with J value 8.0 Hz confirmed the β-configuration of the GlcNAc (S3) moiety. Therefore the glycosidic linkage between S-3 and S-2 was confirmed as β 1→3. Another anomeric proton signal which appeared as a doublet at δ 4.39 (J=8.0 Hz) along with a singlet of amide methyl (-$NHCOCH_3$) at δ 1.88 in ^1H NMR spectrum of Theriose in D_2O was assigned for the presence of β-GalNAc(S-7) moiety. Since it was ascertained by COSY and TOCSY spectrum of Theriose acetate that the positions 2 and 3 of β-Gal (S-2) were available for glycosidic linkages and position 3 of β-Gal(S-2) was already linked with β-GlcNAc(S-3), the leftover H-2 position of S-2 must be linked by β-GalNAc(S-7). The position of linkage between β-GalNAc(S-7) and β-Gal(S-2) was further confirmed by the appearance of H-2 signal of β-Gal (S-2) at δ 3.84 in the ^1H NMR spectrum of Theriose acetate which was also confirmed by COSY and TOCSY spectrum of Theriose acetate at 400 MHz in $CDCl_3$. The large coupling constant of β-GalNAc(S-7) of J=8.0 Hz confirmed the β-glycosidic linkage between β-GalNAc(S-7) and Gal(S-2). Since, none of methine proton of β-GalNAc(S-7) appeared in the linkage region in the TOCSY spectrum of Theriose acetate confirms that β-GalNAc(S-7) was linked at the non-reducing end. Further, In the TOCSY spectrum of Theriose acetate the anomeric proton of β-GlcNAc (S-3) at δ 5.32 showed a cross peak at δ 3.70 in the linkage region suggesting that only one OH was available for glycosidic linkage. Later this signal of δ 3.70 was ascertained as H-3 of β-GlcNAc (S-3) by COSY spectrum of Theriose acetate showing that H-3 of β-GlcNAc (S-3) was glycosidically linked by H-1 of next monosaccharide unit. Further, the presence of another anomeric proton as doublet at δ 4.30 (J=8.0 Hz) was identified due to the presence of β-Gal(S-4) as the next monosaccharide unit in the ^1H NMR spectrum of Theriose in D_2O at 400 MHz which was linked to β-GlcNAc(S-3) , and hence 1→3 linkage between β-Gal(S-4) and β-GlcNAc(S-3) was confirmed. The large coupling constant of β-Gal(S-4) of J=8.0 Hz confirmed the β-glycosidic linkage between β-Gal (S-4) and β-GlcNAc(S-3). Since in the TOCSY spectrum of acetylated Theriose, β-Gal (S-4), the anomeric proton signal at δ 4.39 gave a cross peak at δ 3.75 in the linkage region, showing that only one –OH was available for glycosidic linkage in S-4, which was further confirmed as H-2 of β-Gal (S-4) by COSY spectrum of Theriose acetate in $CDCl_3$. This shows that H-2 of β-Gal (S-4) was available for glycosidic linkage. Another anomeric proton doublet which appeared at δ 4.39 with a singlet of amide methyl (-$NHCOCH_3$) at δ 1.85 in the ^1H NMR spectrum of Theriose in D_2O was assigned for β-GlcNAc(S-5), which was glycosidically linked to H-2 of S-4. The anomeric proton signal at δ 4.75 in the ^1H NMR of Theriose acetate in $CDCl_3$ for GlcNAc S-5 gave a cross peak at δ 3.84 in the TOCSY spectrum of acetylated Theriose showing availability of only one –OH group, which was confirmed as H-4 of β-GlcNAc(S-5) by the COSY spectrum of Theriose acetate, showing that H-4 of it was available for glycosidic linkage. The next anomeric proton signal which appeared as a doublet at δ 4.30 in the ^1H NMR of Theriose in D_2O was assigned to Gal (S-6), which was glycosidically linked to H-4 of S-5. The large coupling constant of anomeric of Gal S-6 J=8.0 Hz confirmed a β glycosidic linkage between S-6 and S-5 at position of H-4 of S-5. Since none of methine proton of β-Gal (S-6) anomeric proton signal at δ 4.53 came in the linkage region in the TOCSY spectrum of Theriose acetate in $CDCl_3$, showed that it was present at the non-reducing end, and hence β-Gal (S-6) was linked with β-GlcNAc(S-5) by 1→4 linkage. All the ^1HNMR assignments for ring proton of monosaccharide units of Theriose were confirmed by HOMOCOSY and TOCSY experiments.

THERIOSE



The positions of glycosidic linkages in the oligosaccharide were confirmed by position of anomeric signals, S.R.G and comparing the signals of ^{1}H and ^{13}C NMR of acetylated and deacetylated Theriose. The glycosidic linkages in Theriose were also confirmed by the cross peaks for glycosidically linked carbons with their protons in the HSQC spectrum of acetylated Theriose. The values of these cross peaks appeared as β-Glc(S-1) H-4 x C-4 at δ 3.90 x 70.0 showed (1→4)linkage between S-2 and S-1, β-Gal(S-2) H-3 x C-3 at δ 3.51 x 82.0 showed (1→3) linkage between S-3 and S-2, β-GlcNAc(S-3) H-3 x C-3 at δ 3.70 x 71.0 showed (1→3) linkage between S-4 and S-3, β-Gal(S-2) H-2 x C-2 at δ 3.84 x 69.0 showed (1→2) linkage between S-7 and S-2, β-Gal(S-4) H-2 x C-2 at δ 3.75 x 69.0 showed(1→2)

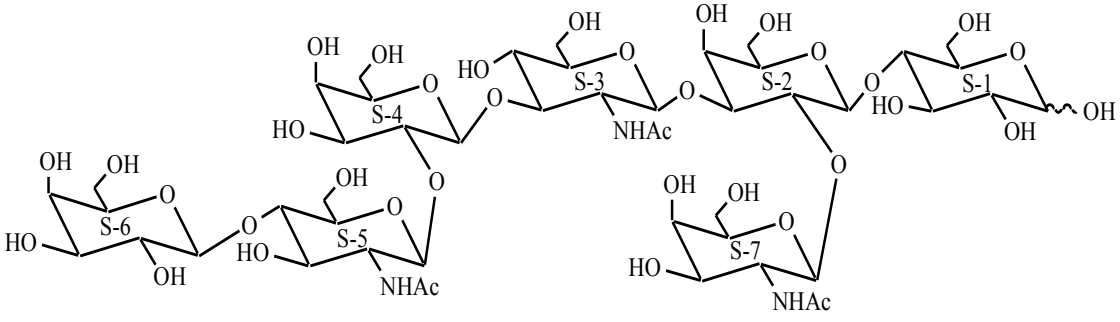
linkage between S-5 and S-4, β-GlcNAc(S-5) H-4 and C-4 at δ 3.84 x 76.0 showed (1→4) linkage between S-6 and S-5. All signals obtained in ^{1}H and ^{13}C NMR of compound Theriose was in conformity by 2D ^{1}H-^{1}H COSY, TOCSY and HSQC experiments. Thus based on the pattern of chemical shifts of ^{1}H, ^{13}C, COSY, TOCSY and HSQC NMR experiments it was interpreted that the compound 'B' Theriose was a heptasaccharide having the following structure as-

β-Gal (1→4) β-GlcNAc(1→2) β-Gal(1→3) β-GlcNAc(1→3)β-Gal(1→4) β-Glc (1→2)

β-GalNAc

The Electronspray Mass Spectrometry data of Theriose not only confirmed the derived structure but also supported the sequence of monosaccharide in Theriose. The highest mass ion peaks were recorded at m/z 1337 assigned to $[M+Na+K]^{+}$ and m/z 1298 assigned to $[M+Na]^{+}$, it also contain the molecular ion peak at m/z 1275 confirming the molecular weight as 1275 which was in agreement with its molecular formula $C_{48}H_{81}O_{36}N_3$. The mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water. The heptasaccharide m/z 1275 (I) fragmented to give mass ion at m/z 1113 (II) [1275-(S-6)], this fragment was raised due to the loss of 162 [β-Glc (S-6)] moiety from heptasaccharide unit. After this, it (II) fragmented to give mass ion peak at m/z 910(III) [1113-(S-5)] which was due to the loss of 203 [β-GlcNAc (S-5)] moiety from hexasaccharide. This pentasaccharide of m/z 910 is then fragmented to give mass ion peak at m/z 748 (IV) [910-(S-4)] which was a tetrasaccharide(IV), arised due to loss of 162 [β-Gal (S-4)] moiety. This tetrasaccharide unit fragmented to give mass ion peak at m/z 545(V) [748-(S-3)], which was due to loss of 203 [β-GlcNAc (S-3)] moiety from tetrasaccharide, which on further fragmentation resulted in m/z 342 (VI) [545- (S-7)] of β-GalNAc (S-7). This disaccharide fragmented to give monosaccharide m/z 180 [342- (S-2)] which was due to loss of 162 [β-Gal (S-2)].

Based on result obtained from chemical degradation , chemical transformation , Electron spray mass spectrometry and ^{1}H , ^{13}C ,NMR , and 2D NMR technique (COSY , TOCSY , AND HSQC) of Theriose acetate and theiose , the structure and sequence of monosaccharides in isolated novel oligosaccharide Theriose was deduced as –



CONCLUSION

In summary the novel milk oligosaccharide named as Theriose (Compound B) was isolated from buffalo milk and its structure was elucidated with the help of ^{1}H, ^{13}C, 2D NMR spectrometry and mass spectrometry as above.

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