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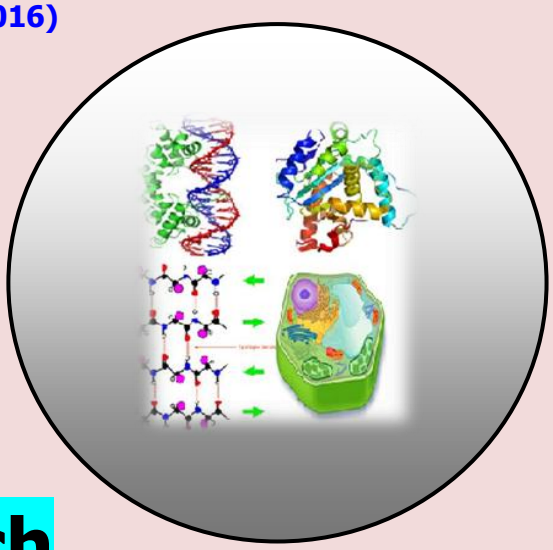

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RESEARCH PAPER

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Isolation and 2D-NMR Studies of Aliose - A Novel Hexasaccharide from Donkey's Milk

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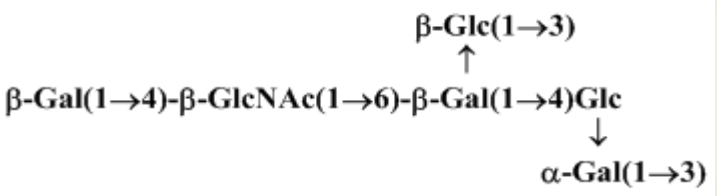
ABSTRACT

One of the most striking features of milk is the diversity and abundance of complex glycans that include free milk oligosaccharides, glycoproteins, glycopeptides and glycolipids. Milk oligosaccharides are indigestible to the infant and play multiple roles in the health of the neonate by stimulating growth of selected beneficial bacteria in the gut, participating in development of the brain, enhancing intestinal epithelial barrier function, exerting potent biological activities such as anti-tumor, immunological, anti-complimentary, anti-cancer, anti-inflammatory, anti-coagulant, hypoglycemic and antipathogenic activities. Donkey milk oligosaccharides have ability to stimulate specific and non-specific immunological resistance and prevention of athereosclerosis. In continuation to our previous studies and keeping above mentioned biological activities of donkey's milk oligosaccharides in mind we have isolated a novel hexasaccharide namely Aliose from Donkey's milk and elucidated its structure by chemical degradation and spectroscopic techniques (like ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, HSQC and Mass). The structure of Aliose was established by comparing the chemical shift (^{1}H NMR and ^{13}C NMR) of anomeric signals and other important signals of isolated milk oligosaccharide with the chemical shifts of known milk oligosaccharides and 2D-NMR and mass of Aliose and structure was deduced as follows-



Keywords: Donkey milk, Oligosaccharides and Aliose.

INTRODUCTION

The enormous biological activities of oligosaccharides such as immunostimulant, anti-tumour, anti-cancer, anti-inflammatory, anti-complementary (Saksena et al., 1999), antiviral, antimicrobial(Yang et al., 2012), antioxidant, hypoglycemic activity, lipid lowering (Halas et al., 2012) and regulation of mineral absorption are reported in medicinal literature(Singh et al., 2017). Milk oligosaccharides consist of mainly five monosaccharide building blocks: glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc) and sialic acid. The oligosaccharides isolated from various milk sources are categorized in two classes i.e. sialylated oligosaccharide and non-sialylated oligosaccharide. Both classes of oligosaccharides have been tested for their varied biological activities(Ranjan et al., 2016). The importance of donkey milk for alimentary and cosmetic

purposes has been popular since Egyptian antiquity, because donkey milk effaces wrinkles in the face, renders the skin more delicate, and preserves its whiteness. Donkey milk is recommended for healing infection and wounds, liver ailments, poisoning, fever, nose bleed and for clearing of edema from the body. The oligosaccharide mixture of Donkey's milk has shown significant stimulation of antibody, delayed type hypersensitivity response to sheep red blood cells in BALB/c mice (Ranjan et al., 2016). Keeping in mind, biological and medicinal importance of Donkey's milk oligosaccharides, Donkey's milk was collected and processed by method of Kobata and Ginsburg (Kobata et al., 1970) yielding oligosaccharide mixture. This oligosaccharide mixture on acetylation and further purification yielded a novel hexasaccharidenamely Aliose. The structure was determined by comparing the chemical shift (^{1}H and ^{13}C NMR) data of anomeric signals and other important signals of isolated milk oligosaccharide with the chemical shifts and anomeric signals of known milk oligosaccharides. In this present study analogies between chemical shifts of certain 'structural reporter group resonances' were used to make proton resonance assignments as well as structural assignments of the oligosaccharide. All chemical shifts of anomeric proton signals of milk oligosaccharide were further confirmed by 2D (^{1}H-^{1}H HOMOCOSY, TOCSY and HSQC) NMR experiments which were earlier assigned with the help of ^{1}H and ^{13}C NMR data. Other techniques like deacetylation, methylation, acid hydrolysis, chemical degradation and electrospray mass spectrometry were also helpful in the elucidation of the structure of hexasaccharide.

MATERIAL AND METHODS

General procedures

Same as described in our previous articles(Singh et al., 2015).

Isolation of donkey milk oligosaccharides

Donkey's milk (12 L) was collected from a domestic donkey and was stored at -20^{0}C. The milk was processed by the method of Kobata et al(Kobata et al., 1969). It was centrifuged for 15 min at 5000 rpm at -4^{0}C. The solidified lipid layer was removed by filtration through glass wool column in cold. Ethanol was added to the clear filtrate to a final concentration of 68% and the resulting solution was left overnight at 0^{0}C. The white precipitate formed, mainly of lactose and protein was removed by centrifugation and washed twice with 68% ethanol at 0^{0}C. The supernatant and washings were combined and filtered through a micro filter (0.24 mm) (to remove remaining lactose) and lyophilized affording crude oligosaccharide mixture (120 g). The lyophilized material responded positively to Morgan-Elson test (Partridge et al., 1948) and thiobarbituric-acid (Warren et al., 1959) assay suggesting the presence of N-acetyl sugars and sialic acid in oligosaccharide mixture. This lyophilized material (mixture of oligosaccharide) was further purified by fractionating it on Sephadex G-25 chromatography using glass triple distilled water as eluent at a flow rate of 5 mi/mm. Each fraction was analyzed by phenol-sulphuric acid reagent (Dubois et al., 1956) for the presence of neutral sugar.

Donkey milk oligosaccharide mixture chromatographed over Sephadex G-25 (1.6 × 40 cm) column

The repeated gel filtration was performed by Sephadex G-25 chromatography of crude donkey milk oligosaccharide mixture. The oligosaccharide mixture (24.2 g) was packed in a column (1.6 × 40 cm) (void volume = 25 mL) equilibrated with glass triple distilled water (TDW) and left for 10-12 h to settle down (19.7 g). The material was applied onto a Sephadex G-25 column and was eluted for separation of protein and glycoprotein from oligosaccharide mixture (low molecular weight component). Presence of neutral sugars was monitored in all eluted fractions by phenol-sulphuric acid test. The sephadex G-25 chromatography of donkey milk oligosaccharide mixture which was monitored by UV spectrophotometry showed five peaks i.e. I, II, III, IV and V. A substantial amount of proteins, glycoproteins and serum albumin were eluted in the void volume which was confirmed by positive colouration with p-dimethylaminobenzaldehyde reagent (Bryant et al., 1953) and phenol-sulphuric acid reagent. Fractions under peaks II, III and IV gave a positive phenol-sulphuric acid test which showed the presence of oligosaccharide mixture in donkey milk. They were pooled together and lyophilized.

Acetylation of oligosaccharide mixture

The pooled fraction (II, III and IV) (12.37 g) which gave positive phenol-sulphuric acid test was acetylated with pyridine (12 mL) and acetic anhydride (12 mL) at 60^{0}C and the solution was stirred overnight. The mixture was evaporated under reduced pressure and the viscous residue was taken in $CHCl_3$ (250 mL) and washed in sequence with 2 N HCl (1 × 25 mL), ice cold 2 N $NaHCO_3$ (2 × 25 mL) and finally with H_2O (2 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness yielding the acetylated mixture (14.71g). The acetylation converted the oligosaccharides into their non-polar acetyl derivatives which were resolved very nicely on TLC.

Purification of acetylated milk oligosaccharide mixture on silica gel column

Separation of the acetylated products (14.71 g) was carried over silica gel using varying proportions of C_6H_{12}:$CHCl_3$, $CHCl_3$ and $CHCl_3$:CH_3OH mixture which was resolved into eleven fractions. Repeated column chromatography of fraction IV led to the isolation of one chromatographically pure compound Aliose(102 mg).

Deacetylation of compoundAliose

The compound Aliose (24 mg) obtained from column chromatography of acetylated oligosaccharide mixture was dissolved in acetone (2 mL) and NH_3 (2 mL) was added and left overnight in a stoppered hydrolysis flask. Ammonia was removed under reduced pressure and the product was washed with $CHCl_3$ (3 × 3 mL) (to remove acetamide) and was finally freeze dried giving the deacetylated oligosaccharide (16.5 mg).

Methylglycosidation/Acid hydrolysis of compound Aliose

The compound Aliose (10 mg) was ref1uxed with absolute MeOH (2 ml) at 70°C for 20 h in the presence of cation exchange !R-l40 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To a solution of methylglycoside of Aliose in 1, 4-dioxane (1 ml), 0.1 N H_2S0_4 (1 ml) was added and the solution was warmed for 45 minutes at 50^0C. The hydrolysis was complete after 26 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, GlcNAc. Their identification was confirmed by comparison with authentic samples (TLC, PC).

Kiliani hydrolysis of compound Aliose

The compound Aliose (5 mg) was dissolved in 2 ml Kiliani mixture (AcOH-H_2O-HCl, 9:13:3) 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 on PC.

Description of isolated compound Aliose

Elemental analysis

Calcd. %C 44.22, %H 6.30, %N 1.35 and found %C 44.21, %H 6.29, %N 1.35.

The molecular formula of compound Aliose was $C_{38}H_{65}NO_{31.}$ For experimental analysis, this compound was dried over P_2O_5 at 100^0 C and 0.1 mm pressure for 8 hr.

The presence of sugar units in compound Aliose have been confirmed by NMR and Mass spectrometry.

^{1}H NMR of Acetylated Aliose in $CDCl_3$

6.25 [d, 1H, J=3.6Hz, α-Glc (S_1), H-1], 5.65 [d, 1H, J=7.8Hz, β-Glc (S'_1), H-1], 5.38 [d, 1H, J=3.0Hz, α-Gal (S_5), H-1], 4.72 [d, 1H, J=7.8Hz, β-Glc (S_6), H-1], 4.517 [d, 1H, J=7.2Hz, β-GlcNAc (S_3), H-1], 4.491 [d, 1H, J=7.2Hz, β-Gal (S_4), H-1], 4.479 [d, 1H, J=7.2Hz, β-Gal (S_2), H-1], 4.07 [H-3, β-Gal (S_4)], 3.93 [H-3, Glc, H-3], 3.86 [Glc, H-4], 2.094 [s, 3H, $NHCOCH_3$ (S_3)].

^{13}C NMR of Acetylated Aliosein $CDCl_3$

89.20 [α-Glc (S_1), C-1],90.60 [α-Gal (S_5), C-1], 91.53 [β-Glc (S'_1), C-1], 95.21 [β-Glc (S_6), C-1] 100.94 [β-Gal (S_2), C-1], 104.20 [β-GlcNAc (S_3), C-1], 104.20 [β-Gal (S_4), C-1].

^{1}H NMR of Aliose in D_2O

5.34 [d, 1H, J=3.6Hz, α-Glc (S_1), H-1], 4.65 [d, 1H, J=7.8Hz, β-Glc (S'_1), H-1], 5.315 [d, 1H, J=3.0Hz, α-Gal (S_5), H-1], 4.616 [d, 1H, J=7.8Hz, β-Glc (S_6), H-1], 4.529 [d, 1H, J=7.2Hz, β-GlcNAc (S_3), H-1], 4.522 [d, 1H, J=7.2Hz, β-Gal (S_4), H-1], 4.522 [d, 1H, J=7.2Hz, β-Gal (S_2), H-1].

^{13}CNMR of AloseD_2O

89.00 [α-Glc (S_1), C-1], 90.01 [β-Glc (S'_1), C-1], 90.01 [α-Gal (S_5), C-1], 94.60 [β-Glc (S_6)C-1], 100.30 [β-Gal (S_2), C-1], 103.01 [β-GlcNAc (S_3), C-1], 103.01 [β-Gal (S_4), C-1].

Mass spectral fragments of compound Aliose

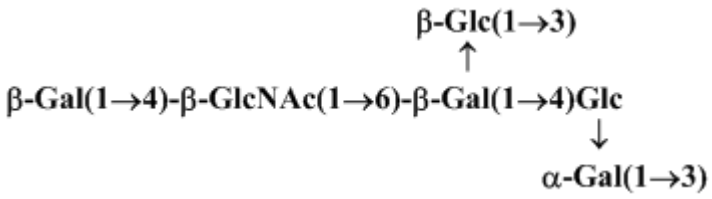
ES-MS m/z: 1070 $[M+K]^+$, 1032 $[M+H]^+$, 995, 973, 925, 869, 827, 792, 785, 767, 732, 709, 707, 676, 659, 609, 599, 573,504 , 462, 445, 402, 385, 384, 342, 180 ,163.

RESULT AND DISCUSSION

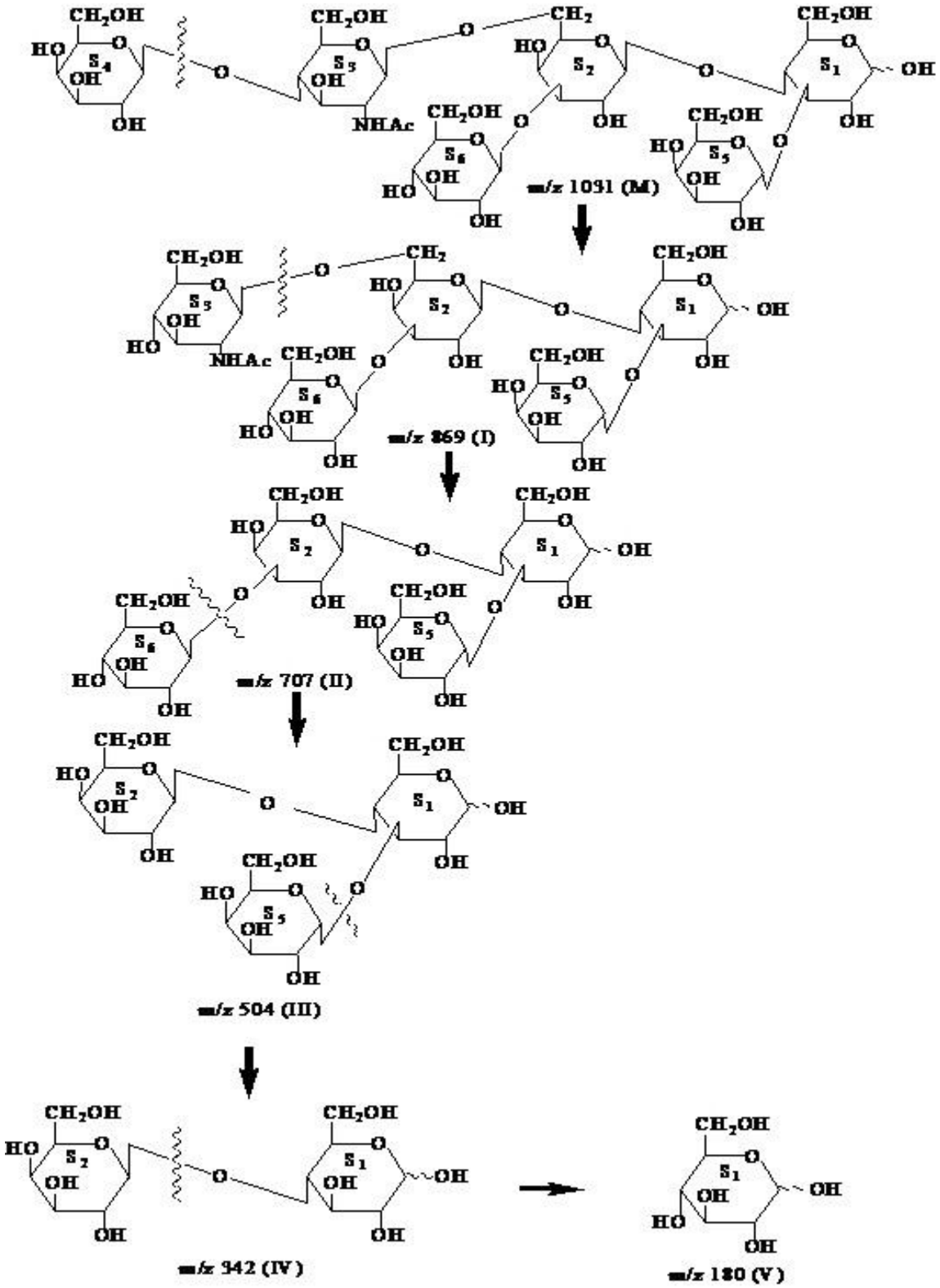
Compound **Aliose**, $C_{38}H_{65}NO_{31}$,$[\alpha]_D$ +22.66 gave positive Phenol-sulphuric acid test, Feigl test, Morgon-Elson test showing the presence of normal and amino sugar(s) in the compound Aliose. The HSQC spectrum of acetylated compound in $CDCl_3$ at 300 MHz exhibited seven cross peaks for six anomeric proton and carbon

signals at δ6.25x89.20, δ5.65x91.53, δ5.38x90.6, δ4.72x95.21, δ4.51x104.20, δ4.49x104.20 and δ4.47x100.94 indicating that the Aliose may be a hexasaccharide in its reducing form giving signals for α and βanomers of glucose in its reducing end. The hexasaccharide nature of acetylated compound Aliose was further confirmed by the presence of seven anomeric carbon and proton at δ89.20 (1C), δ90.60 (1C), δ91.53 (1C), δ95.21 (1C), δ100.94 (1C) and δ104.20 (2C) in ^{13}C NMR spectrum of acetylated aliose in $CDCl_3$ and δ6.25 (1H), δ5.65 (1H), δ5.38 (1H), δ4.72 (1H), δ4.51 (1H), δ4.49 (1H) and δ4.47 (1H) in ^{1}H NMR spectrum of acetylated aliose in $CDCl_3$, respectively. Methylglycosidation of Aliose by $MeOH/H^+$ followed by its acid hydrolysis led to isolation of α and β-methyl glucoside, which confirmed the presence of glucose at the reducing end of the oligosaccharide. It was further confirmed by the presence of two anomeric proton signals at δ5.34 and δ4.65 for α- and β-Glc in ^{1}H NMR of Aliose in D_2O. The six monosaccharides present in compound have been designated as S_1, S_2, S_3, S_4, S_5 and S_6 for convenience starting from reducing end. To confirm the monosaccharide constituents in compound Aliose, it was hydrolysed under strong acidic conditions, in Killiani hydrolysis under strong acid condition, it gave three monosaccharides i.e. glucose, galactose and N-acetylglucosamine, confirming that the hexasaccharide was consist of three types of monosaccharide units i.e. glucose, galactose and N-acetyl-glucosamine. Since the glucose was present in its reducing form which was supported by ^{1}H NMR of Aliose in D_2O which contains two anomeric proton signals for α- and β-Glc at δ5.34 (J=3.0Hz) and at δ4.65 (J=7.8 Hz)(Gronberg et al., 1990 and Chaturvedi et al., 1988). The anomeric proton present at δ5.65 i.e. β-Glc (S_1) contain three cross peaks at δ5.65x4.99, δ5.65x3.88 and δ5.65x3.64 in the TOCSY spectrum of aliose acetate out of which the cross peaks present at δ5.65x3.88 and δ5.65x3.64 were assigned for glycosidic linkage which were later identified for H-3 and H-4 of the reducing glucose by the COSY spectrum of aliose acetate confirming that H-3 and H-4 of reducing glucose were available for glycosidic linkage by the next monosaccharides. Further the presence of another anomeric proton doublet signal at δ4.47 (J=7.2 Hz) was due to presence of β-Gal (S_2) moietyin the Aliose. β-Glc (S_1') H-2 signal appeared as a triplet at δ 3.62 in the upfield region, indicated that both the equatorially oriented hydroxyl groups at C-3 and C-4 of the reducing β-Glc (S_1) were substituted and are involved in glycosidation, suggested the presence of a lactosyl moiety i.e. β-Gal(1→4)Glc with a substitution on 3-position of Glc(S_1)(SRG)(Chaturvedi et al., 1990). The coupling constant of anomeric signal with J value of 7.2 Hz shows the β-configuration of anomeric linkage between $S_2 \rightarrow S_1$. The next anomeric proton doublet which appeared at δ5.38 was due to presence of α-Gal (S_5) moiety. The position of anomeric proton of α-Gal (S_5) at δ5.38, chemical shift of H-5 of S-5 at δ4.097 along with H-2 triplet of S_1 at 3.62also supported the (1→3) glycosidic linkage between $S_5 \rightarrow S_1$ and the presence of α-Gal (S_5) moiety at non-reducing end. The splitting pattern of anomeric signal of α-Gal (S_5) with J value of 3.0Hz shows the α-configuration of anomeric linkage at $S_5 \rightarrow S_1$.It was further confirmed by the upfield shifted values of H-3 of S_1 at δ3.88 and H-5 of S_5 at δ 4.10 in Aliose acetate. The anomeric proton present at δ4.47 i.e. β-Gal (S_2) contain three cross peaks at δ4.47x4.22, δ4.47x3.80 and δ4.47x4.02 in the TOCSY spectrum of aliose acetate out of which the cross peaks present at δ4.47x3.80 and δ4.47x4.02 were assigned for glycosidic linkage which were later identified for H-3 and H-6 of β-Gal (S_2) by the COSY spectrum of aliose acetate confirming that H-3 and H-6 of β-Gal (S_2) were available for glycosidic linkage by the next monosaccharides. Further the presence of another anomeric proton doublet at δ4.51 (J=7.8Hz) along with amide methyl signal at δ2.02 was due to the presence of β-GlcNAc(S_3) moiety. The position of anomeric proton resonance at δ4.51 suggested βGlcNAc(S_3) may be (1→6) linked to β-Gal (S_2) (SRG). The coupling constant of anomeric signals with J value of 7.8 Hz shows the β configuration of anomeric linkage among $S_3 \rightarrow S_2$. It was further confirmed by the presence of H-6 and C-6 resonance of β-Gal (S_2) of aliose acetate at δ4.02 and 76.19 with upfield shifted H-4 value at δ4.097in ^{1}H and ^{13}C NMR of aliose. Presence of another anomeric proton doublet at δ4.72 (J=7.8Hz) was due to the presence of β-Glc (S_6) moiety. The position of anomeric proton at δ4.72 suggested that β-Glc (S_6) may be (1→3) linked to β-Gal (S_2). It was also supported by downfield shifted H-4 at δ4.11 which confirmed that 3 and 6 position of β-Gal (S_2) were involved in glycosidation since the 6 position of (S_2) was already involved in linkage then it was confirmed that β-Glc (S_6) was (1→3) linked to β-Gal(S_2) . The splitting pattern of anomeric signal with J value of 7.8 Hz shows the β configuration of anomeric linkage. It was further confirmed by the H-3 and C-3 resonances of Aliose acetate at δ3.80 and δ73.87.The anomeric proton present at δ4.51 i.e. β-GlcNAc (S_3) contain three cross peaks at δ4.51x4.12, δ4.51x4.93 and δ4.51x3.85 in the TOCSY spectrum of aliose acetate out of which the cross peak present at δ4.51x3.85 was assigned for glycosidic linkage which was later identified for H-4 of β-GlcNAc (S_3) by the COSY spectrum of aliose acetate confirming that H-4 ofβ-GlcNAc (S_3) was available for glycosidic linkage by the next monosaccharide.

Further another anomeric proton signal appeared at δ4.49 (J=7.2Hz) was due to presence of a β-Gal moiety (S_4). The linkage between β-Gal (S_4) and β-GlcNAc (S_3) was confirmed by the chemical shift value of this β-Gal moiety (S_4) (SRG). The splitting pattern of anomeric signal with J value of 7.2Hz shows the β-configuration of anomeric linkage between $S_4 \rightarrow S_3$. This was further confirmed by assignments of the positions of H-4 and C-4 proton of β-GlcNAc (S_3) at δ3.853 and δ72.94 which implies that C-4 of β-GlcNAc(S_3) were involved in the glycosidation in Aliose. The hexasaccharide nature of compound was further confirmed by the 2D-NMR spectral studies of Aliose acetate. The heteronuclear single quantum coherence (HSQC) spectrum of Aliose acetate confirmed the linkages in ^{1}H and ^{13}C NMR spectra by showing cross peaks of α-Glc (S_1) H-3 and C-3 at δ3.868 x 76.25 showed (1→3) linkage of S_5 and S_1 and also its H-4 and C-4 at δ3.68 x 73.45 shows (1→4) linkage of S_2 and S_1, β-Glc (S_1) H-3 and C-3 at δ3.886 x δ77.20 showed (1→3) linkage of S_5 and S_1, β-Gal (S_2) H-3 and C-3, H-6 and C-6 at δ3.800 x δ73.87 and δ4.027 x δ76.19 showed (1→3) & (1→6) linkages of $S_6 \rightarrow S_2$ and $S_3 \rightarrow S_2$ respectively. β-GlcNAc (S_3) H-4 and C-4 at δ3.853 x δ72.94 showed (1→4) linkage of S_4 and S_3 showing in the same chemical region in acetylated and deacetylated spectra. It was further confirmed by the presence of same peaks in COSY and TOCSY spectrum. Thus, based on the pattern of chemical shifts of ^{1}H NMR , ^{13}C NMR, HOMOCOSY, TOCSY and HSQC NMR experiments it was interpreted that the compound was ahexasaccharide having structure as-



The Electrospray Mass Spectrometry data of Aliose not only confirmed the derived structure but also supported the sequence of monosaccharides in Aliose. The highest mass ion peak was recorded at m/z 1070 which was due to $[M+K]^+$, further mass ion peaks at m/z 1032 for $[M+H]^+$ and $[M]^+$ conformed the molecular weight of compound was 1031. Further the mass fragments were formed by repeated H- transfer in the oligosaccharide and was accompained by the elimination of terminal sugar less water. The fragmentation pathway confirmed the sequence of monosaccharides in the oligosaccharide. The hexasaccharide m/z 1031 on fragmentation gave pentasaccharide at m/z 869 (I), which was due to loss of S-4 Sugar unit i.e. Gal (S-4) Sugar unit linked to the S-3 of hexasaccharide. It was supported by its respective fragment at m/z 180 that confirmed the presence of Gal (S-4) at non reducing end. The pentasccharide m/z 869 (I) further fragmented to mass ion peak at m/z 707 (II), which was due to loss of GlcNAc (S-3) Sugar unit i.e. GlcNAc (S-3), its corresponding tetrasaccharide (II) moiety of pentasaccharide i.e. Glc-NAc (S-3) Sugar unit linked to the S-2 of pentasaccharide. The tetrasaccharides m/z 707 (II) on fragmentation gave trisaccharide at m/z 504 (III) which was due to loss of S-6 Sugar unit i.e. Glc (S-6) Sugar unit is linked to the S-2 of tetrasaccharide unit. The trisaccharide m/z 504 (III) on fragmentation gave mass ion peak at m/z 342 (IV) which was due to loss of Gal (S-5) Sugar unit i.e. Gal (S-5) Sugar unit linked to the S-1 of trisaccharide unit. This disaccharide m/z 342 on further fragmentation gave a mass ion peak at m/z 180 (V), which was due to loss of Gal(S-2) Sugar unit i.e. Gal (S-2) Sugar unit linked to the S-1 of disaccharide. The ES-mass of compound Aliose also showed other mass ion peaks at m/z 995 [M - $2H_2O$], 973 [M - $NHCOCH_3$], 925 [973 - CH_2OH - OH]. The mass ion at m/z 1031 fragmented by the loss of other terminal Sugar S-6 163 gave the corresponding pentasaccharide mass ion fragment (I) at m/z 869 which confirmed that two Gal moieties were present at two non reducing ends of the hexasaccharide moiety other mass and fragments corresponds to the moiety m/z 869 appeared at m/z 827 [869 - CH_2CO], 792 [827 - H_2O - OH], 732 [792 - CH_2OHCHO], 785 [827 - CH_2CO], 767[785-H_2O], 709[767-$NHCOCH_3$]. The mass ion peak at m/z 869 further fragmented to give mass ion fragment for tetrasaccharide moiety which arised by the loss of Sugar S-3 other mass ion fragment corresponds to the moiety m/z 707 appeared at m/z 676[707-CH_2OH], 659[676-OH], 599[659-CH_2OHCHO], 609[676-CH_2OH-$2H_2O$], 573[609-$2H_2O$]. The tetrasaccharide mass ion fragment on further fragmentation gave an important trisaccharide fragment at m/z 504.



ES-MS Fragmentation of compound Aliose

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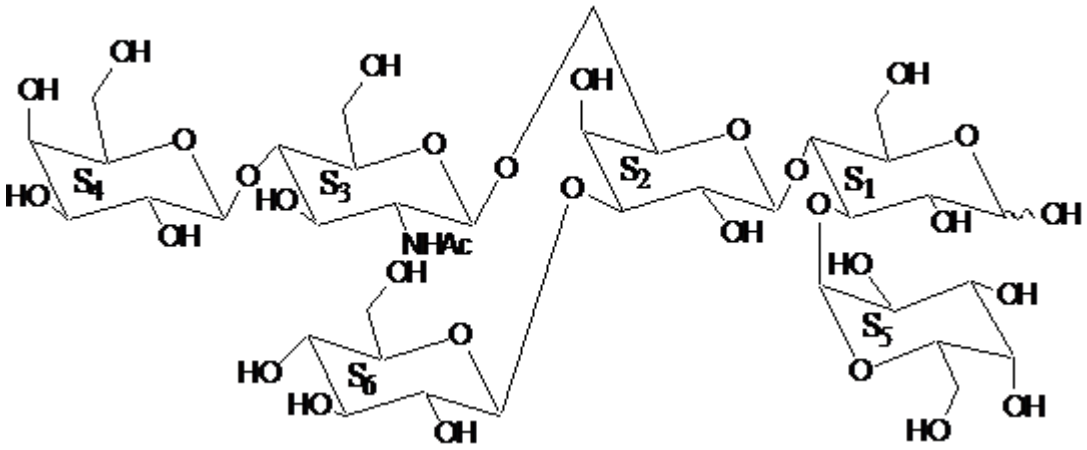
The trisaccharide fragment at m/z 504 on further fragmentation gave a disaccharide fragment at m/z 342 and other mass ion fragment at m/z 462[504-CH_2CO], 445[462-OH], 402[462-CH_2OHCHO], 384[402-H_2O], 385[445-CH_2OHCHO]. The disaccharide fragment at m/z 342 on further fragmentation gave a mass ion peak at m/z 180(V), it was supported by m/z at 163 (180-OH).

Based on the results obtained from chemical degradation/acid hydrolysis, chemical transformation, Electro spray mass spectrometry and 1D-NMR viz.^{1}H NMR, ^{13}C NMR and2D-NMR viz. COSY, TOCSY and HSQC NMR spectra of Aliose acetate and Aliose, the structure and sequence of isolated novelhexasaccharideAliose was deduced as-



Compound Aliose

CONCLUSION

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

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