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SEGREGATION AND STRUCTURAL INTERPRETATION OF NOVEL TETRASACHHARIDES "ISOSE" BY NMR AND MASS SPECROSCOPY

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ABSTRACT: *Carbohydrate are abundantly found in milk and precursor for many organic molecules like amino acids, fats etc. Milk is principal source of nutrition in infant. It is full of bioactive molecules, which act as anti-viral, anti-bacterial, anti-inflammatory, anti-cancer, etc. In traditional medine, "Aurveda and Unani System" of medicine it bother hiccup and dyspnoea, it rises 'pitta and kapha' and decreases body fat. Keeping in mind these biological activity, Gaddi sheep's milk is processed by Kobata and Ginsberg method after that gel filtration, HPLC and column chromatography, which result isolation of novel oligosaccharide named Isose. The structure of isolated and purified Isose was decoded with the help of chemical degradation, chemical transformation and different spectroscopic methods like- NMR (1H, 13C, COSY, TOCSY, HMBC, HSQC etc.), and Mass spectroscopy. The structure of isolated tetrasaccharide "Isose" is explained as-*

$$\beta GlcNAc(1\rightarrow6)\beta GalNAc(1\rightarrow4)\beta GalNAc(1\rightarrow4)Glc$$

KEYWORDS: *Oligosaccharide, HPLC, NMR and Mass Spectrocopy*

INTRODUCTION

Carbohydrates are abundantly found in milk. Milk is the primary source of nutrition for infant mammals, before they are able to digest other types of food, it becomes the primary source of nutrition. This is a white liquid food produced by the mammary glands of mammals. Mother supplies her antibodies to its young once through early lactation milk which contains colostrum this reduces the risk of many disease. Protein and lactose also constitute as major content of milk. It is common practice to consume interspecies milk particularly in human beings. Many of the young once thus suck milk of other mammals. Evidence suggests that milk oligosaccharides are anti-adhesive antimicrobials that serve as soluble decoy receptors, prevent pathogen attachment to infant mucosal surfaces and lower the risk for viral, bacterial and protozoan parasite infections. Modulation of epithelial and immune cell responses are done by milk oligosaccharides that reduces excessive mucosal leukocyte infiltration and activation, lower the risk for necrotizing enterocolitis and provide the infant with sialic acid as a potentially essential nutrient for brain development and cognition. According to 'Ayurveda and Unani' system of medicine, the sheep's milk has various medicinal importance, aggravates hiccup and dyspnoea, elevates pitta and kapha and decreases fat. Studies in traditional medicine suggests that it is a cure against tuberculosis and also helps in the enhancement of platelets count during dengue. Keeping in mind these biological activities, Gaddi sheep's milk was collected and processed by Modified method of Kobata and Ginsburg and then it was purified by Sephadex G-25 Gel column. The acetylation of oligosaccharides mixture followed by the silica gel chromatography led to isolation of a novel oligosaccharide Isose, this gave positive chemical test for normal and amino sugars. While the data was analyzed by various chemical analysis confirmed the position of linkage in oligosaccharide which is further confirmed by different spectral methods (like NMR and Mass spectroscopy).

MATERIAL AND METHODS:

2.1. GENERAL PROCEDURE

2.11. Optical rotations were measured with a PERKIN-ELMER 241 automatic polarimeter in 1cm tube. 1H and 13C NMR spectra of oligosaccharides were recorded in D2O and the spectra of acetylated oligosaccharides were recorded in CDCl3 at 250C on a Bruker AM 300 FT NMR spectrometer. The electrospray mass spectra were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. The sample (dissolved in suitable solvents such as methanol/acetonitrile/water) was 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 50% 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 ethyl acetate-pyridine (2:1) saturated with H2O. Sephadex G –25 (PHARMACIA) was used in gel permeation chromatography. Freeze drying of the compound was done with the help of CT 60e (HETO) lyophylizer 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 diode array detector and G.P. 100 printer plotter. Authentic samples of glucosamine, galactosamine, galactose and glucose were purchased from Aldrich Chemicals.

2.12. Isolation of Compounds by Kobata and Ginsburg Method- 10L Gaddi Sheep milk was collected from a sheep from high altitude region and was stored at -20^{0}C. The milk was processed by the method of Kobata and Ginsburg. 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 (162 g). 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 min/mm. Each fraction was analysed by phenol sulphuric acid reagent for the presence of neutral sugar.

2.2. Isolation and Purification of Isose

Isose (182.00mg) obtained from fraction 47-55 of column chromatography -9. On deacetylation of 24mg of acetylated compound e with NH_3/ acetone it afforded Isose (21mg) as a viscous mass, $[\alpha]_D$ +42.41^0(c, 2, H_2O).

2.3. Deacetylation of Isose

Isose (24.0mg) obtained from column chromatography 2 of acetylated oligosaccharide mixture was dissolved in acetone (2ml) and 3 ml of NH_3 was added and left overnight in a stoppered hydrolysis flask. After 24h ammonia was removed under reduced pressure and the compound was washed with (3 x 5ml) $CHCl_3$ (to remove acetamide) and the water layer was finally freeze dried giving the deacetylated oligosaccharide Isose (21.0mg).

2.31. Methylglycosidation/Acid hydrolysis of Isose–Isose (5mg) was ref1uxed with absolute MeOH (2ml) at 70°C for 18h 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 E in 1,4-dioxane (1ml), 0.1 N H_2SO_4 (1ml) was added and the solution was warmed for 30 minutes at 50^{0}C and solution was left over night. The hydrolysis was complete after 24h. The hydrolysate were neutralized with freshly prepared $BaCO_3$ filtered and concentrated under reduced pressure to afford α-and β-methylglucosides along with the Glc, GalNAc, GlcNAc. Their identification was confirmed by comparison with authentic samples (TLC, PC).

2.32. Killani hydrolysis of Isose: Isose (3mg) was dissolved in 2ml Kiliani mixture (AcOH-H_2O-HCI, 7:11:2) and heated at 100^{0}C for 1h followed by evaporation under reduced pressure. It was dissolved in 2ml of H_2O and extracted twice with 3ml $CHCl_3$. 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, GalNAc and GlcNAc on comparison with authentic samples of glucose, GalNAc and GlcNAc.

2.4. Description of Isose

Isose (182.00mg) obtained from column chromatography. On deacetylation of 24mg of acetylated Isose with NH_3/ acetone it afforded 21.0 mg Isose was obtained as a viscous mass, $[\alpha]_D$ +42.41^0(c, 2, H_2O).

For experimental analysis, this compound was dried over P_2O_5 at 100^0 C and 0.1 mm pressure for 8 hr.

$C_{30}H_{51}N_3O_{21}$	%C	%H	%N	%O
Calculated	45.63	6.51	5.32	42.54
Practically observed	45.61	6.50	5.30	42.53

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

The presence of specific sugar unit in Oviasose is further confirmed by NMR and Mass spectroscopy.

2.41. In D_2O: ^{1}H NMR values of Isose: δ5.191 [d, 1H, J=3.8Hz, αGlc (S_1), H-1], δ4.632 [d, 1H, J=7.8Hz, βGlc (S_1'), H-1], δ4.489 [d, 1H, J=7.8Hz, βGlcNAc (S_4), H-1], δ4.420 [d, 1H, J=7.8Hz, βGalNAc (S_2) & βGalNAc (S_3), H-1], δ3.857 [t, 1H, J=6.1Hz, βGlcNAc (S_4), H-2], δ3.210 [t, 1H, J=5.8Hz, βGlc (S_1'), H-2], δ2.052 [s, 6H, βGalNAc (S_2) & βGalNAc (S_3), NHCO$\underline{CH_3}$] and δ2.044 [s, 3H, βGlcNAc (S_4) NHCO$\underline{CH_3}$].

2.42. In D_2O: ^{13}C NMR values of Isose: δ171.47 [βGalNAc (S_2) & βGalNAc (S_3) NH$\underline{CO}CH_3$], δ169.92 [βGlcNAc (S_4) NH$\underline{CO}CH_3$] δ102.00 [βGlcNAc (S_4) C-1], δ101.60 [βGalNAc (S_2) & βGalNAc (S_3) C-1], δ89.90 [βGlc (S_1'), C-1], δ88.20 [αGlc (S_1) C-1], δ20.81 [βGalNAc (S_3) NHCO$\underline{CH_3}$], δ20.61 [βGalNAc (S_2) NHCO$\underline{CH_3}$] and δ20.37 [βGlcNAc (S_4) NHCO$\underline{CH_3}$].

2.43. In $CDCl_3$: ^{1}H NMR values of Acetylated Isose: δ6.261[d, 1H, J=3.8Hz, αGlc (S_1), H-1], δ5.660 [d, 1H, J=7.8Hz, βGlc (S_1'), H-1], δ4.503 [d, 1H, J=7.8Hz, βGlcNAc (S_4), H-1], δ4.499 [d, 1H, J=7.8Hz, βGalNAc (S_2) H-1], δ4.439 [d, 1H, J=7.8Hz, βGalNAc (S_3) H-1 δ2.052 [s, 6H, βGalNAc (S_2) & βGalNAc (S_3), NHCO$\underline{CH_3}$] and δ2.044 [s, 3H, βGlcNAc (S_4) NHCO$\underline{CH_3}$]

2.44. In $CDCl_3$: ^{13}C NMR values of Acetylated Isose: δ171.47 [βGalNAc (S_2) & βGalNAc (S_3) NH$\underline{CO}CH_3$], δ169.92 [βGlcNAc (S_4) NH$\underline{CO}CH_3$] δ104.34 [βGalNAc (S_2) & βGalNAc (S_3) C-1], δ104.20 [βGlcNAc (S_4) C-1], δ91.54 [βGlc (S_1'), C-1], δ89.20 [αGlc (S_1) C-1], δ20.81 [βGalNAc (S_3) NHCO$\underline{CH_3}$], δ20.61 [βGalNAc (S_2) NHCO$\underline{CH_3}$] and δ20.37 [βGlcNAc (S_4) NHCO$\underline{CH_3}$].

2.45. ES mass of Isose: m/z 850 , m/z 790 , m/z 586, m/z 387, m/z 180, m/z 714 , m/z 758, m/z 716 , m/z 597, m/z 789, m/z 528 , m/z 461, m/z 430 , m/z 372, m/z 510, m/z 468, m/z 449, m/z 390, m/z 372 m/z 352, m/z 310, m/z 271, m/z 330, m/z 294, m/z 276, m/z 211, m/z 162 and m/z 144.

RESULT AND DISCUSSION

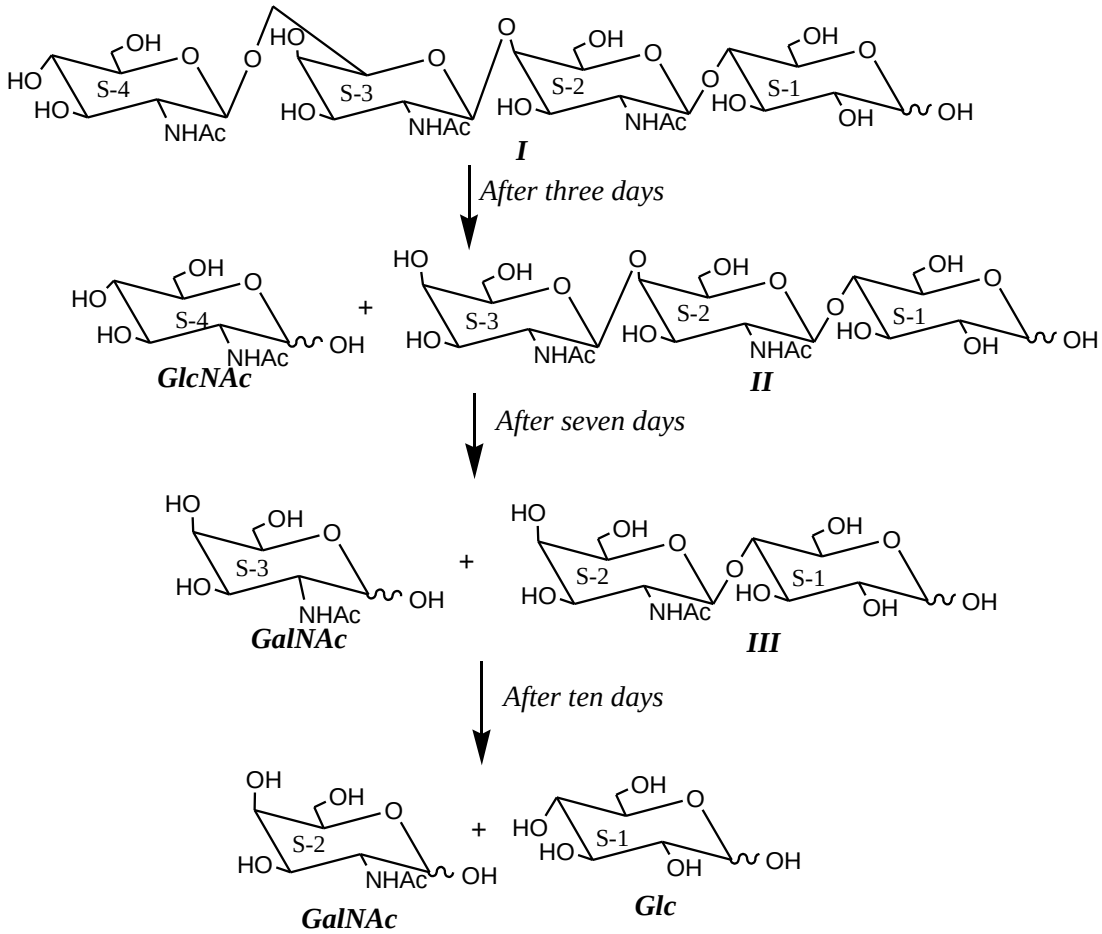
Isose , $C_{30}H_{51}N_3O_{21}$, $[\alpha]_D$ +42.41, gave positive Phenol- sulphuric acid test, Feigl test, Morgon-Elson test showing the presence of normal and amino sugar(s) in the compound. The ^{1}H NMR spectrum of acetylated compound at 300 MHz exhibited five cross peaks for four anomeric proton signals at δ 6.261 x 89.20, δ 5.660 x 91.54, δ 4.999 x 104.20, δ 4.439 x 104.34, and x 104.34 indicating that the Isose may be a tetrasaccharide, in its reducing form giving signals for α and β anomers of glucose in its reducing end. The tetrasaccharide nature of acetlated compound Isose was further confirmed by the presence of five-anomeric carbon and proton at δ 89.20(1C), δ 91.54(1C),δ 104.20(1C), and δ 104.34(2C), in ^{13}C NMR and δ 6.261(1H), δ 5.660(1H), δ 4.503(1H), δ 4.499(1H) and δ 4.439(1H) in ^{1}H NMR, respectively. Methylglycosidation of Isose 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 δ 5.191 and δ 4.632 for α- and β-Glc in ^{1}H NMR of Isose in D_2O. The four monosaccharides present in compound have been designated as S_1, S_2, S_3 and S_4 for convenience starting from reducing end. To confirm the monosaccharide constituents in compound, it was hydrolysed under strong acidic conditions. In Killiani hydrolysis under strong acid condition, it gave three monosaccharides i.e. glucose, N-acetylgalactosamine and N-acetylglucosamine, confirming that the tetrasaccharide is consist of three types of monosaccharide units i.e. glucose, N-acetylgalactosamine and N-acetyl-glucosamine. To confirm the

monosaccharide constituents and their sequence in Isose, it was hydrolysed under mild acidic conditions (Mannich-Siewert method) followed by paper chromatography and TLC. In this hydrolysis after three days paper chromatogram showed three spots, mobility of one spot was identical in mobility with authentic sample of GlcNAc, and the other spot with the lowest mobility was identical with unreacted compound E (I), further the compound with the intermediate mobility may be the Trisaccharide(II). Further after seven days two new spots were observed of which one was identical in mobility with authentic sample of GalNAc and other with lower mobility may be the disaccharide (III) which was having faster mobility with trisaccharide (II).The hydrolysis was partially completed in ten days and showing a new spot, which was found identical with authentic sample of Glc showing that after hydrolysis of disaccharide (III) the spot of Glc observed and spot of GalNAc was merged with already existing spot of GalNAc observed earlier. The hydrolysis was completed in fifteen days and showing three spots, which were found identical with authentic sample of GlcNAc ,GalNAc and Glc on TLC and PC may be confirm in that the sequence of monosaccharide in tetrasaccharide as-

GlcNAc-GalNAc-GalNAc-Glc.

The hydrolyzates were isolated for these compounds as GlcNAc, GalNAc and Glc and it was compared with authentic sample GlcNAc, GalNAc and Glc on paper chromatography. The results obtained from this type of acid hydrolysis confirmed that the sequence of monosaccharide in this tetrasaccharide was-

GlcNAc-GalNAc-GalNAc-Glc.



Scheme -1 Mannich-Siewert Hydrolysis of Isose (Compound E)

Since the glucose was present in its reducing form which was supported by ^{1}H NMR of Isose which contains two anomeric proton signals for α- and β-Glc at δ 5.191 (J=3.9) and at δ 4.632 (J=7.8). Further the presence of another anomeric proton doublet signal at δ 4.420(J=7.8) along with signal of amide methyl

group at δ 1.964, was due to presence of β-GalNAc moiety in the Isose. β-Glc (S_1) H-2 signal as a triplet at δ3.210 in the ^{1}H NMR of Isose showed that the equatorial orientation of hydroxyl group at C-4 of the reducing β-Glc (S_1') was substituted and involved in glycosidation, suggested the presence of β-GalNAc(1→4)Glc moiety at reducing end[116-135]. The splitting pattern of anomeric signal with J value of δ7.8 shows the β-configuration of anomeric linkage at S_2→S_1. It was further supported by the presence of β-Glc H-4 proton resonance at δ3.967 in acetylated derivative of Isose. Another anomeric proton signal, which appeared at δ4.420(J= 7.8) along with signal of amide methyl group at δ 1.964 was due to presence of β-GalNAc (S_3) moiety. The coupling constant of anomeric signal with J value of δ7.8 shows the β-configuration of anomeric linkage at S_3→S_2. The presence of H-4 and C-4 resonances of β-GalNAc (S_2) of Isose acetate, at δ 3.954 and δ 76.58 confirm that β-GalNAc (S_3) was (1→4) linked to β-GalNAc (S_2), respectively. Further the presence of another anomeric signal at δ 4.489(J=7.8) along with signal of amide methyl group at δ 1.877 was due to presence of β-GlcNAc (S_4) moiety. The position of anomeric proton at δ 4.489(J=7.8) confirmed that β-GlcNAc (S_4) (1→6) linked to β-GalNAc (S_3) (SRG). The splitting pattern of anomeric signal with J value of δ7.8 shows the β-configuration of anomeric linkage at S_4→S_3. It was also confirmed by the presence of H-6 and C-6 resonances of β-GalNAc (S_3) of Isose acetate, at δ 3.961 and δ 77.00 respectively. This was confirmed on the basis of assignments made by 2D NMR spectra of acetylated compound.

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

Moieties	**^{1}H NMR**	**^{13}C NMR**	**Coupling Constt.(J)**
α-Glc (S_1)	5.191	88.20	3.8
β-Glc (S'_1)	4.632	89.90	7.8
β-GalNAc (S_2)	4.420	101.60	7.8
β-GalNAc (S_3)	4.420	101.60	7.8
β-GlcNAc (S_4)	4.489	102.00	7.8

Table 2: ^{1}H NMR values of acetylated Isose in $CDCl_3$

Moieties	**H-1**	**H-2**	**H-3**	**H-4**	**H-5**	**H-6**	**-CH_3**
α - Glc (S_1)	6.261	5.0532	5.416	3.967			
β - Glc (S'_1)	5.660	5.130	5.365	3.872			
β - GalNAc (S_2)	4.499	3.854	5.080	3.954			2.052
β - GalNAc (S_3)	4.439	3.806	5.053	4.400	5.212	3.961	2.052
β - GlcNAc (S_4)	4.503	3.857	5.285	4.518			2.044

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

Table 3: ^{13}C NMR values of acetylated Isose in $CDCl_3$

Moieties	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**	**-$CONH_2$**	**-CH_3**
α - Glc (S_1)	89.20	62.79	63.00	77.43				
β - Glc (S'_1)	91.54	61.85	63.29	77.21				
β - GalNAc (S_2)	104.34	68.83	68.15	76.58			171.47	20.61
β - GalNAc (S_3)	104.34	62.79	68.98	62.00	61.85	77.00	171.47	20.81
β - GlcNAc (S_4)	104.20	68.94	65.00	66.98			169.92	20.37

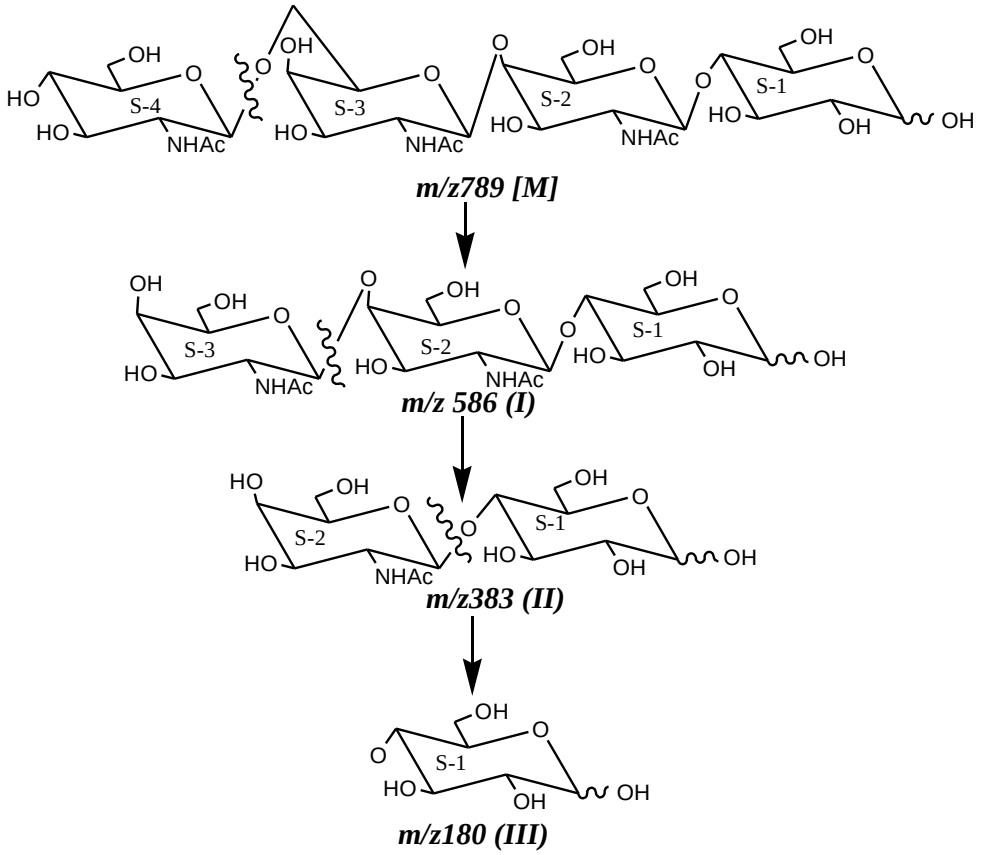
The glycosidic linkages were assigned by the cross peaks for glycosidically linked carbons with their protons in HSQC spectrum of Isose acetate. The values of these cross peaks were as α-Glc (S_1) H-4 and C-4 at (δ3.967 x 77.43) showed (1→4) linkage of S_2 and S_1, β-Glc (S_1) H-4 and C-4 at (δ3.872 x 77.21) showed (1→4) linkage of S_2 and S_1 ,β-GalNAc (S_2) H-4 and C-4 at (δ3.954 x 76.58) shows (1→4) linkage of $S_3 \rightarrow S_2$. β-GalNAc(S_3) H-6 and C-6 at (δ3.961 x 77.00) shows (1→6) linkage of S_4 and S_3. The tetrasaccharide nature of compound was further confirmed by the spectral studies of acetylated derivative of compound. Ring hydrogens involved in linkage at δ3.872 (4-position) for $S_1 \rightarrow S_2$, δ3.954(4-position) for $S_2 \rightarrow S_3$, δ3.961(6-position) for $S_3 \rightarrow S_4$, showing same chemical shift in acetylated and deacetylated spectra. These studies were made on the basis of HOMOCOSY, TOCSY and HSQC connectivities. It was further confirmed by the presence of same peaks in COSY and TOCSY spectrum.

Based on the pattern of chemical shifts of ^1H , ^{13}C, HOMOCOSY, TOCSY and HSQC NMR experiments it was interpreted that the compound was tetrasaccharide having structure as-

βGlcNAc(1→6)βGalNAc(1→4)βGalNAc(1→4)Glc

The result obtained from the ES mass spectrum further substantiated the structure of Isose which was derived by its ^1H and ^{13}C NMR. Spectra. The highest mass ion peak were recorded m/z 850 which was due to $[M+Na+K]^+$.Other mass ion peak recorded at m/z 790 was due to $[M+H]^+$, confirmed that the molecular weight of compound was 789. 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 tetrasaccharide (scheme: 2 & 3).

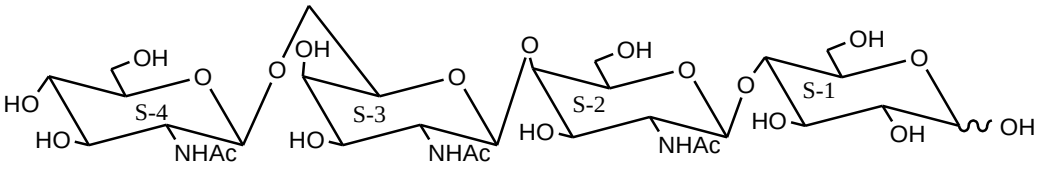
The result obtained from ES mass spectrum further sustained the structure of Isose which was derived by its ^1H and ^{13}C NMR spectra. The highest mass ion peak was recorded at m/z 850 for [M+Na+K] and other was at m/z 790 [M+H], further confirmed the molecular weight of compound was 789. Further the 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 tetrasaccharide (scheme 3). The tetrasaccharide on fragmentation gave a mass ion peak at m/z 586(I), which was due to loss S-4 sugar unit i.e. GlcNAc (S-4) sugar unit linked to the S-3 of tetrasaccharide unit. The trisaccharide (I), on fragmentation gave a mass ion peak at m/z 383(II), which was due to loss S-3 sugar unit i.e. GalNAc (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(III), which was due to loss of S-2 sugar unit i.e. GalNAc (S-2) sugar unit linked to the S-1 of disaccharide. The other supporting mass fragments obtained at m/z 714 (789- $NHCOCH_3$, OH), m/z 758 (789-CH_2OH), m/z 716 (758-CH_2CO), m/z 597 (758- CH_2OHCHO, $NHCOCH_3$, H_2O), confirmed the tetrasaccharide nature of compound. The tetrasaccharide m/z 789 on fragmentation gave trisaccharide m/z 586 (M-S_4), which was further confirmed by its other fragments ions at m/z 528 (586-$NHCOCH_3$), m/z 461 (528-CH_2OH,$2H_2O$), m/z 430 (461-CH_2OH), m/z 372 (430-$NHCOCH_3$), m/z 510 (586-CH_2CO,$NHCOCH_3$), m/z 468 (510-CH_2CO), m/z 449 (468-H_2O), m/z 390 (449-CH_2OH,$2H_2O$) and m/z 372 (390-H_2O). The trisaccharide m/z 586 on fragmentation gave disaccharide m/z 383, which was further confirmed by its other fragments ions at m/z 352 (383-CH_2OH), m/z 310 (352- CH_2CO), m/z 271 (310-CH_2OH), m/z 330 (387-$2H_2O$,OH), m/z 294 (330-$2H_2O$), m/z 276 (294- H_2O),and m/z 211 (276-CH_2OH,H_2O,OH and 271-CH_2OHCHO). The disaccharide m/z 347 on fragmentation gave monosaccharide m/z 180, it was supported by m/z 162 (180-H_2O) and m/z 144 (162-H_2O).



Scheme : 2-ES Mass Fragmentation of Compound E (Isose)

Based on the results obtained from chemical degradation chemical transformation, mass spectrometry and ^{1}H , ^{13}C, HOMOCOSY, TOCSY , HSQC NMR, the structure of the isolated novel tetrasaccharide, Isose was deduced as-

βGlcNAc(1→6)βGalNAc(1→4)βGalNAc(1→4)Glc



CONCLUSION

From the above discussion, we conclude that the structure of isolated from Gaddi sheep milk is novel oligosaccharide named as Isose (tetrasaccharide). 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, 2DNMR (COSY, TOCSY and HSQC) spectroscopy and mass spectroscopy.

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