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"GADOSE" ISOLATION AND STRUCTURE ELUCIDATION OF NOVEL OCTASACCHARIDE FROM GADDI SHEEP'S MILK

By

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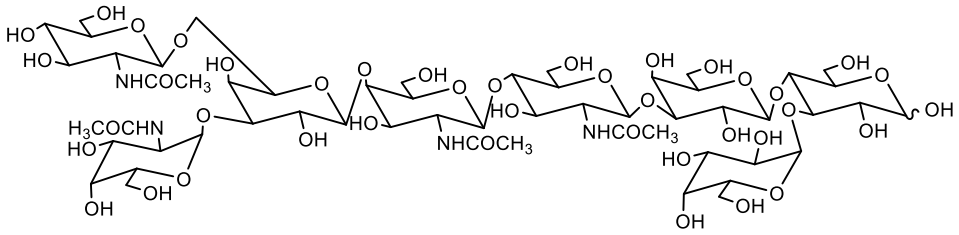
ABSTRACT

Milk is an essential white fluid in glycobiology which contains number of important nutrients like: carbohydrate, protein, fats etc. Carbohydrate present in milk is either in free form or as glyco-conjugates. Milk is one of the rich sources of biologically active oligosaccharides in new born which protect from various disease. Milk is full of various biological activities such as antitumor, anti-inflammatory, anti-cancer, antimicrobial activities. To discovered more novel biologically active milk oligosaccharides, Gaddi Sheep milk was collected and processed by Modern Kobata and Ginsburg method proceed by gel filtration and column chromatography which resulted in the isolation of novel milk oligosaccharide namely Gadose. The structure of isolated novel oligosaccharide 'Gadose" as an Octasaccharide was elucidated with the help of HPLC, NMR (1H, 13C, 2D NMR: COSY, TOCSY and HSQC) techniques and mass spectrometry and further confirmed by chemical degradation. The structure of Gadose is-



KEYWORDS: Gaddi's Sheep Milk, Silica Gel Chromatography, Advanced Spectroscopy.

1. INTRODUCTION

Oligosaccharides are the main constituent of milk. Milk is a white liquid eatable secreted by the exocrine glands of mammals, which is the rudimentary form of nutrition for infants, before they are able to digest other complementary food. Early lactation milk also known as colostrum, is full of mother's antibodies offer to its young onesand almost reduce the chances of the varied

diseases.It enriched with number of macromolecules like protein and milk sugar. Ingestion of inter species milk is quite common, especially in humans, several of that suction the milk of alternative mammals. An aggregative body of confirmed that milk oligosaccharides are anti-adhesive antimicrobials that play as dispersible decoy receptors that shield to infant mucosal surfaces from infective agents and lower the hazards of microorganisms and protozoan parasite infections. Milk oligosaccharides would possibly regularize epithelial and immune cell respond alongside diminution of immoderate mucosal leukocyte infiltration and activation, reduces the treat for disease like necrotizing enterocolitis and supplied sialic acid that is essential nutrient for brain development and cognizance in infants. In conformity with 'Ayurveda and Unani' system of medicine, the sheep's milk has varied medicinal values aggravates hiccup and dyspnoea, elevates tyrannid and kapha and also reduces bodyfat. It’s used against tuberculosis in ancient medicinal system and jointly helps to increase of platelets count throughout dengue fever. Remembered these bioactive properties, Gaddi sheep's milk was collected and processed by Improved method of Kobata and Ginsburg and so it absolutely was purified by Sephadex G-25 Gel column. It is conjointly confirmed by HPLC. Further the acetylation of oligosaccharides mixture followed by the silica gel chromatography led to isolation of a unique oligosaccharide Gadose that gave positive chemical test for traditional and amino sugars. From the quantitative and qualitative analysis of data, confirmed the position of linkage in oligosaccharides that is re-confirmed by different spectral ways (like 1D, 2D and 3D NMR and Mass spectroscopy).

2. MATERIAL AND METHODS:

2.1. GENERAL PROCEDURE

2.12. Optical rotations were deliberate with a PERKIN-ELMER 241 automatic polarimeter in 1cm tube.1D and 2D NMR spectra of oligosaccharides were observed in D_2O whereas the spectra of acetylated oligosaccharides were noted in $CDCl_3$ at 25^0C on a Bruker AM 300 FT nuclear magnetic resonance spectrometer. The ES-MS was observed on a triple quadrupole mass spectrometer, MICROMASS QUATTRO II. A syringe pump at the speed of 5µl per min was used to introduce the sample (dissolved in specific solvents such as methanol/acetonitrile/water). The cone voltage at 40 Volt with ESI capillary was set at 3.5 KV. The spectra were recorded in 6s scans and also the print outs square measure averaged spectra of 6-8 scans. The Carbon, Hydrogen and Nitrogen analysis were observed through an elemental analyzer, CARLO-ELBA

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1108. The sugars were visualized on TLC with 50% aqueous sulphuric acid reagent and with acetyl acetone and p-dimethyl amino benzaldehyde reagents on Paper Chromatography. The absorbent used for TLC was silica gel G (SRL) while in CC silica gel (SRL, 60-120 mesh). Paper Chromatogram was performed with the help of solvent system ethyl acetate-pyridine (2:1) saturated with H_2O on Whatman No.1 filter paper. The Gel permeation chromatography was performed by Sephadex G-25 (PHARMACIA). Freeze drying of the compound was through with the assistance of CT 60e (HETO) lyophylizer and further by Remi instruments C-23 JJRCI 763, cooled centrifugation machine. To identified the homogeneity of the segregated compound by reverse phase HPLC system was used equipped with Perkin Elmer 250 solvent delivering system, 235 diode array detector and G.P. 100 printer plotter. Standard samples were purchased from Aldrich Chemicals of glucosamine, galactosamine, galactose and glucose.

2.22. 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 -20^0C. The milk was processed by the method of Kobata and Ginsburg. It was centrifuged for 15 min at 5000 rpm at -4^0C. The solidified lipid layer was removed by filtration through glass wool column in cold. Ethanol was added to the clear filtrate to a final concentration of 68% and the resulting solution was left overnight at 0^0C. The white precipitate formed, mainly of lactose and protein was removed by centrifugation and washed twice with 68% ethanol at 0^0C. The supernatant and washings were combined and filtered through a micro filter (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 analyzed by phenol sulphuric acid reagent for the presence of neutral sugar.

2.3. Isolation and Purification of Gadose

89.0 mg Gadose obtained from column chromatography. On deacetylation of 26.4mg of acetylated compound k with NH_3/acetone it afforded 20.5 mg of Gadose obtained as a viscous mass, $[\alpha]_D$ $+113.53^0$ (c, 4, H_2O).

2.4. Deacetylation of Gadose:

Gadose (26.4mg) obtained from column chromatography of acetylated oligosaccharide mixture was dissolved in acetone (2ml) and 3 ml of NH_3 was added and left overnight in a stoppered hydrolysis

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flask. After 24 h 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 Gadose (20.5mg).

2.41. Methylglycosidation/Acid hydrolysis of Gadose–

Gadose (5mg) was ref1uxed with absolute MeOH (2 ml) 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 K in 1,4-dioxane (1ml), 0.1 N H_2SO_4 (1 ml) was added and the solution was warmed for 30 minutes at 50^0C and solution was left over night. The hydrolysis was complete after 24h. 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. Their identification was confirmed by comparison with authentic samples (TLC, PC).

2.42. Killani hydrolysis of Gadose-

Gadose (3 mg) was dissolved in 2ml 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 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, galactose, GalNAc and GlcNAc on comparison with authentic samples of glucose, galactose, GalNAc and GlcNAc.

2.5. Description of Gadose:

Gadose (326.00mg) obtained from column chromatography. On deacetylation of 29.2mg of acetylated Gadose with NH_3/acetone it afforded 23.4 mg Gadose as a viscous mass, $[\alpha]_D$ $+115.39^0$ (c, 4, H_2O).

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

$C_{54}H_{91}N_3O_{42}$	%C	%H	%N	%O
Calculated	44.60	6.31	2.89	46.21
Practically Obsevered	44.65	6.34	2.90	46.25

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

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

2.51. In D_2O: ^1H NMR values of Gadose:

δ5.271 [d, 1H, J=3.6Hz, αGlc (S_1), αGalNAc (S_7) & αGal (S_8), H-1], δ4.721 [d, 1H, J=7.8Hz, βGlc (S_1'), H-1], δ4.627 [d, 1H, J=7.9Hz, βGlcNAc (S_3), H-1], δ4.600 [d, 1H, J=7.8Hz, βGlcNAc (S_6), H-1] and δ4.501 [d, 1H, J=3.8 Hz, αGal (S_2), βGalNAc (S_4) and αGal (S_5), H-1],

2.52. In D_2O: ^{13}C NMR values of Gadose:

δ171.20 [βGlcNAc (S_4) NH<u>CO</u>CH_3], δ169.10 [βGlcNAc (S_6) NH<u>CO</u>CH_3], δ168.20 [βGalNAc (S_7) $NHCOCH_3$], δ168.10 [βGlcNAc (S_3) $NHCOCH_3$], δ101.66 [βGal (S_2), βGal (S_5) & βGlcNAc (S_6) C-1], δ100.66 [βGlcNAc (S_3) C-1], δ99.10 [βGalNAc (S_4) C-1], δ91.50 [αGal (S_8) C-1], δ90.16 [αGalNAc (S_7) C-1], δ89.20 [βGlc (S_1') C-1], δ86.00 [αGlc (S_1) C-1], δ20.05 [αGalNAc (S_4) NHCO<u>CH_3</u>], δ20.03 [αGalNAc (S_7) NHCO<u>CH_3</u>] and δ20.01 [βGlcNAc (S_3) and βGlcNAc (S_6) NHCO<u>CH_3</u>].

2.53. In $CDCl_3$: ^1H NMR values of Acetylated Gadose:

δ6.220 [d, 1H, J=3.6Hz, αGlc (S_1) H-1], δ5.646 [d, 1H, J=7.8Hz, βGlc (S_1'), H-1], δ5.353 [d, 1H, J=3.9Hz, αGalNAc (S_7) H-1], δ5.298 [d,1H, J=4.2Hz, αGal (S_8), H-1], δ4.635 [d, 1H, J=8.4Hz, βGlcNAc (S_6), H-1], δ4.650[d, 1H, J=7.8Hz, βGlcNAc (S_3), H-1], δ4.571 [d, 1H, J=3.8 Hz, βGal (S_2), H-1], δ4.558 [d, 1H, J=7.9Hz, βGal (S_5), H-1], δ4.539 [d, 1H, J=3.8 Hz, βGalNAc (S_4), H-1], δ2.056 [s, 3H, βGalNAc (S_4), NHCO<u>CH_3</u>], δ2.046 [s, 3H, βGlcNAc (S_6), NHCO<u>CH_3</u>], δ2.036 [s, 3H, βGlcNAc (S_3), NHCO<u>CH_3</u>] and δ1.983 [s, 3H, αGalNAc (S_7), NHCO<u>CH_3</u>].

2.54. In $CDCl_3$: ^{13}C NMR values of Acetylated Gadose:

δ170.43 [βGalNAc (S_4) NH<u>CO</u>CH_3], δ170.05 [αGalNAc (S_7) NH<u>CO</u>CH_3], δ169.51[βGlcNAc (S_6) NH<u>CO</u>CH_3], δ169.50 [βGlcNAc (S_3) NH<u>CO</u>CH_3], δ101.88 [βGal (S_2) and βGal (S_5), C-1], δ101.68 [βGlcNAc (S_3) and βGlcNAc (S_6), C-1], δ101.10 [βGalNAc (S_4) C-1], δ92.20 [αGalNAc (S_7) C-1], δ91.56 [βGlc (S_1') C-1], δ91.20 [αGal (S_8) C-1], δ87.20 [αGlc (S_1) C-1], δ20.82 [βGalNAc (S_4)

NHCO$\underline{CH_3}$] δ20.56 [αGalNAc(S_7) NHCO$\underline{CH_3}$], δ20.43 [βGlcNAc (S_6) NHCO$\underline{CH_3}$] and δ20.33 [βGlcNAc (S_3) NHCO$\underline{CH_3}$].

2.55. ES mass of Gadose:

m/z 1501$[M+Na]^+$, m/z 1479$[M+H]^+$, m/z 1478$[M^+]$, m/z 1460, m/z 1448, m/z 1446, m/z 1416, m/z 1401, m/z 1398, m/z 1368 , m/z 1312, m/z 1275, m/z1210, m/z 1180, m/z1120, m/z1109, m/z 1102, m/z 1072, m/z 1063, m/z 1027, m/z 1010, m/z 945, m/z 910, m/z 945, m/z 884, m/z 880 , m/z 852 , m/z 824, m/z 805, m/z 802, m/z 745, m/z 742, m/z 707 , m/z 704, m/z 687, m/z 643, m/z 582, m/z 559, m/z 548 , m/z 504, m/z 499, m/z 455, m/z 422, m/z 393 , m/z 384, m/z 375 , m/z 364, m/z 357, m/z 345 , m/z 342, m/z 303, m/z 281, m/z 264, m/z 261 , m/z 222, m/z 203, m/z 183, m/z 180, m/z 156, m/z 150 and m/z 144.

3. Result and Discussion:

Gadose $C_{54}H_{91}N_3O_{42}$, $[\alpha]_D$ +115.39, gave positive Phenol-sulphuric acid test, Feigl test, Morgon-Elson test showing the presence of normal and amino sugar(s) in the compound. The HSQC spectrum of acetylated compound at 400 MHz exhibited ten cross peaks for nine anomeric proton signals at δ 6.220 x 87.20, δ5.646 x 91.56, δ4.571 x 101.88, δ4.650 x 101.68, δ4.539 x 101.10, δ4.558 x101.88, δ4.635 x 101.68, δ5.353 x 92.20 and δ5.298 x 91.20 indicating that the Gadose may be an octasaccharide in its reducing form giving signals for α and β anomers of glucose in its reducing end. Methylglycosidation of Gadose 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 also confirmed by the presence of two anomeric proton signals at δ 5.271 and δ 4.712 for α- and β-Glc in ^1H NMR of deacetylated Gadose. The octasaccharide nature of acetylated compound Gadose was further confirmed by the presence of nine anomeric carbon and proton at δ87.20 (1C), δ90.20 (1C), δ92.20 (1C), δ91.56 (1C), δ101.10 (1C) and δ101.68 (2C) and δ101.88 (2C) in ^{13}C NMR and δ6.220 (1H), δ5.646 (1H), δ4.571 (1H), δ4.650 (1H), δ4.539 (1H), δ4.558 (1H), δ4.635 (1H) δ5.353 (1H) and δ5.298 (1H) in ^1H NMR, respectively. The octasaccharide nature of Gadose was also confirmed by presence of nine anomeric proton and carbon signals at δ5.271 (3H), δ4.712 (1H), δ4.501 (3H), δ4.627 (1H), δ4.600 in ^1H NMR and δ86.00 (1C), δ89.20 (1C), δ91.50(1C), δ90.16 (1C), δ99.10 (1C) and δ100.55 (2C) and δ101.66 (2C) in ^{13}C NMR spectrum, respectively of Gadose in D_2O at 500MHz. The ^1H and ^{13}C NMR

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values of anomeric proton and ring proton with anomeric carbon and ring carbon are given in table-1, 2 and 3:

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

Moieties	^{1}H NMR	Coupling Constt.(J)	^{13}C NMR
α-Glc (S_1)	5.271	3.6	86.00
β-Glc (S'_1)	4.712	7.8	89.20
β-Gal (S_2)	4.501	7.6	101.66
β-GlcNAc (S_3)	4.627	7.9	100.50
β-GalNAc (S_4)	4.501	7.8	99.10
β-Gal (S_5)	4.501	7.8	101.66
β-GlcNAc (S_6)	4.600	7.8	101.66
α-GalNAc (S_7)	5.271	3.6	91.50
α-Gal (S_8)	5.271	3.6	90.16

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

Moieties	H-1	H-2	H-3	H-4	H-5	H-6	-CH_3
α - Glc (S_1)	6.220	5.113	4.122	3.827			
β - Glc (S'_1)	5.646	5.100	4.016	3.797			
β - Gal (S_2)	4.571	4.997	3.862	4.804	4.101	3.799	
β - GlcNAc (S_3)	4.650	3.834	4.587	4.057			2.036
β - GalNAc (S_4)	4.539	3.901	4.404	4.013			2.056
β - Gal (S_5)	4.558	5.206	3.827	4.291	4.117	4.013	
β- GlcNAc (S_6)	4.635	3.797	4.260	4.404			2.046
α - GalNAc (S_7)	5.353	3.766	4.353	5.488	4.090		1.983
α - Gal (S_8)	5.298	4.940	5.488	5.220			

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

Moieties	C-1	C-2	C-3	C-4	C-5	C-6	-$CONH_2$	-CH_3
α - Glc (S_1)	87.20	70.83	71.72	73.27				
β - Glc (S'_1)	91.56	72.47	71.75	72.66				
β - Gal (S_2)	101.88	68.76	62.05	72.48	70.85	72.29		
β - GlcNAc (S_3)	100.68	63.82	67.15	61.50			169.50	20.33
β - GalNAc (S_4)	101.10	62.40	71.20	62.29			170.43	20.82
β - Gal (S_5)	101.88	73.27	61.80	72.66	65.50	62.55		
β- GlcNAc (S_6)	101.68	63.00	68.90	73.00			169.51	20.43
α -GalNAc (S_7)	92.20	61.20	68.70	69.24			170.05	20.56
α - Gal (S_8)	91.20	66.05	68.76	64.50	63.60			

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The eight monosaccharides present in compound 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 reducing end. To confirm the monosaccharide constituents in compound, it was hydrolyzed under strong acidic conditions. In Killiani hydrolysis under strong acid condition, it gave four monosaccharides i.e. glucose, galactose, N-acetylgalactosamine and N-acetyl-glucosamine, confirming that the octasaccharide is consist of four types of monosaccharide units i.e. glucose, galactose, N-acetylgalactosamine and N-acetyl-glucosamine. Since the glucose was present in its reducing form which was supported by ^{1}H NMR of Gadose in D_2O which contains two anomeric proton signals for α- and β-Glc at δ 5.271(J=3.6) and at δ 4.712(J=7.8). Since it showed H-2 signal of β-Glc (S_1') as a triplet at δ3.32, indicated that the equatorially oriented hydroxyl groups at C-4 of the reducing β-Glc (S_1') was substituted and involved in glycosidation, suggested the presence of a lactosyl moiety i.e β-Gal (1→4) Glc.

The presence of another anomeric proton doublet signal at 4.571 was due to presence of β-Gal (S_2) moiety with J value of 7.6Hz in the acetylated Gadose. The anomeric proton value of β-Gal at δ4.501 (J=δ7.6Hz) in D_2O confirmed (1→4) linkage between β-Gal (S_2) and β-Glc (S_1'). It was also supported by the presence of β-Glc H-4 proton resonance at δ3.797 in acetylated derivative of Gadose. The next anomeric proton value of α-Gal at δ5.271 (J=δ3.6 Hz) confirmed (1→3) linkage between α-Gal (S_8) and β-Glc (S_1'). It was also supported by the presence of β-Glc H-3 proton resonance at δ4.016 in acetylated derivative of Gadose. Further the presence of next anomeric proton doublet at δ4.627(J=7.9) along with signal of amide methyl group at δ 2.036with presence of H-2 signal at δ3.834 was due to presence of β-GlcNAc moiety which is represented as S_3, which is also supported in ^{13}C NMR with C-2 at δ63.82 with δ169.50 and δ20.33 for -$CONH_2$ and -CH_3. The position of anomeric proton resonance at δ4.627 suggested that GlcNAc may be (1→3) linked (SRG) to β-Gal(S_2). The coupling constant of anomeric signal with J value of δ7.9Hz shows the β-configuration of anomeric linkage, amongst S_3→S_2. This linkage was further confirmed by the presence of H-3& C-3resonance of β-Gal (S_2), in^{1}H NMR of Gadose acetate at δ3.862 &δ 62.05, respectively. The presence of another GalNAc moiety was suggested by the presence of another anomeric proton signal S_4 at δ4.539along with signal of amide methyl group at δ 2.056with presence of H-2 and C-2 signals at δ3.901 and δ62.82, was due to presence of β-GalNAc(S_4) moiety. The position of anomeric proton resonance at δ4.539 suggested that β-GalNAc may be

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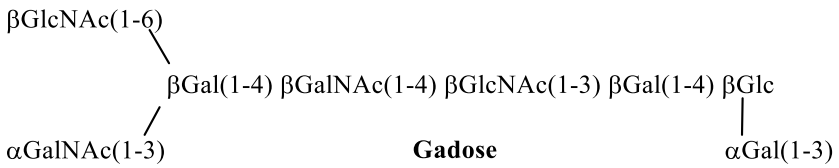
(1→4) linked to β-GlcNAc(S_3). The coupling constant of anomeric signal with J value of 7.8 Hz shows the β-configuration of anomeric linkage, between S_4→S_3. It was further confirmed by the presence of H-4& C-4 resonance of β-GlcNAc (S_3), of Gadose acetate at δ4.057&δ61.50, respectively. The next anomeric proton signal, which appeared at δ4.558(J=δ7.8 Hz) was due to presence of β-GalNAc (S_5) moiety. The (1→4) linkage between β-Gal (S_5) and β-GalNAc (S_4) is confirmed by the presence of H-4 & C-4 resonance of β-GalNAc (S_4), of Gadose acetate at δ4.013 &δ62.29 respectively. The next two anomeric proton signal, which appeared at δ4.635 (J=δ 7.8Hz) along with signal of amide methyl group at δ 2.046 and δ5.353(J=δ 3.6Hz) along with signal of amide methyl group at δ 1.983 was due to presence of β-GlcNAc (S_6) and α-GalNAc (S_7) moieties, respectively. The linkages between β-GlcNAc (S_6) and α-GalNAc (S_7) to β-Gal(S_5) was confirmed by the position of anomeric proton values of β-GlcNAc (S_6-δ4.600 & S_7-δ5.271) with downfield shifted value of H-4 and H-6 of β-Gal(S_5) (SRG). The coupling constant of anomeric signals with J value of δ7.8Hz andδ3.6 Hz shows the β- configuration and α-configuration of anomeric linkages between S_6→S_5 and S_7→S_5, respectively. It was further confirmed by the chemical shift of β-Gal(S_5) of H-3, H-6, C-3 and C-6 resonances in acetylated spectrum of Gadose which appeared at δ3.827, δ4.013, δ61.80 and δ62.55 respectively, with upfield shifted H-4 value of β-Gal(S_5) at δ4.291(SRG).

The various values of ring carbons and hydrogen are in accordance with ^{13}C and ^{1}H values of their respective monosaccharides, which also support the derived structure. These studies are analyzed on the basis of ^{1}H, ^{13}C, COSY, TOCSY, HMBC and HSQC NMR spectrum.

Based on the pattern of chemical shifts NMR experiments it was interpreted that the compound was octasaccharide having structure as-



The result obtained from the ES mass spectrum further substantiated the structure of Gadose which was derived by its ^{1}H and ^{13}C NMR spectra. The highest mass ion peak was recorded m/z 1501 which was due to $[M+Na]^+$, which confirmed that the molecular weight of compound was

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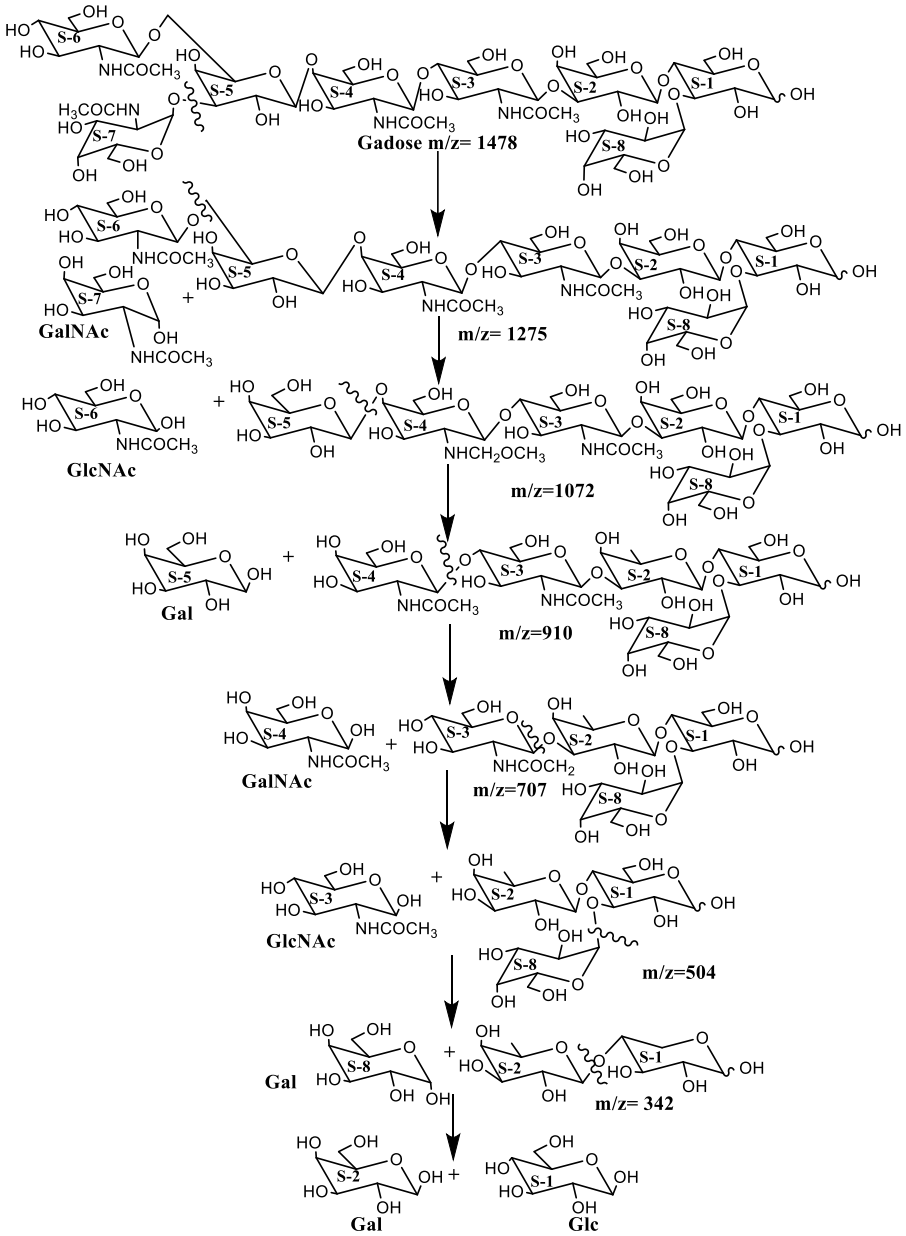
1478. Further the mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water. The fragmentation pathway confirmed the sequence of monosaccharide units in the octasaccharide. The octasaccharide fragment mass ion peak at m/z 1478 [M] on fragmentation gave the heptasaccharide mass ion peak at m/z 1275, which was obtained by the loss of S_7 sugar unit i.e. GalNAc(S_7) sugar unit linked to S_5 of the oligosaccharide, which was supported by its respective fragment at m/z 221 and m/z 239, this confirmed the presence of GalNAc (S_7) at the non-reducing end.



Mass fragmentation of Gadose

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The heptasaccharide mass ion peak fragmented to give mass ion peak at m/z 1072, which was due to the loss of S_6 sugar unit i.e. GlcNAc(S_6) sugar unit linked to S_5 of the oligosaccharide which was also supported by its complimentary fragment at m/z 221 and m/z 239, this confirmed the presence of GlcNAc (S_6) at the non-reducing end. Further the hexasaccharide mass ion fragmented by the loss of other sugar i.e. Gal (S_5), gave the corresponding pentasaccharide mass ion fragment at m/z 910. This pentasaccharide mass ion fragment on further fragmentation gave tetrasaccharide segment at m/z 707, by loss of sugar (S_4) [GalNAc]. This on further fragmentation gave a trisaccharide segment at m/z 504 by loss of sugar (S_3) [Gal]. This trisaccharide mass ion fragment on further fragmentation gave an important disaccharide segment at m/z 342, by loss of sugar (S_8) [Gal], which on further fragmentation give a monosaccharide fragment at 180 by loss of sugar (S_2) [Gal]. The octasaccharide mass ion peak at m/z 1478 in the spectrum of compound also showed other supporting mass ion peaks. The other important signals obtained at m/z 1139[M-S_1,S_8], m/z1113 [M-S_6,S_8 or M-S_7,S_8],], m/z 992[M-S_1,S_2,S_8], m/z 910 [M-S_6,S_7,S_8, or M-S_5,S_6,S_7], m/z 790[M-S_1,S_2,S_3,S_8], m/z 748[M-S_5,S_6,S_7,S_8], m/z 504[M-S_4,S_5,S_6,S_7,S_8,], m/z342 [M- S_3,S_4,S_5,S_6,S_7,S_8 or M-S_2,S_3,S_4,S_5,S_6,S_7], m/z 221[M-S_1,S_2,S_3,S_4,S_5,S_6,S_8or M-S_1,S_2,S_3,S_4,S_5,S_7,S_8]. and m/z180 [M-S_2,S_3,S_4,S_5,S_6,S_7,S_8].The other supporting mass fragments obtained at m/z 1443 [M-H_2O, OH], m/z 1416 [M-CH_3OH,HCHO], m/z 1398 [1416-H_2O], m/z 1349[1398-HCHO, OH], m/z 1300 [1349-CH_3OH, OH], m/z 1275 [1349-CH_2CO], m/z 1269 [1349- CH_3OH, HCHO, H_2O], m/z 1240 [1300-CH_2OHCHO], m/z 1234 [1300-HCHO, $2H_2O$], m/z 1209 [1269-CH_2OHCHO], confirmed the octasaccharide nature of compound. The octasaccharide m/z 1478 on fragmentation gave heptasaccharide m/z 1275 (M-S_7), which was further confirmed by its other fragments ions at m/z 1323 [1419- CH_2OHCHO, H_2O], m/z 1269 [1349- CH_3OH, HCHO, H_2O], m/z 1207 [1268-CH_2OHCHO], m/z 1113 [1207-OH, CH_3CHO, CH_3OH], m/z 1038 [1113-$NHCOCH_3$,OH] and m/z 1055 [1113 -$NHCOCH_3$]. The heptasaccharide m/z 1275 on fragmentation gave hexasaccharide m/z 1072 (1275-S_6), which was further confirmed by its other fragments ions at m/z 1032 [1072-CH_2CO] m/z 974 [1032- $NHCOCH_3$], m/z 945 [1032- CH_3CHO, HCHO, OH], m/z 925 [974-HCHO, OH], m/z 917 [974-CH_2CO, CH_3], m/z 910 [945-H_2O, OH], m/z 901 [945-CH_3CHO], m/z 858 [917- CH_2CO, OH], m/z 849 [901-OH,CH_3CHO], m/z 798 [858-CH_2OHCHO], m/z 783 [798- CH_3] and m/z 741 [798-CH_3,CH_2CO]. The hexasaccharide m/z 1072 on fragmentation gave pentasaccharide m/z 910 (1072-S_5), which was further confirmed by its other fragments ions at m/z 790 [869-HCHO,CH_2OH,H_2O], m/z 772 [790-H_2O], m/z 722 [772-H_2O,CH_3OH], m/z 742 [772-

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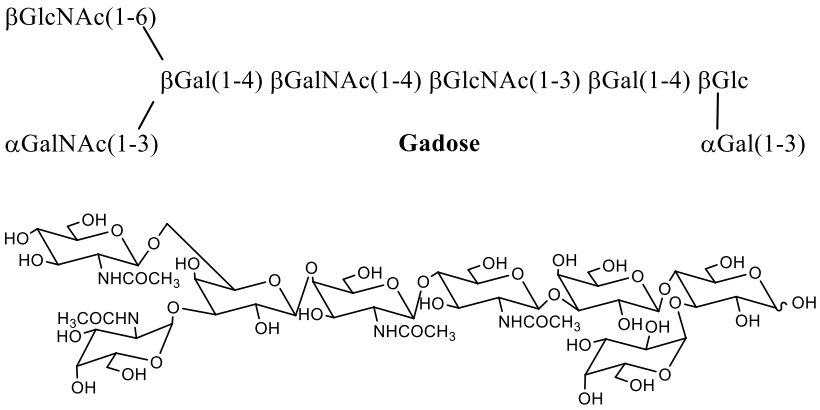
HCHO], m/z 707 [742-H_2O,OH], m/z 692 [742-H_2O,CH_3OH], m/z 650 [692-CH_2CHO], m/z 618 [650-CH_3OH], m/z 550 [618-2H_2O,CH_3OH], m/z 480 [550-2HCHO], m/z 465 [480-CH_3] and m/z 406 [465-CH2CHO,OH].The pentasaccharide m/z 910 on fragmentation gave tetrasaccharide m/z 707 (910-S_4), which was further confirmed by its other fragments ions at m/z 672 [707-H_2O,OH], m/z 640 [672-CH_3OH], m/z 620 [670-CH_3OHH_2O], m/z 590 [640-H_2O,CH_3OH], m/z 569 [620-OH,CH_3OH], m/z 512 [590-2HCHO,H_2O], m/z 512 [568-$CHCOCH_3$]and m/z 454 [512-$NHCOCH_3$].The tetrasaccharide m/z 707 on fragmentation gave trisaccharide m/z 504 (707-S_3), which was further confirmed by its other fragments ions at m/z 489 [504-CH_3], m/z 471 [504-H_2O], m/z 446 [504-HCHO, H_2O], m/z 440 [504-CH_3OH], m/z 406 [440-2OH], m/z 357 [406-H_2O] and m/z 342 [406-2CH_3OH].The trisaccharide m/z 504 on fragmentation gave disaccharide m/z 342 (504-S_8), which was further confirmed by its other fragments ions at m/z 324 [342-H_2O], m/z 300 [342- CH_2CO], m/z 292 [342- CH_3OH, H_2O], m/z261 [342-CH_3OH, H_2O, CH_2OH], m/z 202 [261-$NHCOCH_3$] and m/z 182 [342-CH_2OHCHO]. The disaccharide m/z 342 on fragmentation gave monosaccharide m/z 180 (342-S_2), which was further confirmed by its other fragments ions at m/z 162 (180-H_2O) and m/z 144 (162-H_2O).

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



4. Bio-activity of Gadose:

4.1. Gadose increase the growth of Saccharomyces *cerevisiae* by 75%, which was found in the skin, oral cavity, oropharynx, duodenal mucosa, digestive tract, and vagina of healthy humans. Saccharomyces cerevisiae is employed as a probiotic in humans and animals. It is used for prevention or treatment of several gastrointestinal diseases like traveler's diarrhea, etc.

4.2. Gadose suppressed the growth of Rhodotorula *mucilaginosa* which is most commonly found in patients who are immunosuppressed and/or are using foreign-body technology such as central venous catheters. R. *mucilaginosa* is the most frequent species causing fungemia, which is responsible for up to 79 % of infections and lethality.

5. Conclusion:

From the above discussion, we conclude that the structure of isolated from Gaddi sheep milk is novel oligosaccharide named as Gadose (Octasaccharide). 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. Different medicinal values of Gaddi Sheep's Milk are reported; Gadose also shows their unique medicinal values, which gives a milestone in pharma and health industry for preparation of skin ointments, mouth wash, vaginal cleaning gels, treatment of intestinal infections etc.

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