Journal of
Dairy & Veterinary Sciences
ISSN: 2573-2196

Research Article
Volume 16 Issue 5 - August 2024
DOI: 10.19080/JDVS.2024.16.555950

Dairy and Vet Sci J

Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR

Desh Deepak A P Singh Chauhan[1], Sarita Chauhan[1]*, Manisha Shukla[2], Anil Mishra[2] and Desh Deepak[2]

[1]*Department of Chemistry, JNM PG College, Lucknow, UP India*

[2]*Department of Chemistry, University of Lucknow, Lucknow, UP India*

Submission: July 05, 2024, **Published:** August 26, 2024

***Corresponding author**: Desh Deepak, Department of chemistry, University of Lucknow, Lucknow U.P. India Email: deshdeepakraju@rediffmail.com

Abstract

Since time immemorial the milk was used as a food supplement for the growth of neonates and humans. Recent advancements and ancient Indian literature of Ayurveda and Charak sanghita have shown that cow milk has immunostimulant brain developer and strengthening of the heart muscle properties. Later it was discovered that all the medicinal properties residing in the cow milk was due to its oligosaccharide contents. Keeping the view in mind we have collected the Rathi cow milk and processed it by modified method of Kobata and Ginsburg for obtaining its oligosaccharide mixture. It was further acetylated and purified by using different chromatographic techniques which resulted in the isolation of a novel tetrasaccharide 'Hisose'. Its structure was elucidated by chemical transformation, chemical degradation and NMR experiments like 1H, 13C, HSQC, COSY, TOCSY and HMBC along with Mass spectrometry.

Keywords: Milk, Oligosaccharides, Hisose, NMR and Mass spectrometry

Introduction

The oligosaccharide content of milk of any animal species differs due to their habitat and food they eat. In our continued studies on milk of various cow species, we have investigated the oligosaccharide constituents of Cow milk which belong to different geographical area of India. In this context we have collected the milk of various cow species which belong to arid zone of our country i.e. Rajasthan and Gujrat state of India. As we are aware that the cow milk is well defined for its medicinal properties in ancient Indian literature of Ayurveda and Charak Samhita [1]. It is defined as 'Amrata' and is beneficial for heart, bones and eyes [2]. The recent researches have shown that oligosaccharide found in the milk of cows belonging to Rajasthan state have structural diversities. They are found in the form of glycoside instead of oligosaccharides and hence they are of non-reducing nature [3]. It was also reported that the monosaccharide contents of these oligosaccharides are present in their furanose form instead of commonly reported pyranose form [4] Keeping in mind the above specialties we have collected the milk of Rathi cow from Panchmukhi district of Rajasthan state and processed it, by modified method of Kobata and Ginsburg [4] which was further purified by various chromatographic techniques which resulted in the isolation of four compounds which were investigated for their structure elucidation. In our previous searches we have reported two novel compounds; A (Rathose) [5] and B (Thisose) [6] and elucidated their structures from milk of Rathi cow. Further in our continued studies for search of more novel medicinally important oligosaccharides, we are reporting another novel tetrasaccharide in this communication. The structure of this novel tetrasaccharide named as Hisose was elucidated with the help of recent physicochemical experiments of ^{1}H NMR ^{13}C NMR and 2-Dimensional NMR experiments of COSY [8], TOCSY [9], HSQC [9] and HMBC [10] along with Mass spectrometric data along with traditional system of structure elucidation of oligosaccharides by chemical degradation and chemical transformation.

Material & Methods

General procedure were same as given in our previous communication [4].

Isolation of Rathi Cow Milk Oligosaccharides by Modified Method of Kobata and Ginsburg

The process for isolation of oligosaccharide from Rathi Cow milk by modified method of Kobata and Ginsburg was the same as

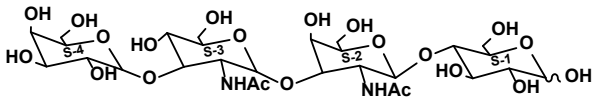
given in our earlier communication [5].

Acetylation of Oligosaccharide Mixture

Acetylation of Oligosaccharide Mixture was described as our earlier [5].

Deacetylation of Compound 'c', HisoseAcetate [5]

Compound 'c' (45 mg) was obtained from column chromatography of acetylated oligosaccharide mixture of Rathi cow milk. 40 mg of Compound 'c' was dissolved in acetone (4 ml) and 4 ml of NH_4OH was added to it and left overnight in a stopped hydrolysis flask. After 24 hrs, ammonia was removed under reduced pressure and the compound was washed thrice with $CHCl_3$ (10 ml) (to remove acetamide) and the water layer was finally freeze dried giving the deacetylated natural oligosaccharide 'C' (31 mg).



Compound C, HISOSE

Methyl Glycosidation/Acid Hydrolysis of Compound C [5]

Compound 'C' (10 mg) was refluxed with absolute MeOH (2 ml) at 70°C for 18 hr in the presence of cation exchange IR-l20 (H) resin. The rseaction mixture was filtered while hot and filtrate was concentrated. To this reaction mixture of methylglycoside C, 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°C. The hydrolysis was complete after 24 hrs. (TLC) The hydrolysate was neutralized with freshly prepared BaCO3and further filtered and concentrated under reduced pressure to afford α-and β-methylglucosides along with the Glc, Gal, GlcNHAc and GalNHAc. Identification of monosaccharides in compound 'C'was confirmed by comparison with authentic samples (TLC, PC) of α-and β-methylglucosides alonssg with the Glc, Gal,GlcNHAc and GalNHAc.

Kiliani Hydrolysis of Compound C, Hisose [5]

Compound 'C' (5 mg) was dissolved in 2 ml Kiliani mixture (AcOH-H_2O-HCI, 7:11:2) and heated at 100°C 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, GlcNHAcand GalNHAc on comparison with authentic samples of Glc, Gal, GlcNHAc and GalNHAc.

Description of compound 'C', Hisose:

Compound C (30mg) = -9.23. For experimental analysis, this compound was dried over P2O5 at 100°C and 0.1 mm pressure for 8 hr. It gave positive Phenol-sulphuric acid test [11], Feigl test [12] and Morgan-Elson test [13].

C28H48O21N2	%C	%H	%N
Calculated	44.92	6.41	3.74
Found	44.92	6.40	3.43

1.1. ^{1}H NMR of Compound 'c', Hisose Acetate in $CDCl_3$ at 300 MHz.

δ6.21[d, 1H, J=3.9Hz, α-Glc(S-1) H-1], δ5.62[d, 1H, J=8.1Hz, β-Glc(S-1) H-1], δ4.42[d, 2H, J=6.6 Hz, β-GalNHAc(S-2) &β-GlcNHAc(S-3) H-1], δ4.40[d,1H, J=3.6, α-Gal(S-4)],δ3.89[m, 1H, β-Glc(S-1) H-4], δ3.80[m, β-GalNHAc(S-2) andβ-GlcNHAc(S-3) H-3].

1.2. ^{13}C NMR of Compound c, Hisose Acetate in $CDCl_3$ at 300 MHz.

δ101.16[1C, α-Gal(S-4) C-1], δ100.90[2C, β-GalNHAc(S-2) & β-GlcNHAc(S-3) C-1], δ91.52[1C, β-Glc(S-1) C-1], δ88.94[1C, α-Glc(S-1) C-1]

1.3. ^{1}H NMR of Compound 'C', Hisose in D_2O at 300 MHz.

δ5.42[d, 1H, J=3.6Hz, α-Glc(S-1) H-1], δ5.05 [d,2H, J=3.6, α-Gal(S-4)],δ4.38[d, 1H, J=8.1Hz, β-Glc(S-1) H-1], δ4.30[d, 1H, J=6.9 Hz, β-GlcNHAc(S-2 & S-3) H-1], δ1.94[s, 6H, (NHCOCH3), β-GalNHAc(S-2)&GlcNHAc (S-3)].

ES MASS Fragment of Compound 'C', Hisose

771 [M+Na]+, 748[M]+, 712[748-2H2O], 695[712-2H2O, H+], 694[695-H+], 687[748-CHOHCHO], 654[687-2OH, H+], 653[652-H+], 608[654-CHO,OH], 586[748-(S-4)], 549[586-2H2O, H+],508[549-CHCO], 507[508-H+], 465[507-CH3OH], 407[465-NHCOCH3], 406[407-H+], 383[586-(S-3)], 366[383-OH], 365[366-H+],319[365-CHO, OH], 318[365-HCHO, H2O],303[365-2CH2OH], 180[383-(S-2)].

Discussion of Compound 'C', Hisose

Compound C, Hisose $C_{28}H_{48}O_{21}N_2$ $[\alpha]\frac{25}{D}$ = -9.23 gave positive Phenol-sulphuric acid test [10], Feigl test and [11] Morgan-Elson test [13] showing the presence of normal and amino sugars moietie (s) in the Compound C. The name of the compound Hisose was originated from the name of the animal i.e. Rathi Cow and was designated as 'C' while it's acetylated form was proposed as 'c' (Figure 1).

The HSQC spectrum of acetylated Compound 'c' showed four cross peaks for five anomeric protons and carbons in their respective region at δ6.21×88.94, δ5.62×91.52, δ4.40×101.16 and δ4.42×100.90 suggested presence of five anomeric protons and carbon in Compound 'c' in its reducing form.The reducing nature of Hisose 'C' was confirmed by its methylglycosidation MeOH/H+ followed by its acid hydrolysis [5], which resulted into isolation of

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

α and β methyl glucosides along with Gal, GalNHAc, GlcNHAcand Glc suggesting the presence of glucose at the reducing end [5], all four monosaccharides were denoted as S-1, S-2, S-3and S-4 for convenience. The monosaccharides constituents in Hisose were also confirmed by its Kiliani hydrolysisunder strong acidic condition, under observation by paper chromatography and TLC. In the hydrolysis of compound 'C' four spots were found and they were found identical in mobility with the authentic samples of Glc, Gal, GlcNHAcand GalNHAc by co-chromatography (Paper chromatography/TLC). Thus the Compound C was made up of four monosaccharides units i.e. Glc, Gal,GlcNHAc and GalNHAc (Scheme 1).

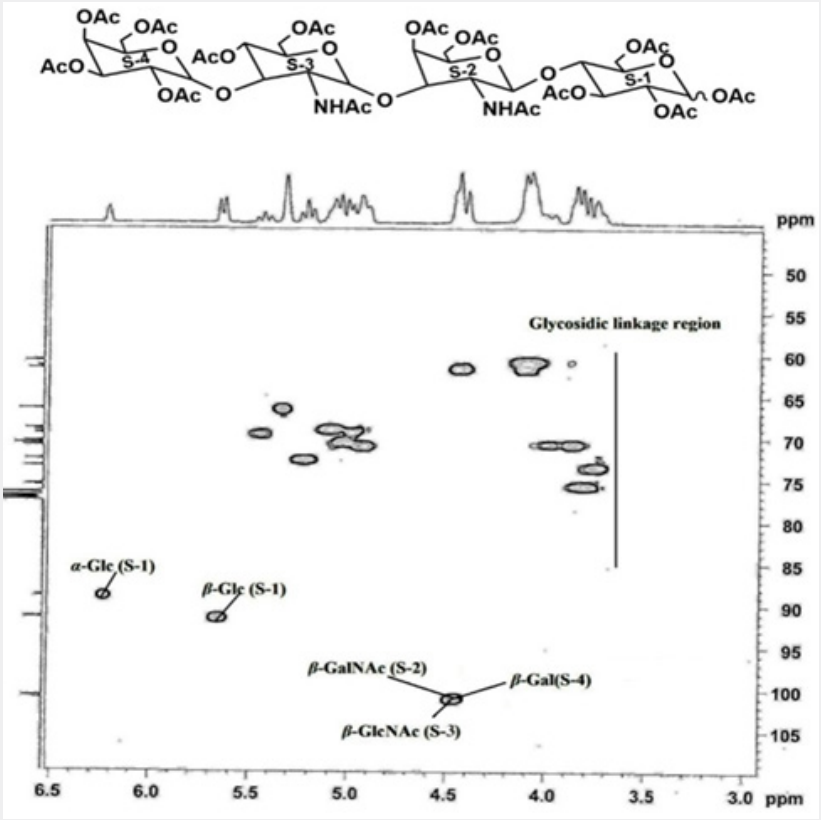


Figure 1: HSQC Spectrum of Hisose acetate in $CDCl_3$ at 300 MHz.

Further the presence of four doublets of five anomeric protons at δ6.21(1H), δ5.62(1H), δ4.42(2H) and δ4.40(1H) in the ^{1}H NMR of Hisose Acetate 'c' in $CDCl_3$ at 300 MHz. confirmed the reducing nature of tetrasaccharide Hisose (Figure 2).

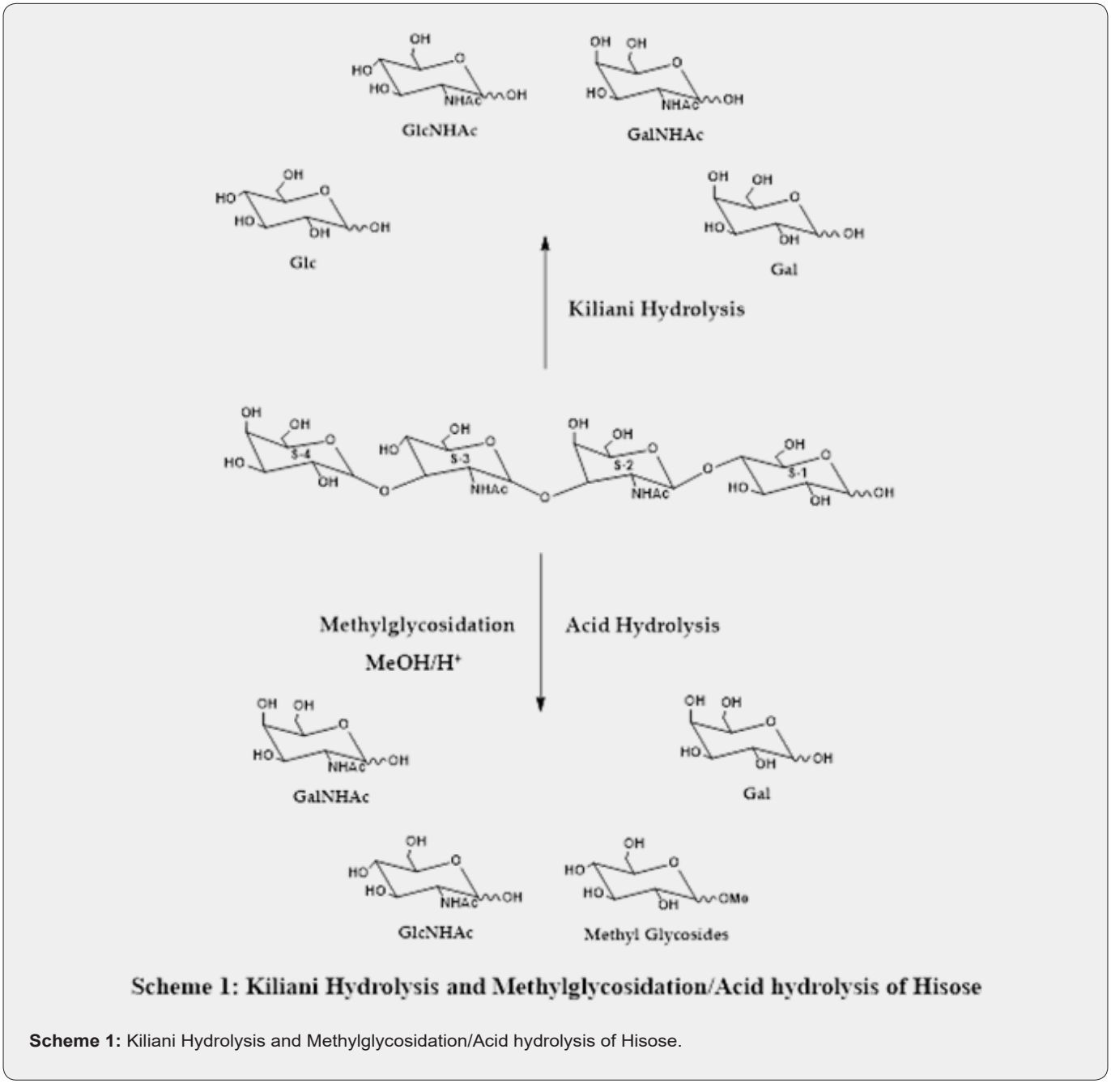
Presence of five anomeric peaks for five anomeric carbon at δ88.94 (1C), δ91.52 (1C), δ101.16 (1C) and δ100.90 (2C) in the ^{13}C NMR spectrum of acetylated Hisose 'c' in $CDCl_3$ at 300 MHz. also confirmed Hisose as a tetrasaccharide in its reducing form (Figure 3).

The tetrasaccharide nature of Hisose was further supported by the presence of four anomeric proton doublets for five anomeric protons at δ5.42 (1H), δ4.38 (1H), δ4.30 (2H) and δ5.05 (1H) in ^{1}HNMR spectrum of Hisose in D_2O at 300 MHz.The molecular formula of Hisose $C_{28}H_{48}O_{21}N_2$ was also in agreement with mass ion peak obtained from ES-MS spectrum of Hisose which showed the highest mass ion peak at 748 m/z [M]+ for a tetrasaccharide. ^{1}H and ^{13}C NMR spectra justify the five anomeric signals for tetrasaccharide with total integral intensity of four anomeric protons/carbons. The 1H NMR spectrum of Hisose in D_2O at 300 MHz contain two doublets at δ5.42 (J= 3.6Hz) and δ4.38 (J=8.1Hz) confirmed the presence of Glc at the reducing end in the tetrasaccharide. Simultaneously ^{1}H and 13C NMR spectrum of Hisose acetate showed downfield shifted α and β anomeric proton and carbon of reducing monosaccharides (S-1) i.e. Glc (S-1) at δ6.21 (J=3.9 Hz), δ5.62 (J=8.1Hz) and δ88.94, δ91.52 respectively (Figures 4,5) (Table 1-5).

The anomeric proton signal present at δ5.62in TOCSY Spectrum of Hisose Acetate assigned to β-Glc (S-1) contains three cross peaks at δ5.62×3.89, δ5.62×5.50 and δ5.62×5.05 which was later identified for H-4, H-3 and H-2 of reducing Glc respectively by COSY spectrum of Compound 'c' acetate. The signal assigned to H-4 of S-1 at δ3.89 in the 1H NMR of Hisose Acetate suggested that the position H-4 of S-1 was available for glycosidic linkage

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

by next monosaccharide unit. Further the H-4 of S-1 showed long range coupling with and anomeric carbon of next monosaccharide (S-2) at δ3.89×100.90 in the HMBC spectrum of Hisose acetate which confirmed the (1→4) linkage between S-2 and S-1. The anomeric carbon of at S-2 at δ100.90 had its complimentary signal at δ4.40 in HSQC spectrum of Hisose acetate in CDCl3 at 300 MHz. The chemical shift values of anomeric carbon and anomeric proton at δ100.90 and δ4.42 were having resemblance with literature value of anomeric chemical shift value of GalNHAc hence S-2 monosaccharide was confirmed as GalNHAc [14]. Further the coupling constant of anomeric proton signal (S-2) at δ4.40 with larger J value of 6.6Hz confirmed the β-configuration of the glycosidic linkage between (S-2) and (S-1) in Hisose Acetate. Moreover the presence of β-GalNHAc as next monosaccharide in Hisose was also confirmed by appearance of anomeric proton signal at δ4.30 (6.0Hz) along with a singlet of three protons at δ1.94 in 1H NMR spectrum of Hisose in D2Oat 300MHz. The anomeric proton signal at δ4.42 assigned for β-GalNHAc (S-2) showed three cross peaks at δ4.42×3.80, 4.42×4.12 and 4.42×5.25 in TOCSY spectrum of Hisose acetate which was later identified as H-3, H-2 and H-4 of β-GalNHAc (S-2) respectively by COSY spectrum of acetylated Hisose at 300 MHz in $CDCl_3$ (Figure 6).



Scheme 1: Kiliani Hydrolysis and Methylglycosidation/Acid hydrolysis of Hisose

Scheme 1: Kiliani Hydrolysis and Methylglycosidation/Acid hydrolysis of Hisose.

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

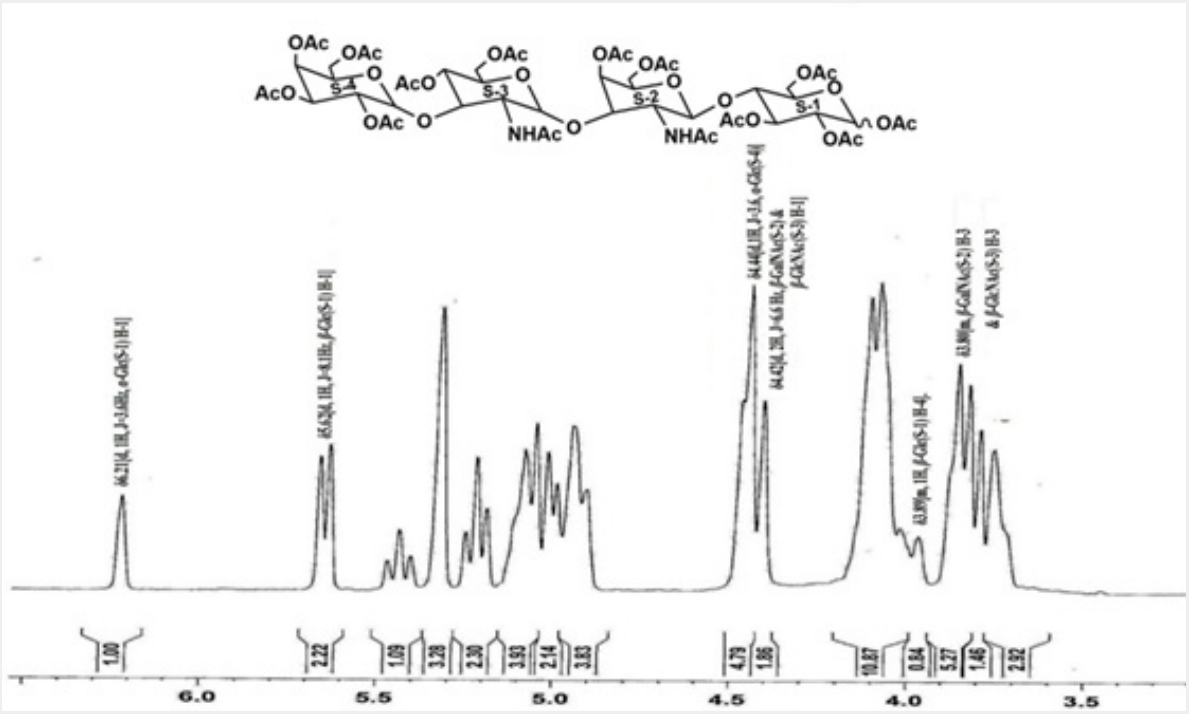


Figure 2: 1H NMR Spectrum of Hisose acetate in $CDCl_3$ at 300 MHz.

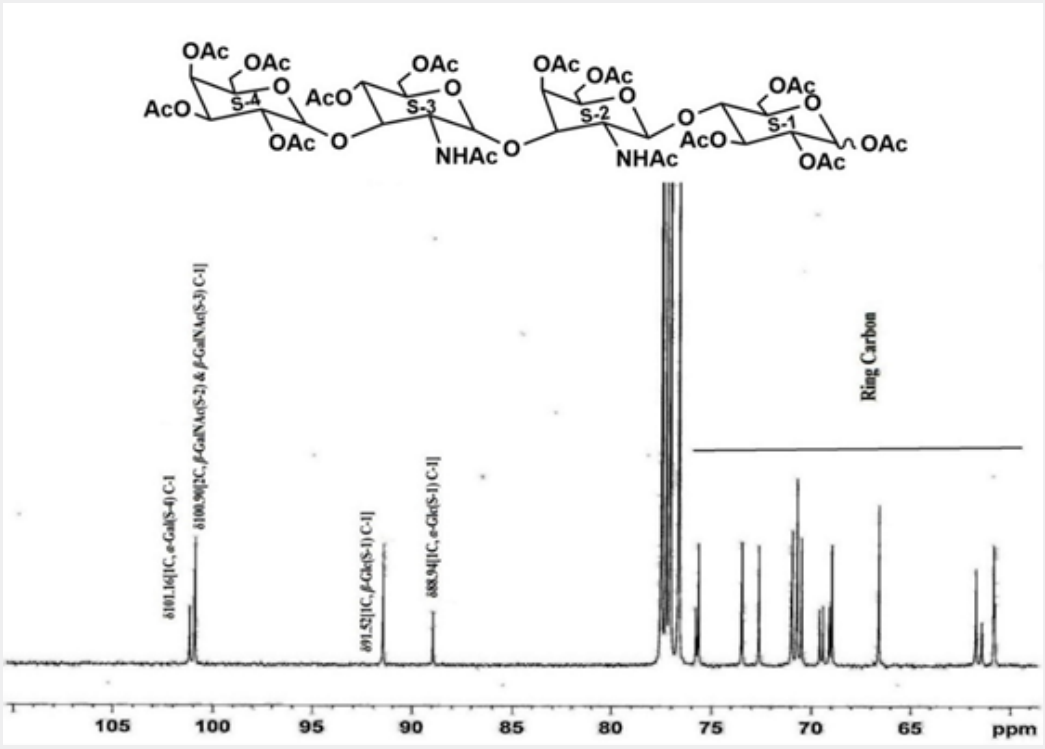


Figure 3: 13C NMR Spectrum of Hisose acetate in $CDCl_3$ at 300 MHz.

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

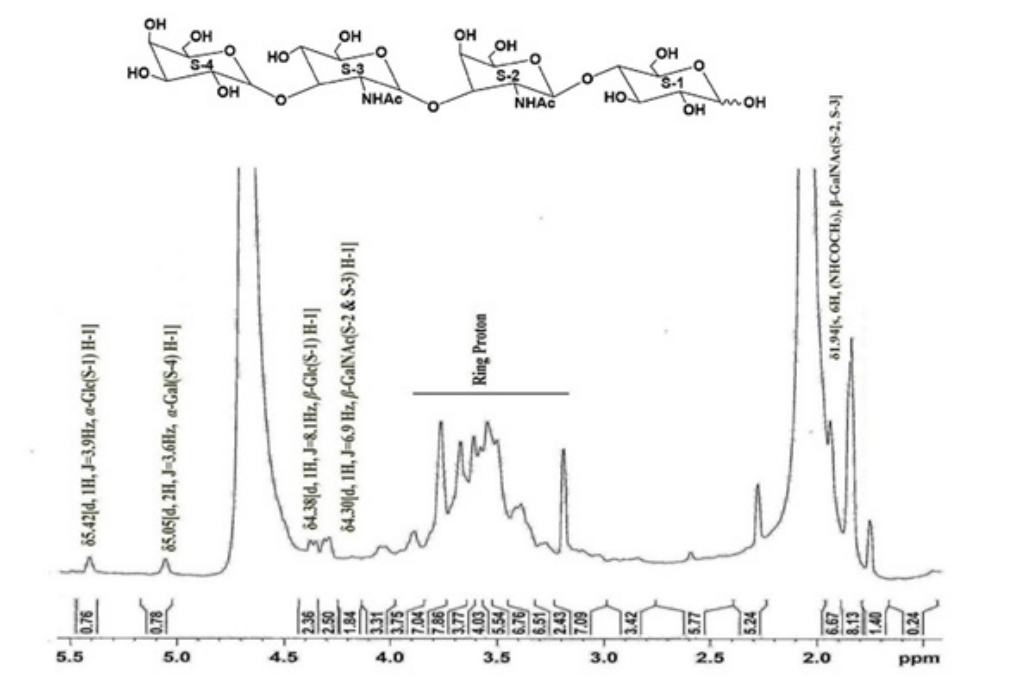


Figure 4: 1H NMR Spectrum of Hisose in D2O at 300 MHz.

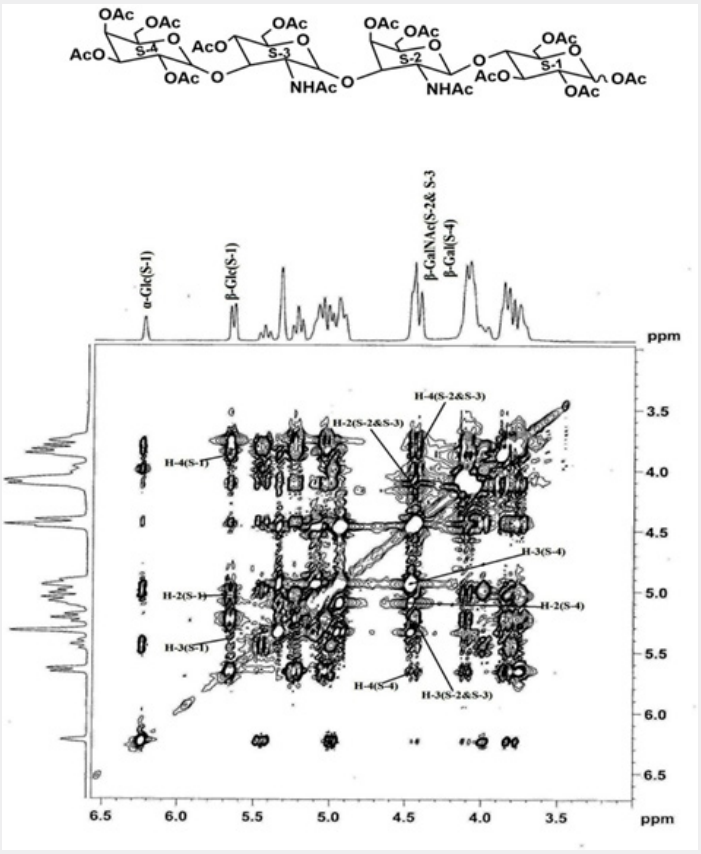


Figure 5: TOCSY Spectrum of Hisose acetate in $CDCl_3$ at 300 MHz.

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

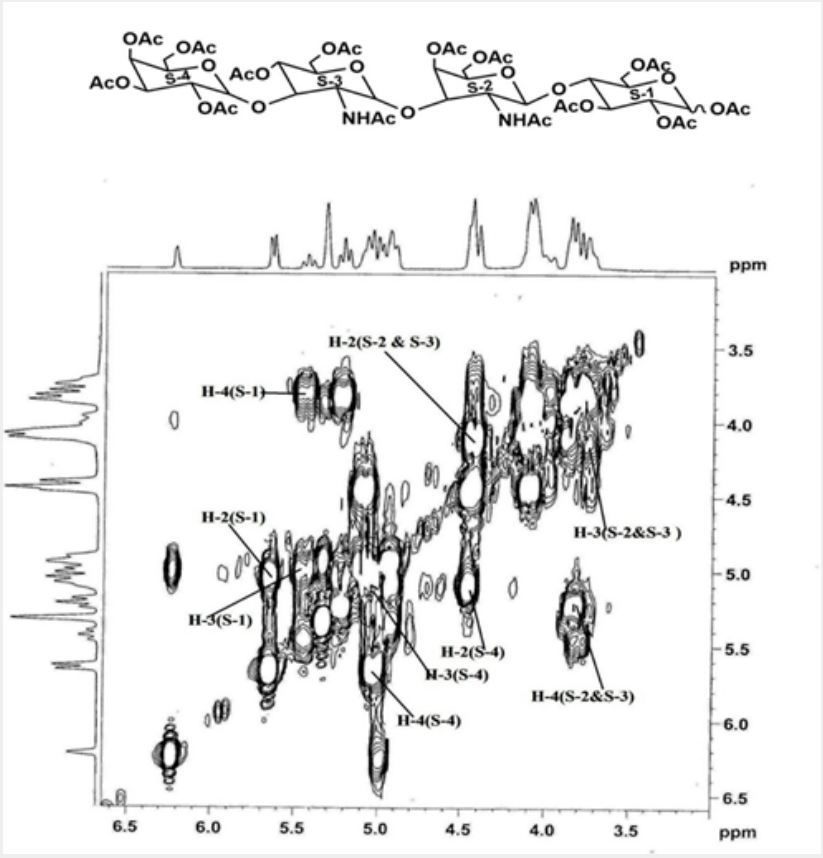


Figure 6: COSY Spectrum of Hisose acetate in $CDCl_3$ at 300 MHz.

Table 1: Anomeric proton/carbon assignments in Hisose Acetate by HSQC Spectrum.

Anomeric Proton x Anomeric Carbon	Cross-peaks of Anomeric Proton x Anomeric Carbon
H-1(S-1) × C-1(S-1α)	δ 6.21 × 88.94
H-1(S-1) × C-1(S-1β)	δ 5.62 × 91.52
H-1(S-2) × C-1(S-2)	δ 4.42 × 100.90
H-1(S-3) × C-1(S-3)	δ 4.42 × 100.90
H-1(S-4) × C-1(S-4)	δ 4.40 ×101.16

Table 2: Anomeric Proton Values of Compound C, Hisose in D_2O and $CDCl_3$ at 300MHz,

Moieties	Anomeric proton value in D_2O		Anomeric proton value in $CDCl_3$	
	^{1}HNMR (δ)	Coupling constant (J)	^{1}HNMR (δ)	Coupling constant (J)
α-Glc(S-1)	5.42	3.6Hz	6.21	3.9Hz
β-Glc(S-1)	4.38	8.1Hz	5.62	8.1Hz
β-GalNHAc(S-2)	4.3	6.0Hz	4.42	6.6Hz
β-GlcNHAc(S-3)	4.3	6.0Hz	4.42	6.6Hz
α-Gal(S-4)	5.05	3.3Hz	4.4	3.6Hz

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

Table 3: Assignment of ring protons of Hisose acetate by TOCSY Spectrum in ppm.

	S-1	S-2	S-3	S-4
Anomeric Protons	5.62	4.42	4.42	4.4
Ring Protons	3.89	3.8	3.8	5
	5.05	4.12	4.12	5.15
	5.5	5.25	5.25	5.7

Table 4: Assignment of Methine protons by COSY Spectrum of Hisose acetate in ppm.

		S-1	S-2	S-3	S-4
Anomeric Proton	H1	5.62	4.42	4.42	4.4
Ring Protons	H2	5.05	4.12	4.12	5.15
	H3	5.5	3.8	3.8	5
	H4	3.89	5.25	5.25	5.7

Table 5: Assignment of Glycosidic linkages by HMBCSpectrum of Hisoseacetate.

Sugar	Linkage	Type of Linkage
S1-S2	δ 3.89×100.90	β-Glc(S-1) [1→4]β-GalNHAc(S-2)
S2-S3	δ3.80×100.90	β-GalNHAc(S-2) [1→3]β-GlcNHAc(S-3)
S3-S4	δ3.80×101.16	β-GlcNHAc(S-3)[1→3] α-Gal(S-4)

The chemical shift of the cross peak δ4.42×4.12 of β-GalNHAc assigned to S-2, also confirmed that in sugar (S-2), H-2 position was due to $NHCOCH_3$ at C-2 and further the chemical shift present at δ4.50×3.80 assigned for H-3 position of β-GalNHAc (S-2) suggested the H-3 of S-2 was available for glycosidic linkage by next monosaccharide unit. Further the signal present at δ3.80 gave a cross peak at δ3.80×100.90 in HMBC spectra of Hisose Acetate which was between H-3 of β-GalNHAc (S-2) and C-1 of S-3 confirmed the 1→3 linkage between S-2 and S-3 (Figure 7).

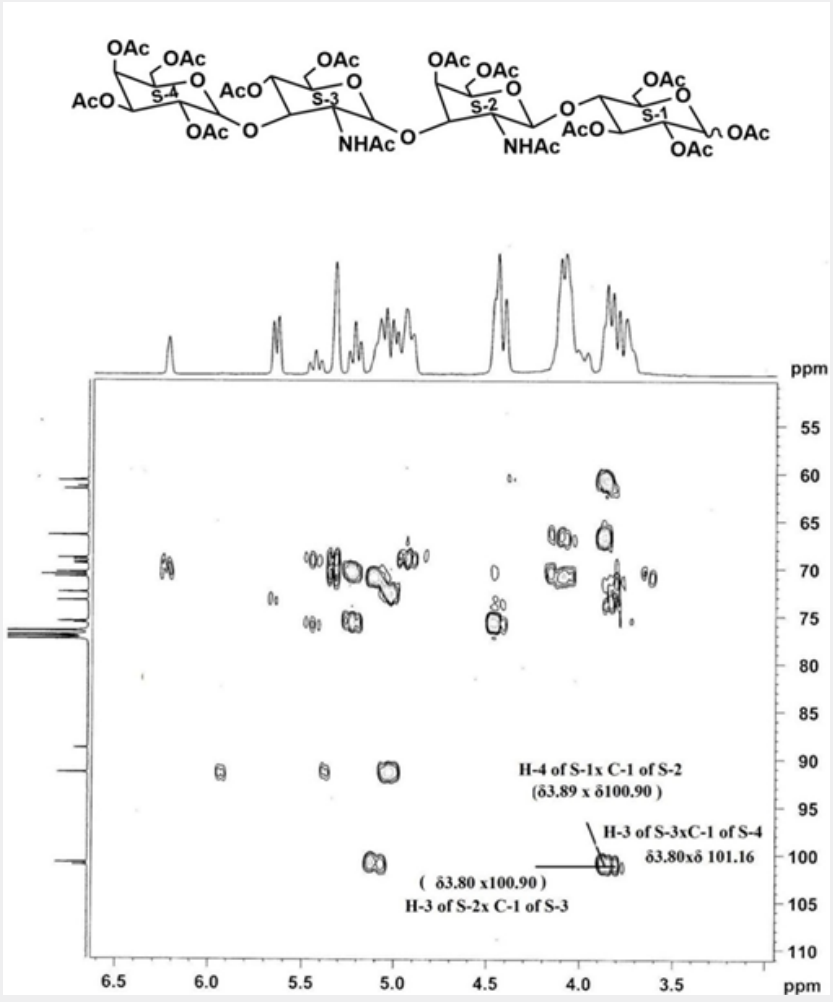


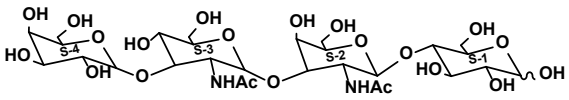
Figure 7: HMBC Spectrum of Hisose acetate in $CDCl_3$ at 300 MHz.

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

The anomeric carbon of at S-3 at δ100.90 had its complimentary signal at δ4.42 in HSQC spectrum of Hisose acetate in $CDCl_3$ at 300 MHz. The chemical shift values of anomeric carbon and anomeric proton at δ100.90 and δ4.42 were having resemblance with literature value of anomeric chemical shift value of GlcNHAc hence S-3 monosaccharide was confirmed as GlcNHAc [15]. Further the coupling constant of anomeric proton signal (S-3) at δ4.42 with larger J value of 6.6Hz confirmed the β-configuration of the glycosidic linkage between (S-3) and (S-2) in Hisose Acetate. Moreover the presence of β-GlcNHAc as next monosaccharide in Hisose was confirmed by appearance of another anomeric proton signal at δ4.30 (6.0Hz) along with a singlet of three protons at δ1.94 in ^1H NMR spectrum of Hisose in D_2O at 300MHz. The anomeric proton signal at δ4.42 assigned for β-GlcNHAc (S-3) contain three cross peaks at δ4.42×3.80, 4.42×4.12 and 4.42×5.25 in TOCSY spectrum of Compound Hisose acetate which was later identified as H-3, H-2 and H-4 of β-GlcNHAc (S-3) respectively by COSY spectrum of acetylated Hisose at 300 MHz in $CDCl_3$. The chemical shift of the cross peak δ4.42×4.12 of β-GlcNHAc (S-3), also confirmed that in sugar (S-3), H-2 position was due to $NHCOCH_3$ at C-2 and further the chemical shift present at δ4.42×3.80 assigned to H-3 position of β-GlcNHAc (S-3) suggested the H-3 of S-3 was available for glycosidic linkage by next monosaccharide unit. Further the signal present at δ3.80 gave a cross peak at δ3.80 × 101.16 in HMBC spectra of Hisose Acetate 'c' which was between H-3 of β-GlcNHAc (S-3) and C-1 of S-4 confirmed the 1→3 linkage between S-4 and S-3. The anomeric carbon of S-4 at δ101.16 had its complimentary signal at δ4.40 (3.6 Hz) in HSQC spectrum of Hisose Acetate. The chemical shift values of anomeric carbon and anomeric proton at δ101.16 and δ4.40 were having resemblance with literature value of anomeric chemical shift value of Gal hence S-4 monosaccharide was confirmed as Gal [14]. Further the coupling constant of anomeric proton signal (S-4) at δ4.40 with smaller J value of 3.6 Hz confirmed the α-configuration of the glycosidic linkage between S-3 and S-4 in Hisose Acetate. The anomeric proton signal at δ5.05 in 1H NMR spectrum of Hisose in D2O was confirmed the presence of α-Gal (S-4) moiety. The anomeric proton signal at δ4.40 assigned for α-Gal (S-4), gave three cross peaks at δ4.40×5.7, δ4.40×5.0 and δ4.40×5.15 in its TOCSY spectrum, which was later identified as 5.15 (H-2), 5.0 (H-3) and 5.7 (H-4) of α-Gal (S-4) respectively by COSY spectrum of Hisose acetate. Since this anomeric proton does not show any cross peak in the linkage region, it was confirmed that α-Gal (S-4) was present at non-reducing end which was confirmed by the TOCSY and COSY spectrum of Hisose acetate in $CDCl_3$ at 300 MHz. in the TOCSY spectrum of Hisose acetate does not contain any methine protons in glycosidic linkage region i.e. δ3-4 ppm showed that none of -OH group of α-Gal (S-4) were involved in glycosidic linkage hence, confirmed that α-Gal was present at non-reducing end and none of their -OH group were available for glycosidic linkage, which was confirmed by the TOCSY and COSY.

All the ^1H NMR assignments for ring protons of monosaccharide units of Hisose were confirmed by COSY and TOCSY experiments. The positions of glycosidation in the oligosaccharide were confirmed by position of anomeric signals, Structure Reporter Groups [15] (S.R.G.) and comparing the signals in ^1H and ^{13}C NMR of acetylated and deacetylated oligosaccharide. The glycosidic linkages in Hisose were assigned by the cross peaks for glycosidically linked carbons with their protons in the HSQC and HMBC spectrum of acetylated Hisose which were in conformity with the assigned structure and their position were confirmed by 2D NMR viz. . All signals obtained in ^1H and ^{13}C NMR of compound Hisose were in conformity with the assigned structure and their position were confirmed by 2D NMR viz. COSY, TOCSY, HSQC experiments. Thus based on the pattern of chemical shifts of ^1H NMR, ^{13}C NMR, COSY, TOCSY and HSQC experiments it was interpreted that the Compound 'C' was a tetrasaccharide having following structure as:

Gal-α-(1→3)-GlcNHAc-β-(1→3)-GalNHAc-β-(1→4)Glc



The ESI Mass Spectrometry data of Hisose not only confirmed the derived structure but also confirmed the sequence of monosaccharide in Hisose. The highest mass ion peaks were recorded at m/z 771 and 748 which were due to [M+Na] + and [M]+ respectively, confirming the molecular weight of Hisose as 748 and agreed with its molecular formula $C_{28}H_{48}O_{21}N_2$ (Figure 8).

Further the mass fragments were formed by repeated H transfer and the elimination of terminal sugar less water [16]. The Tetrasaccharide m/z 748 (I) fragmented to give mass ion at m/z 586 (II) [748-Gal], this fragment was arised due to the loss of terminal Gal (S-4) moiety from tetrasaccharide indicating the presence of α-Gal (S-4) at the non-reducing end (Scheme 2).

It was further fragmented to give mass ion peak at m/z 383 (III) [586-GlcNHAc] which was due to loss of β-GlcNHAc (S-3) moiety from trisaccharide. The disaccharide unit again fragmented to give mass ion peak at m/z 180 (VI) [383-(S-2)], which was due to loss of β-GalNHAc (S-2) moiety from disaccharide. The other fragmentation pathway in ES Mass spectrum ossf Compound C m/z 748 shows the mass ion peak at 712[748-2H_2O], 695[712-2H_2O, H+], 694[695-H+], 687[748- CHOHCHO], 654[687-2OH, H+], 653[652-H+], 608[654-CHO,OH], 586[748-(S-4)], 549[586-2H_2O, H+],508[549-CHCO], 507[508-H+], 465[507-CH_3OH], 407[465-$NHCOCH_3$], 406[407-H+], 383[586-(S-3)], 366[383-OH], 366[365-H+],319[365-CHO, OH], 318[365- HCHO, H_2O], 303[365-2CH_2OH], 180[383-(S-2)] (Scheme 3).

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

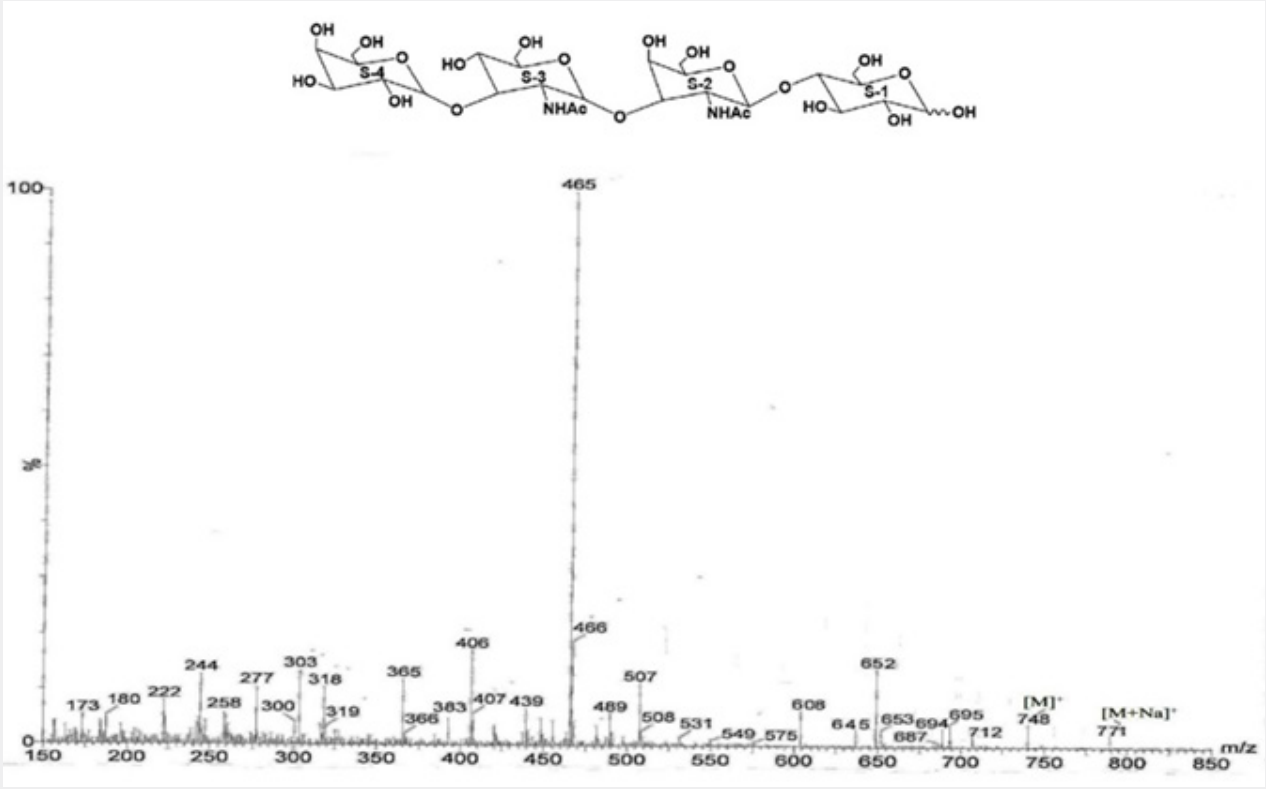
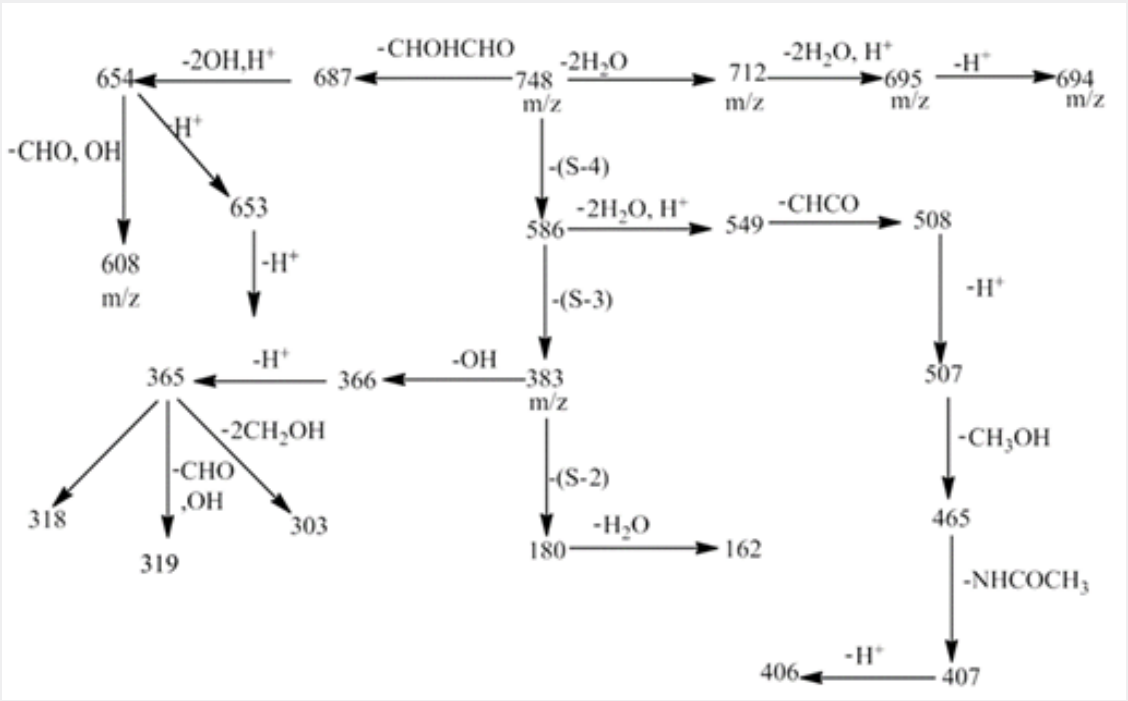
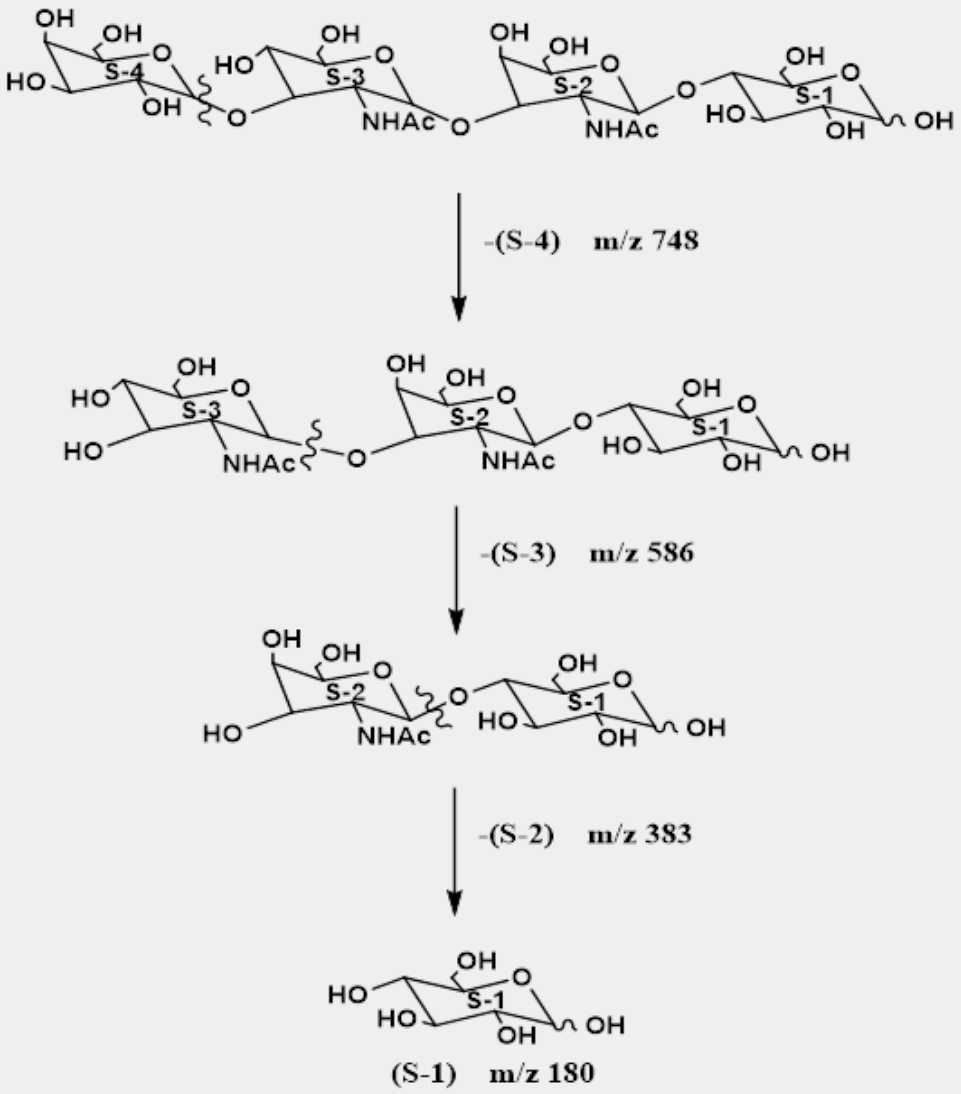


Figure 8: ES-MS Spectrum of compound C, Hisose.



Scheme 2: Mass Fragments of Compound C, Hisose.

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950



Scheme 3: Mass Fragmentation of Compound C, Hisose.

Conclusion

With a view to isolate medicinally important oligosaccharides from cow milk, a novel tetrasaccharide 'Hisose' was isolated from Rathi cow milk consisting of Glc, GalNHAc and Gal with 1→4 and 1→3 glycosidic linkages with α and β configurations.

Acknowledgement

Authors are thankful to Prof. Anil Mishra, Head Department of Chemistry for providing lab and library facilities for completing this work.

Conflict of Interest

Authors do not have any conflict of interest with any researcher or scientist.

References

1. Mana D, Kozhiyott Mohanan A, Venkatesha RN (2021) Milk and Milk Products in Ayurveda: A Review. Biol Life Sci Forum 6(1): 13.
2. Ling ER, Kon SK, Porter JW (1961) The Composition of Milk and the Nutritive Value of its Components. Milk: the Mammary Gland and its Secretion. 17(2): 195-263.

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950

3. Sharma M, Shukla M, Deepak D (2024) Recognition of Lost Glycosidic Linkage by Reverse HMBC Experiments in Milk Oligoglycoside/ Oligosaccharide. Trend Carbohyd Chem 16(2): 56-70.
4. Kumar K, Singh R, Deepak D (2018) DFT Studies and Structure Elucidation of Novel Oligosaccharide from Camel Milk. Chem Biol Interface 8(2): 106-114.
5. Chauhan DDAPS, Shukla M, Chauhan S, Deepak D (2024) Isolation of Biologically Active Oligosaccharide 'Rathose' from Rathi Cow Milk and Their Structure Elucidation by 2D NMR. J Biol Chem Res.
6. Chauhan DDAPS, Shukla M, Chauhan S, Deepak D (2024) Isolation and Structure Elucidation of an Undefined Hexasaccharide 'Thisose' from Milk of Rathi Cow. J Biol Chem Res.
7. Davis DG, Bax A (1985) Assignment of complex proton NMR spectra via two-dimensional homonuclear Hartmann-Hahn spectroscopy. J Am Chem Soc 107: 2820-2821.
8. Bax A, Summers AS (1987) New Insights into the Solution Behavior of Cobalanins. Studies of the base-of form of Coenzime B12 using Modern two-Dimensional NMR Methods. J Am Chem Soc 109(2): 566-574.
9. Kay L, Keifer PA, Saarinen T (1992) Pure absorption gradient enhanced heteronuclear single quantum correlation spectroscopy with improved sensitivity. J Am Chem Soc 114: 10663-10665.
10. Schoefberger W, Schlagnitweit J, Muller N (2011) Recent Development in Hetronuclear Multiple Bond Correlation Experiments. Annual Reports on NMR Spectrosc 72: 1-60.
11. Partridge SM (1949) Aniline Hydrogen Phthalate as a Spraying Reagent for Chromatography of Sugars. Nature 164(4167): 443-443.
12. Feigl F (1975) Spot Tests in Organic Analysis. Elsevier.
13. Warren L (1960) Thiobarbituric Acid Spray Reagent for Deoxy Sugars and Sialic Acids. Nature 186(4720): 237-237.
14. Bush CA (1988) High Resolution NMR in the determination of structure in complex carbohydrates. Bull Magn Reson 10(3/4): 73-78.
15. Vliegenthart JFG, Dorland L, Halbeek H (1983) High-Resolution, 1H-Nuclear Magnetic Resonance Spectroscopy as a Tool in the Structural Analysis of Carbohydrates Related to Glycoproteins. Adv Carbohydrate Chem Biochem 41: 209-374.

DOI:10.19080/JDVS.2024.16.555950

How to cite this article: Desh Deepak A P Singh C, Sarita C, Manisha S, Anil M, Desh D. Isolation and Structure Elucidation of a New Tetrasssaccharide 'Hisose' from Rathi Cow's Milk by 2D NMR. Dairy and Vet Sci J. 2024; 16(5): 555950. DOI: 10.19080/JDVS.2024.16.555950