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Structure Elucidation and Confirmation of Monosaccharide Units by Splitting Pattern of Epicentre Four of Glc and GalNHAc in a Novel Trisaccharide Friuose from Friesian Cow Milk

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Abstract

In search for more and more biologically active milk oligosaccharides, milk of different cow species was investigated for their oligosaccharide contents. It was found that the oligosaccharides isolated from this milk carry different stereoscopic structures which varied in their inter glycosidic linkages, configurations and conformations of the monosaccharides present therein. In our present study we have isolated a novel tri-saccharide Friuose from Friesian cow milk comprised of Glucose and Galactose with α Glycosidic linkage. The structure of oligosaccharide was elucidated with high resolution 800 MHz NMR experiments. The identification of monosaccharide contents of Friuose were not only done by traditional methods, Structure Reporter Group (SRG), but also justified by the splitting pattern of epicentre four of Glucose and Galactose. This is the first report for identification of monosaccharides based on J values of epicentre four of the monosaccharides present in a novel oligosaccharide. The structure of Friuose was established as under- (Image 1).

Key Words: Friesian Cow Milk; 1D NMR; 2DNMR (COSY, TOCSY, HSQC, HMBC) Experiments; Friuose

Introduction

Oligosaccharides are biologically active components of plants, bacteria and milk. Milk is a mammalian fluid essential for development of neonates. It also provides immunity and strength to new born. Besides the human milk, cow milk is the most efficacious and medicinally important intake for neonates which not only develops the immune system, it strengthens the cardiac muscles and brain gangliosides and it also improves eye muscles as well [1]. It is important to note that oligosaccharide content of cow milk depends on the fodder and geographical environment around them. In India a large number of cow species are found along with the cow of foreign origin. Oligosaccharides found in cow milk constitute Glucose, Galactose, GlcNHAc, GalNHAc etc which are linked together by α-and β-glycosidic linkages positioned at different ring OH groups of monosaccharide building blocks, resulting in straight and branched chain oligosaccharide structures [2]. Specific conformation and position of glycosidic linkages result in the specific biological activities. In literature of ancient medicinal systems [3], the cow milk is defined as '*Amrita*' the panacea and is beneficial for development of bones, improves milk output in lactating women, more over cow milk is beneficial for gut health improvement, considering the medicinal efficacies of cow milk and the milk of Friesian cow was collected in bulk (10L) from Hardoi district of Uttar Pradesh in India and was processed by modified method of Kobata and Ginsburg, incorporating de-protonation micro-filteration and lyophilisation, as a result crude oligosaccharide mixture 260g was obtained. This oligosaccharide mixture was acetylated by acetic anhydride and pyridine and it was further purified on silica column chromatography which resulted in the isolation of a new Trisaccharide comprised of Glc, and GalNHAc. Besides the identification of Glc and GalNHAc by TLC/PC and position of anomeric proton/carbon with the literature value of Glc and GalNHAc (structure reporter group theory) [5], it was also identified by splitting pattern of epicentre4of Glc and GalNHAc. This is the first report of a trisaccharide Friuose in which the reducing Glc is attached to two GalNHAc moieties with α glycosidic linkages and the identification of Glc and GalNHAc by splitting pattern of 4th epicentre of monosaccharide constituents. The Structure of the novel oligosaccharide was elucidated by combining the result obtain from the NMR experiments of ^{1}H, ^{13}C and 2D NMR (HSQC, TOCSY, COSY, HMBC) [6-8], Mass spectrometry, chemical degradation, chemical transformation.

Material & Methods

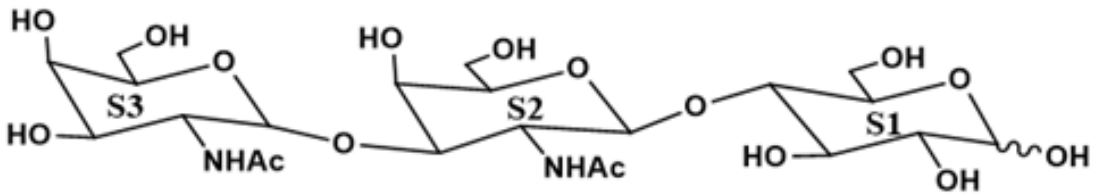
General procedures were the same as given in our earlier communication [9], besides the NMR experiments were performed on 800MHz and 300MHz Bruker NMR machine.

General procedure for isolation of milk oligosaccharides [9]

10L of cow milk was collected in 4 days in normal milking condition from a single domestic cow of district Hardoi Uttar Pradesh, India. Milk was fixed immediately by addition of an equal amount of ethanol (10L). The preserved milk was filtered by a glass wool column and then it was centrifuged on C-25 centrifuging machine at 5000rpm at -4°C, after centrifugation, the solidified layer was removed by filtration through glass wool column. After filtration lipid layer was discarded and supernatant was precipitated by addition of a maximum of 68% ethanol which was again separated by centrifugation and removal of protein and lactose by centrifugation, supernatant was filtered through a micro filter (0.2μ) to remove remaining lactose. Further it was then lyophilized to obtain the oligosaccharide mixture (260gm).

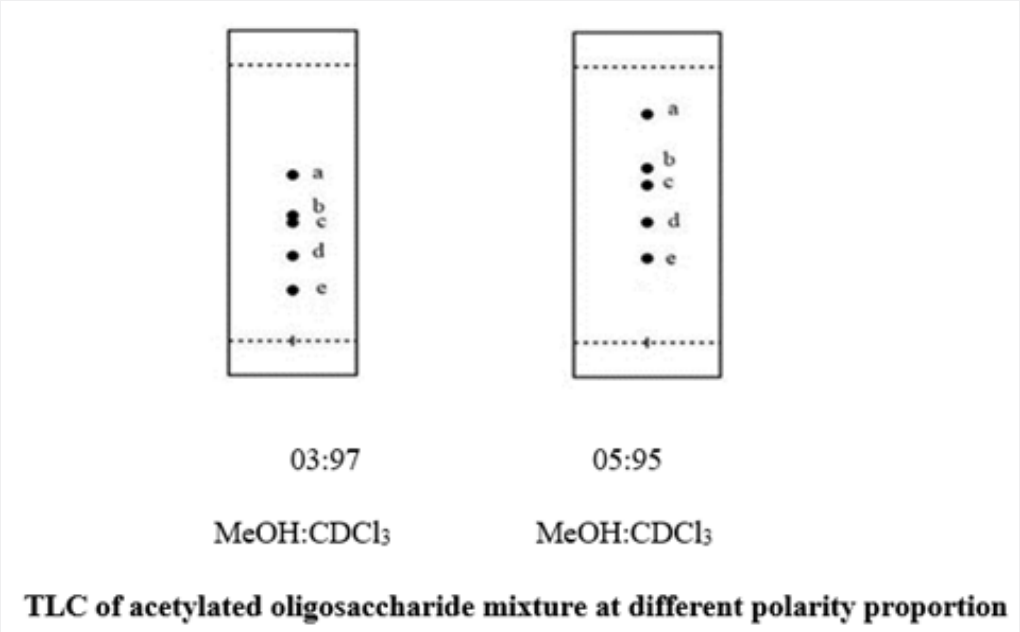
Acetylation of oligosaccharide mixture [9]

Acetylation of oligosaccharide mixture 10g oligosaccharides mixture was acetylated with pyridine (10 ml) and acetic anhydride (10ml) at 60°C and solution was stirred overnight. The mixture was evaporated under reduced pressure and viscous residue was taken in $CHCl_3$ (3×100ml) and washed twice with ice cold water and was evaporated to dryness yielding 10.2g of acetylated mixture. The acetylation converted the free sugars into their nonpolar acetyl derivatives which resolved nicely on TLC, giving 6 spots i.e. a, b, c, d, e, and f. The six compounds were finally purified by silica column chromatography using varying proportions of chloroform and methanol as eluents (Image 2).



GalNHAc-α-L(1→3)-GalNHAc-α-L(1→4)-Glc

Image 1



TLC of acetylated oligosaccharide mixture at different polarity proportion

Image 2

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Purification of acetylated milk oligosaccharides on silica gel column

Separation and purification of the acetylated oligosaccharide mixture (10.0gm) was carried out over silica gel (500g) using varying proportion of solvent such as $CHCl_3$, $CHCl_3$: MeOH as eluents, collecting fraction of 250mL each. All these fractions were checked on TLC and those showing similar spots were taken together for further investigations. Repeated column chromatography of acetylated oligosaccharide mixture resulted in isolation of 62mg of compound 'a'.

Deacetylation of compound 'GalNHAc' [9]

Compound 'a' (30mg) was dissolved in acetone (3mL) and 3mL of NH_3 was added in it and was left overnight in a Stoppard hydrolysis flask. After 24h ammonia was removed under reduced pressure and the compound was dissolved in water and washed thrice with $CHCl_3$ (3×5mL) (to remove acetamide) and water layer was freeze dried providing the deacetylated natural oligosaccharide 'A' (24mg).

Methyl glycosidation/acid hydrolysis of Compound 'A':

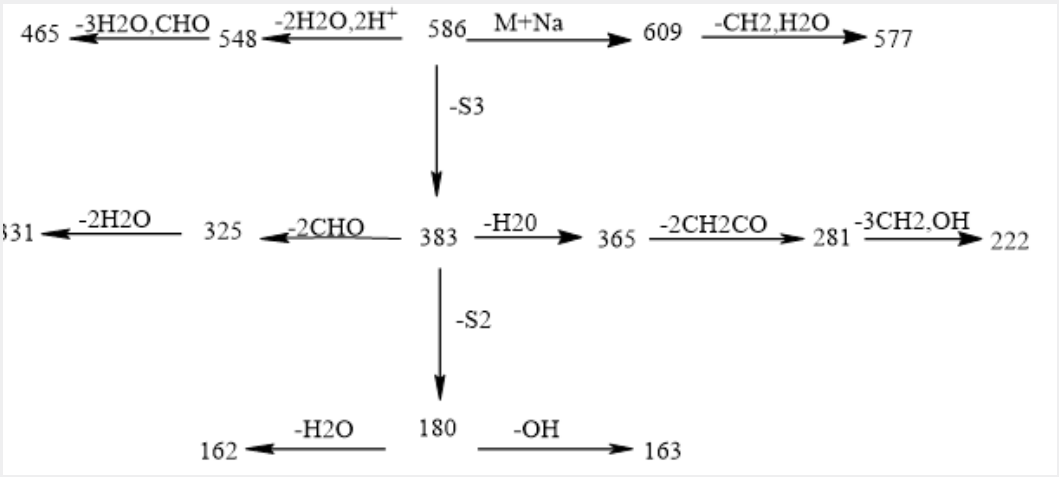
Compound 'A' (10mg) was refluxed with absolute MeOH (2ml) at 65°C for 18h in the presence of cation exchange IR-l20 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To this reaction mixture of 'A', 1, 4-dioxane (1ml) and 0.1N H_2S0_4 (1ml) was added, and the solution was warmed for 30 minutes at 50°C. The hydrolysis was complete after 24h. (TLC) The hydrolysate was neutralized with freshly prepared $BaCO_3$ and further filtered and concentrated under reduced pressure to afford α-and β-methyl glucosides along with the Gal and GalNHAc. Identification of monosaccharides in compound 'A' was confirmed by comparison with authentic samples (TLC, PC) of α-and β-methyl glucosides along with the GalNHAc.

Kiliani hydrolysis of compound A, friuose [9]

Compound 'A' (5mg) was dissolved in 2ml Kiliani mixture (AcOH-H_2O-HCI, 7:11:2) and heated at 100°C for 1hr 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 and was evaporated under reduced pressure to afford Glc, GalNHAc on comparison with authentic samples of Glc, and GalNHAc (Scheme 1).

Description of compound-A, Friuose:

Compound a (62mg) was obtained from fraction 61-75 of column chromatography-6. On deacetylation of 32 mg of substance with NH_3/ acetone it afforded substance 'A' (24.0mg). For experimental analysis, this compound was dried over P_2O_5 at 100°C and 0.1mm pressure for 8h. It gave positive phenol-sulphuric acid test [10], Feigl test [11], Morgan-Elson test [12] (Table 1).



Scheme 2: Mass Fragmentation of Friuose.

Table 1:

$C_{22}H_{38}O_{16}N_2$	%C	%H	%N
Calculated	46.92	6.41	4.74
Found.	46.92	6.4	4.73

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i. **^{1}H NMR of Friuose acetate in $CDCl_3$ at 800MHz**

δ6.25[d, 1H, J=3.1Hz, α-Glc(S-1) H-1)], 5.68[d, 1H, J=8.1Hz, β-Glc(S-1) H-1], 4.47 [d, 1H, J=2 Hz, α-GalNHAc (S-2), H-1)], 4.47[d, 1H, J=2 Hz, α -GalNHAc (S-3) H-1].δ3.80(J=3.4 and 3.2 Hz) [m, 1H, β-Glc(S-1) H-4], δ3.80 (J=3.4 and 3.2 Hz)[α-GalNHAc(S-2) H-3].value of epicentre -4 Glc (S1) H-4, 3.38 (J=6.4 and 6.2 Hz.

ii. **^{13}C NMR of Friuose acetate in $CDCl_3$ at 200MHz**

δ88.94[1C, α-Glc(S-1), C-1], 91.50[1C, β-Glc(S-1), C-1], 100.93[1C, α-GalNHAc (S-2), C-1], 101.19[1C, α -GalNHAc (S-3), C-1].

iii. **^{1}H NMR of Friuose in D_2O at 300MHz**

δ5.61[d, 1H, J=3.1Hz, α-Glc(S-1) H-1], 5.38 [d,2H, J=8.1, βGlu (S-1)], 4.32[d, 1H, J=2Hz, α -GlcNHAc (S-2) H-1], 4.39[d, 1H, J=2Hz, α-GlcNHAc (S-3) H-1].

iv. **ES-MS fragments of friuose**

609[M+Na]$^{+}$, 586[M]$^{+}$, 577[609-CH_2H_2O], 548[586-$2H_2O$,$2H^{+}$], 465[548-$3H_2O$, CHO], 406[465-$3CH_2OH$], 383[586-S3], 365[383-H_2O], 325[383-2CHO], 281[365-$2CH_2CO$], 222[281-$3CH_2OH$], 180[383-S3], 162[180-H_2O].

Result and Discussion of Compound A, Friuose

Compound A, Friuose ($C_{22}H_{38}O_{16}N_2$), gave positive phenol-sulphuric acid test [10], Feigl test [11] and Morgan-Elson test [12] showing the presence of normal and amino sugars moieties in the compound A. The Oligosaccharide mixture of Friesian cow milk which was acetylated and chromatographed by silica gel column chromatography resulted in isolation of compound 'a' which was later deacetylated with NH_3 and acetone to obtain natural oligosaccharide 'A' The name of the compound Friuose originated from the source i.e.; Friesian cow and designated as 'A' while its acetylated derivative was named as 'a'.

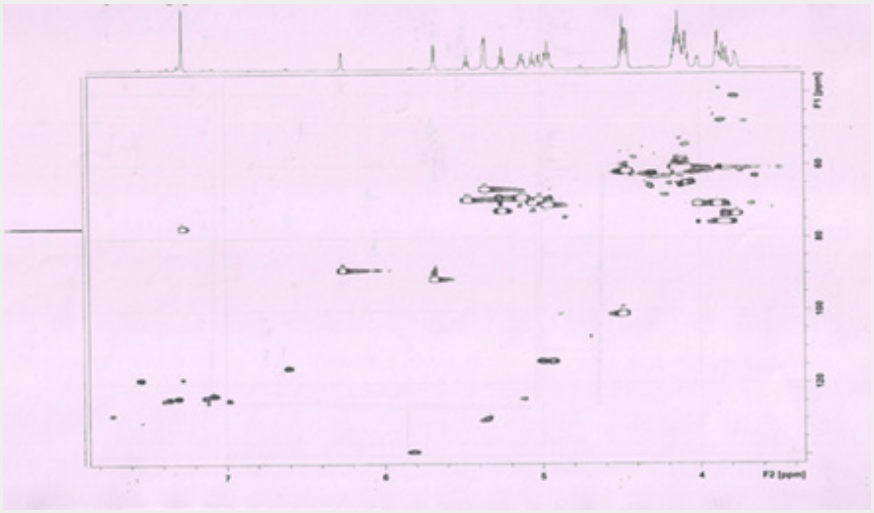

Figure 1: HSQC spectrum of Friuose Acetate in $CDCl_3$ at 800MHz.

The HSQC spectrum of acetylated Friuose showed the presence of four cross peaks (Figure 1) of anomeric protons and carbons in their respective region at **δ6.25× 88.94, δ5.68×91.50, δ4.47×100.93, δ4.43×101.19** suggesting the presence of four anomeric protons and carbons into it. Further the 1H NMR of acetylated Friuose at 300 MHz showed four doublets of one proton i.e. **δ6.25(1H), 5.68 (1H), δ4.47 (1H), δ4.43(1H)** (Figure 2). The trisaccharide nature of friuose was further confirmed by the presence of four anomeric carbons peaks at **δ88.94(1C), 91.50(1C), 100.93(1C), 101.19(1C)**, in the acetylated spectrum of Friuose at 75MHz (Figure 4). The ^{1}H NMR of natural friuose at **300 MHz** in D_2O showed four anomeric proton signals at **δ5.61, 5.38, 4.32, 4.39**, (Figure 3) along with two, three proton singlet at 1.91 and 1.81 confirming the presence of three monosaccharide units in oligosaccharide in their reducing form, out of which two of the monosaccharides had -NHAc group in them. Further the anomeric proton doublet presents at δ6.25 and δ5.68 in ^{1}H NMR of acetylated Friuose showed downfield shifted α and β anomeric protons confirming that the oligosaccharides was present in its reducing form and also suggested that compound Friuose was a trisaccharide in reducing form. Thus ^{1}H and ^{13}C NMR spectra justify the four anomeric signals for trisaccharide with total integral intensity of three anomeric proton carbons.

Table 2: Chemical shift for Anomeric Proton/ Carbon by HSQC Spectra of Ring proton of Friuose Acetate.

Sugar	^{1}H	^{13}C
S1	6.25	88.94
S1	5.68	91.50
S2	4.47	100.93
S3	4.43	101.19

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Table 3: ^{13}C Values of anomeric carbon of Friuose Acetate.

Moieties	^{13}C NMR(δ)
S1	88.94ppm
S1	91.50ppm
S2	100.93ppm
S3	101.19ppm

Table 4: ^{1}H NMR Values of Anomeric Protons.

Moieties	^{1}H NMR(δ)	Coupling Constant(J)
S1	6.25	3Hz
S1	5.68	8.1Hz
S2	4.47	2Hz
S3	4.43	2Hz

f1 234

The reducing nature of compound was further confirmed by its methyl glycosylation (MeOH/H^+) followed by its acid hydrolysis, which led to isolation of α- and β- methyl glucosides, along with GalNHAc suggesting the presence of glucose at the reducing end, along with GalNHAc unit. For convenience the monosaccharides present in oligosaccharide 'A' were designated as S-1, S-2and S-3. The monosaccharides present in the compound 'A' were also confirmed by its Kiliani hydrolysis under strong acidic conditions which were monitored on paper chromatography (PC) and TLC. On completion of Kiliani hydrolysis it gave two spots of Glc, and GalNHAc, which were identified by their comparison with the authentic samples of Glc and GalNHAc confirming that the oligosaccharide Friuose was composed of two types of monosaccharide units. Further, the anomeric proton signals present at **δ6.25 and 5.68 in ^{1}H NMR** of Friuose acetate was assigned to α- and β-anomeric protons of Friuose acetate 'a' at 800 MHz in $CDCl_3$.The HSQC spectrum of 'a' showed a cross peak at δ5.68× 91.50 for monosaccharide (S-1) which resembled with the literature value of Glc [13] hence the S-1 was reconfirmed as Glucose.

Further, the anomeric proton presents at **δ 5.68**, showed three cross peaks at **δ5.68× (3.80, 5.05, 5.50,)** in TOCSY spectrum of Friuose acetate (Figure 5), which were later confirmed as (H2 5.05, H3 5.50, H4 3.80) of S-1 by COSY spectrum of Friuose acetate (Figure 6). The chemical shift of H-4 of S-1 at δ3.80 suggested that the position of H-4 of S-1 was available for glycosidic linkage by the next monosaccharide unit. Further, the signal for H-4- of S-1 at **δ3.80** gave a cross peak with C1 of S-2 at δ3.80×100.93, in the HMBC spectrum (Figure 7) of Friuose acetate confirming a 1→4 glycosidic linkage between S-2 and S-1. The anomeric carbon signal at δ100.93 gave a cross peak at **δ100.93 × 4.47** in the **δ100.93×4.47** resemble with the literature value of anomeric proton/carbon value of GalNHAc [13]. Hence, the monosaccharide S-2 was confirmed as GalNHAc. The anomeric proton doublet at δ4.47(J= 2Hz) showed that glycosidic linkage between S2→S1 was a ɑ-glycosidic linkage, it is also confirmed that GalNHAc was present in its $_4C_1$ L conformation more over the splitting pattern of H4 of S1(J=6.4 and 6.2 Hz) also confirmed that Glc, S1 was Glucose. Further, the anomeric proton signal present at **δ4.47** in the TOCSY spectrum of Friuose acetate 'a' exhibited three cross peaks at (δ4.47×3.80, 4.47×4.10, 4.47×5.30). These peaks were later identified by COSY spectrum as (H2 4.10, H3 3.80, and H4 5.30) (Figure 6). The chemical shift of H3 at δ3.80 suggested that H3 of (S-2) was available for glycosidic linkage with the next monosaccharide unit. Further the signal present at δ3.80 gave a cross peak at δ3.80×101.19 in the HMBC Spectrum of Friuose acetate which was between H3 of S-2 and C1 of S-3 confirming a (1→3) linkage between S-2 and S-3. The HSQC spectrum of Friuose acetate at 800 MHz in $CDCl_3$ gave a cross peak between H1 of (S-3) and C1 of (S-3) at **δ4.43 ×101.19**, confirming the anomeric proton/carbon value of S-3 unit at **δ4.43 ×101.19.** The anomeric carbon/proton value at **δ101.19** and **δ4.43** resembled with literature value of GalNHAc [13] confirmed that S-3 was a GalNHAc. The anomeric proton doublet for GalNHAc S-3 present at δ4.43 showed a coupling constant of 2 Hz and hence the configuration of GalNHAc (S-3) was α. It also confirmed that GalNHAc, S3 had a $^4C_1$ L conformation. Besides this splitting pattern of H4 of S-2 J= 3.2 and 3.4 Hz also confirm the sugar S2 was GalNHAc. The anomeric proton signal at **δ4.43** showed three cross peaks at (5.10, 5.30, 5.55) in TOCSY spectrum of Friuose acetate. These were later confirmed by the COSY spectrum as (H2 5.10, H3 5.50, and H4 5.55). The chemical shift of Ring Proton values of monosaccharide S3 confirmed that none of its ring protons were involved in glycosidic linkage by next monosaccharide unit and hence the monosaccharide S3 was present at the non-reducing end of Friuose.

Table 5: ^{1}H NMR Values along with Coupling Constant Values.

Moieties	^{1}H NMR(δ)	Coupling Constant(J)
S1	6.25	3Hz
S1	5.68	8.1Hz
S2	4.47	2Hz
S3	4.43	2Hz

Table 6: TOCSY Correlation of Anomeric Protons of Friuose Acetate.

Order of S1	Order of S2	Order of S3
3.8	3.8	4.43
5.05	4.1	4.05
5.5	4.47	5.1
5.68	5.3	5.55

Table 7: COSY Correlation of Anomeric Protons of Friuose Acetate.

Sugar	S1	S2	S3
H1	5.68	4.47	4.43
H2	5.05	4.1	4.05
H3	5.5	3.8	5.1
H4	3.8	5.3	5.55

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Table 8: HMBC Values of Linkages in Friuose Acetate.

Sugar	Linkage	Type of Linkage
S1-S2	1→4	ɑGalNHAc(S-2)1→4Glc (S-1)
S2-S3	1→3	ɑGalNHAc(S-3)1→3 GalNHAc (S-2)

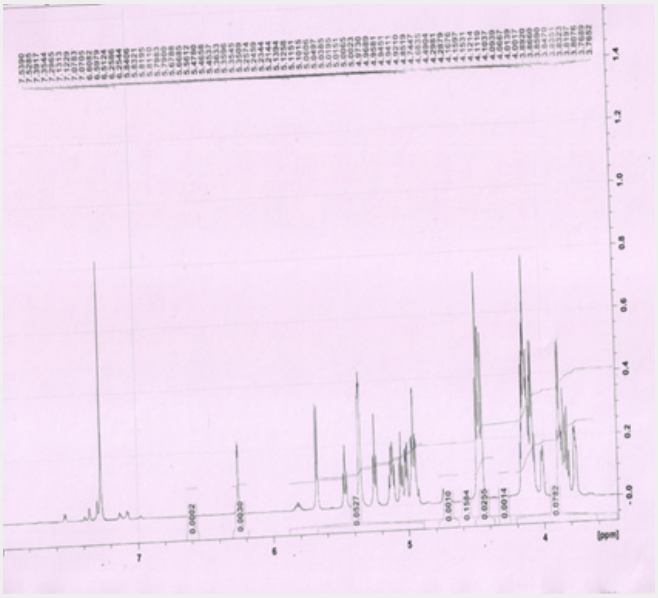

Figure 2: ^{1}H NMR spectrum of Friuose Acetate in $CDCl_3$ at 800MHz

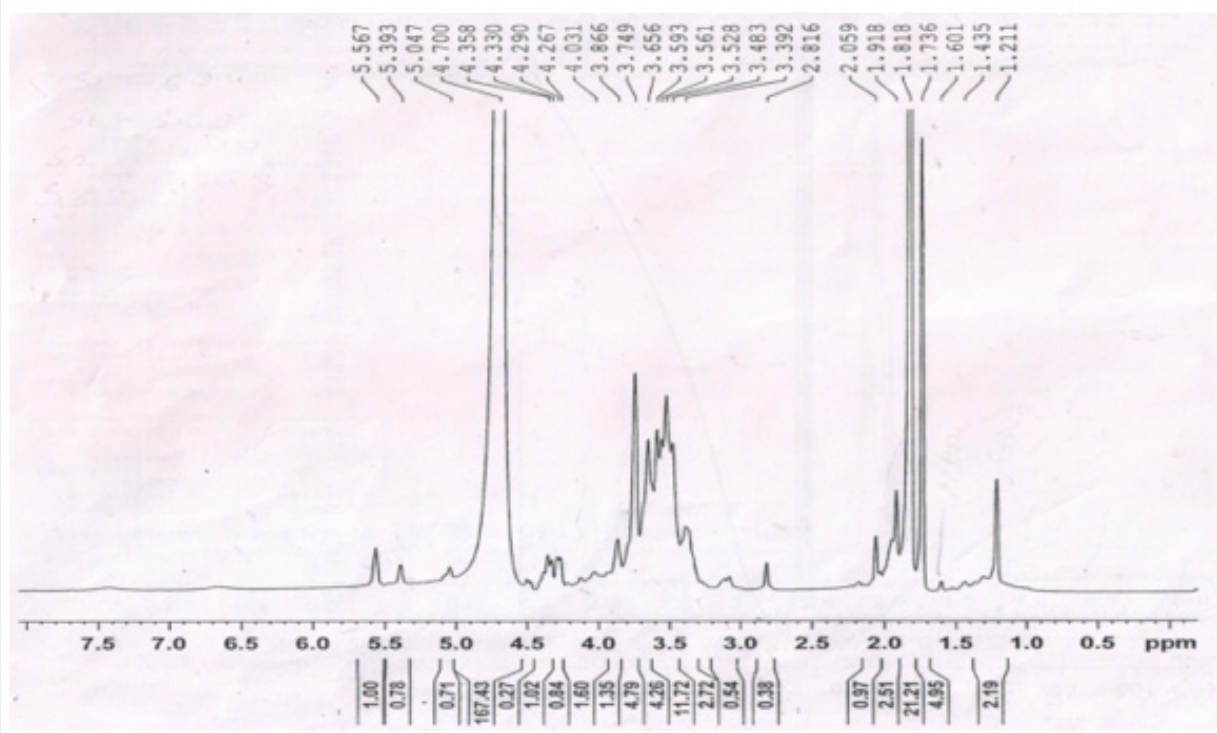

Figure 3: ^{1}H NMR spectrum of Friuose in D_2O at 300MHz

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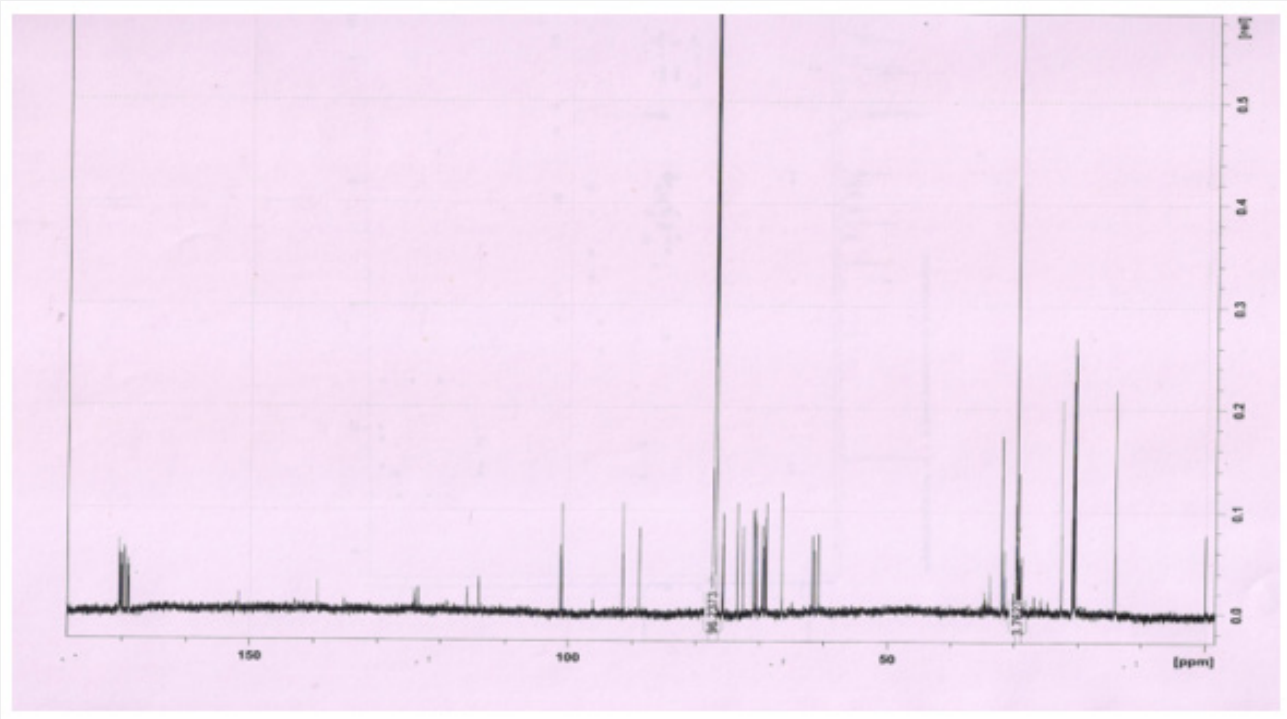

Figure 4: ^{13}C NMR spectrum of Friuose Acetate in $CDCl_3$ at 200MHz

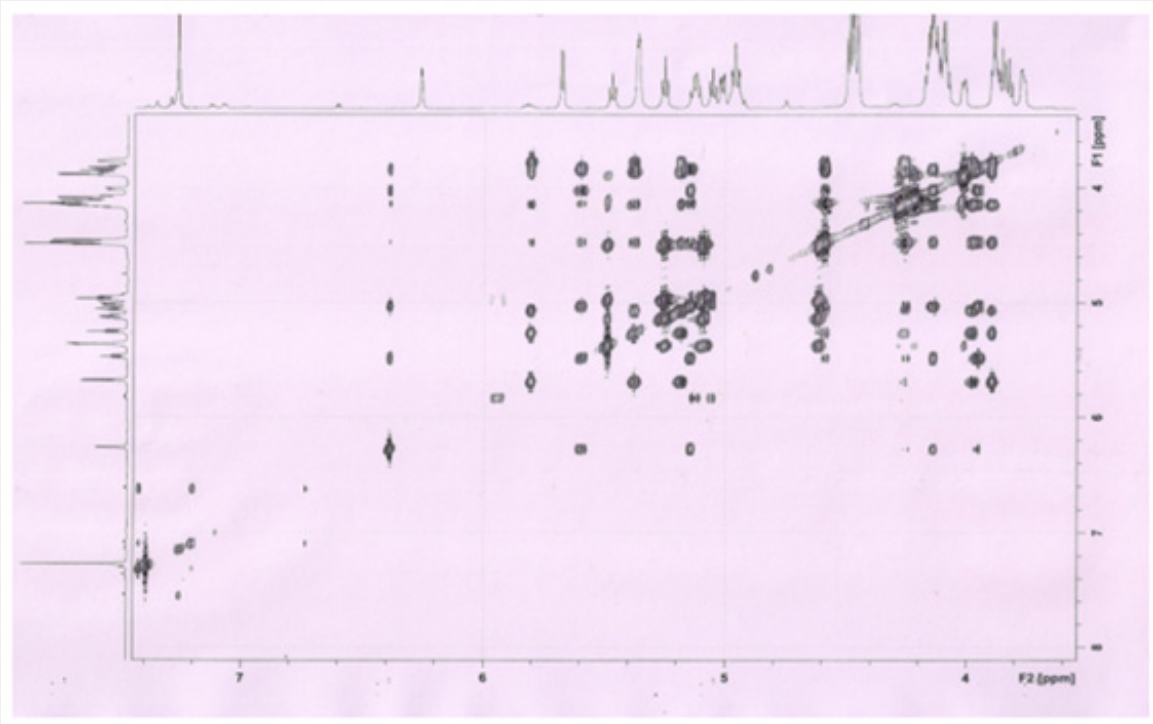

Figure 5: TOCSY spectrum of Friuose Acetate in $CDCl_3$ at 800MHz

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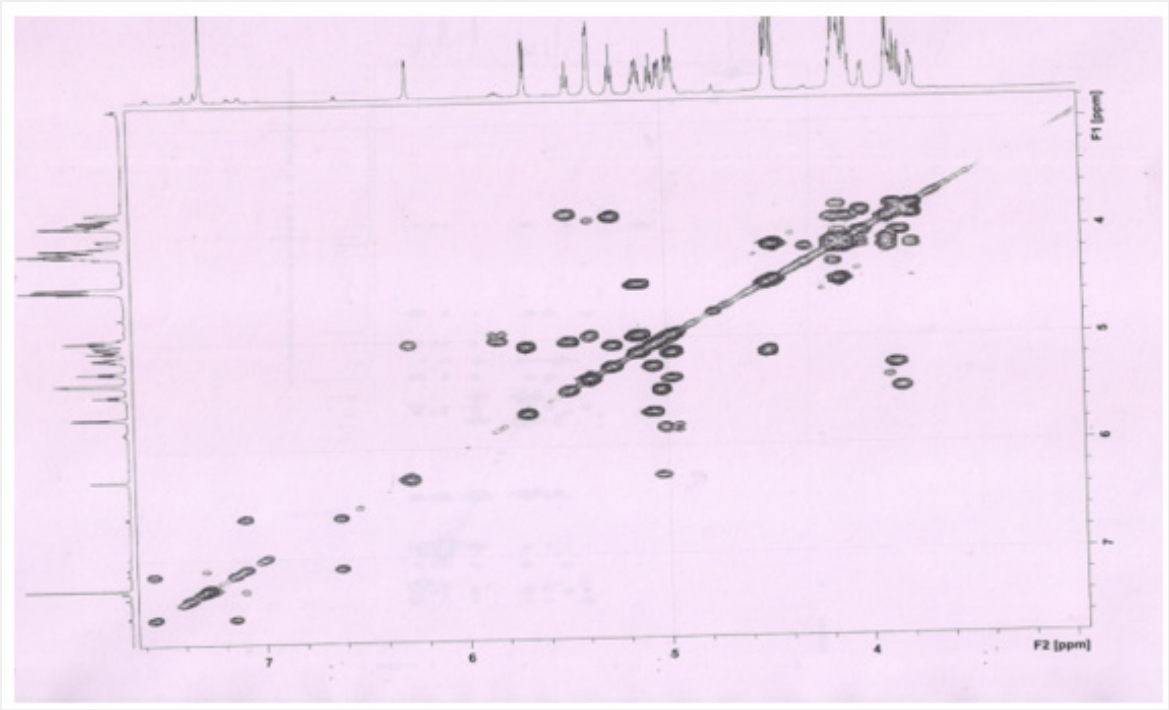

Figure 6: COSY spectrum of Fiuose Acetate in $CDCl_3$ at 800MHz

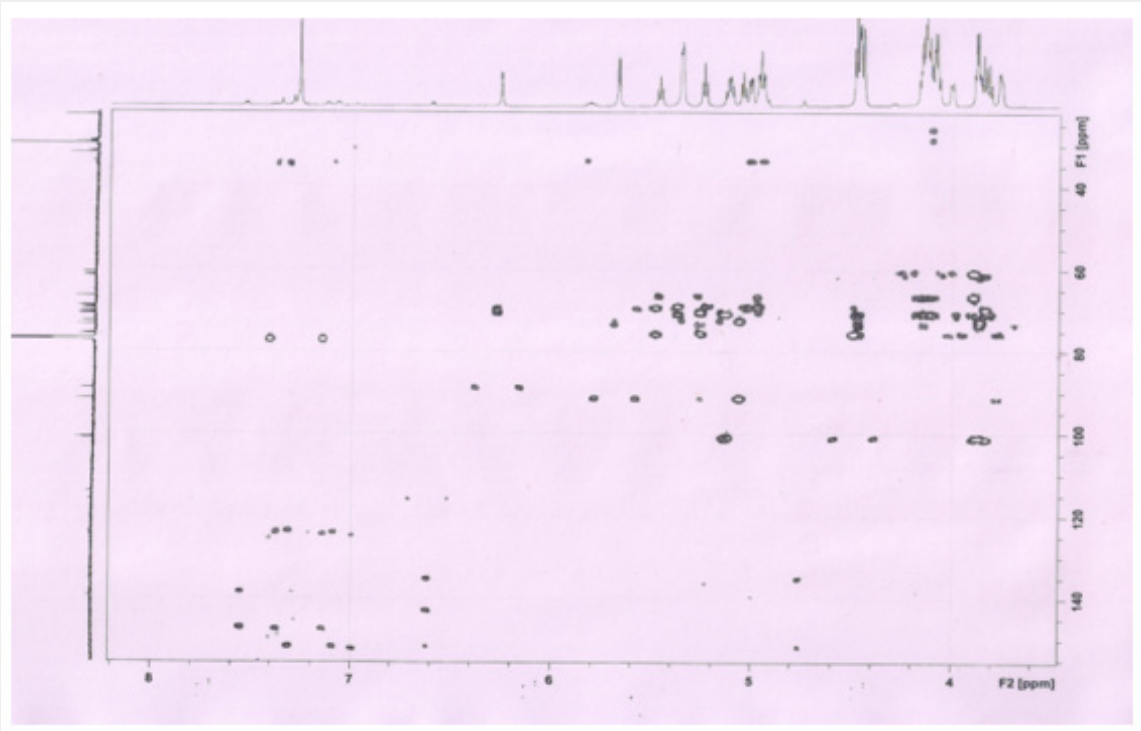

Figure 7: HMBC spectrum of Friuose Acetate in $CDCl_3$ at 800MHz

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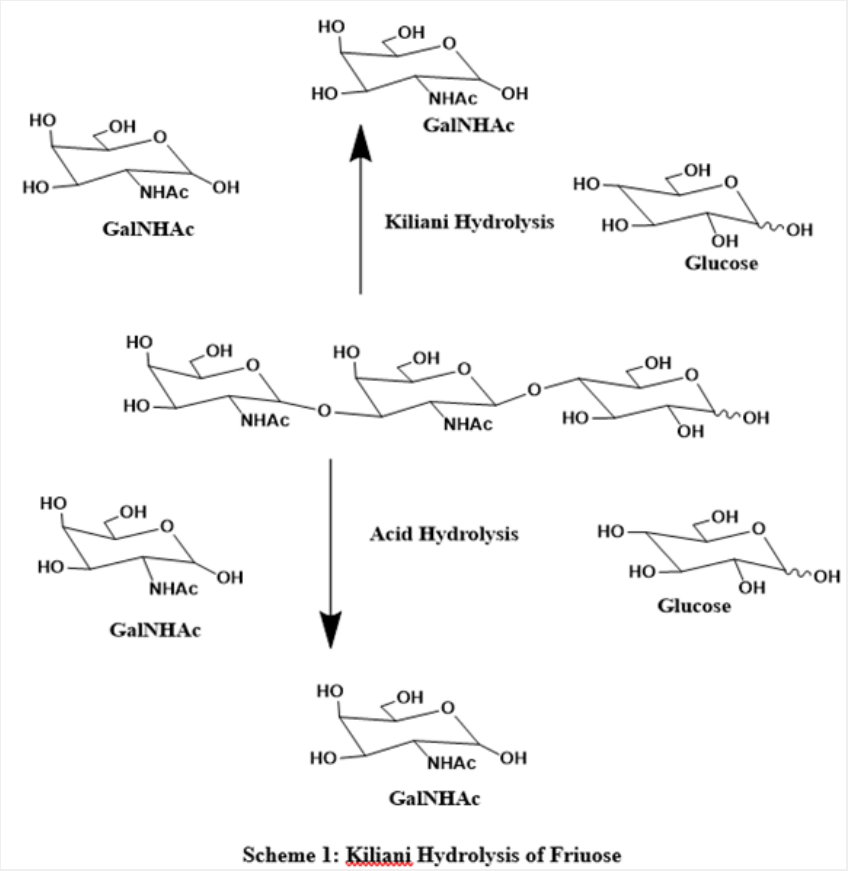

Scheme 1: Kiliani Hydrolysis of Friuose

Image 3

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

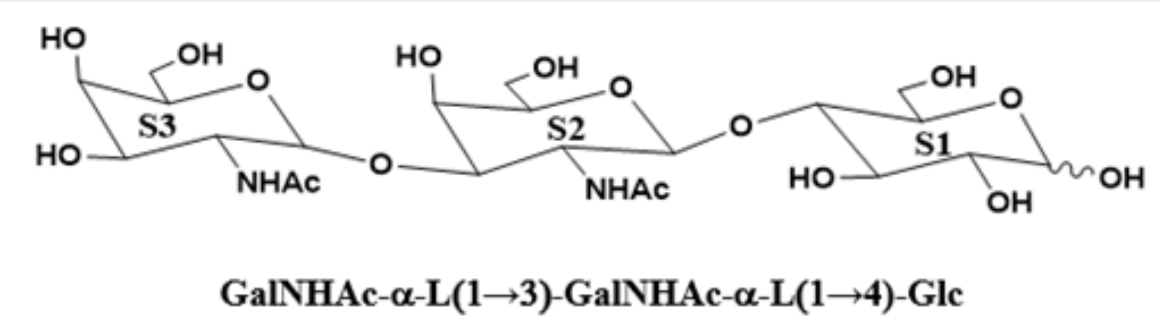

Image 4.

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The ESI Mass spectrometry data of Friuose (Figure 8) not only confirmed the derived structure but also confirmed the sequence of monosaccharides in Friuose [14]. The highest mass ion peaks were recorded at m/z 609 and 586 which were due to $[M+Na]^+$ and $[M]^+$ respectively, confirming the molecular weight of Friuose as 586 in agreement with its molecular formula $C_{22}H_{38}O_{16}N_2$. Further, the mass fragments were formed by repeated H transfer with elimination of terminal sugar less water. The triasaccharide, m/z 586 (I) fragmented to give mass ion at m/z 383 (II), this fragment arose due to the loss of terminal GalNHAc (S-3) moiety from trisaccharide indicating the presence of GalNHAc (S-3) at the non-reducing end. This disaccharide further fragmented to give mass ion peak at m/z

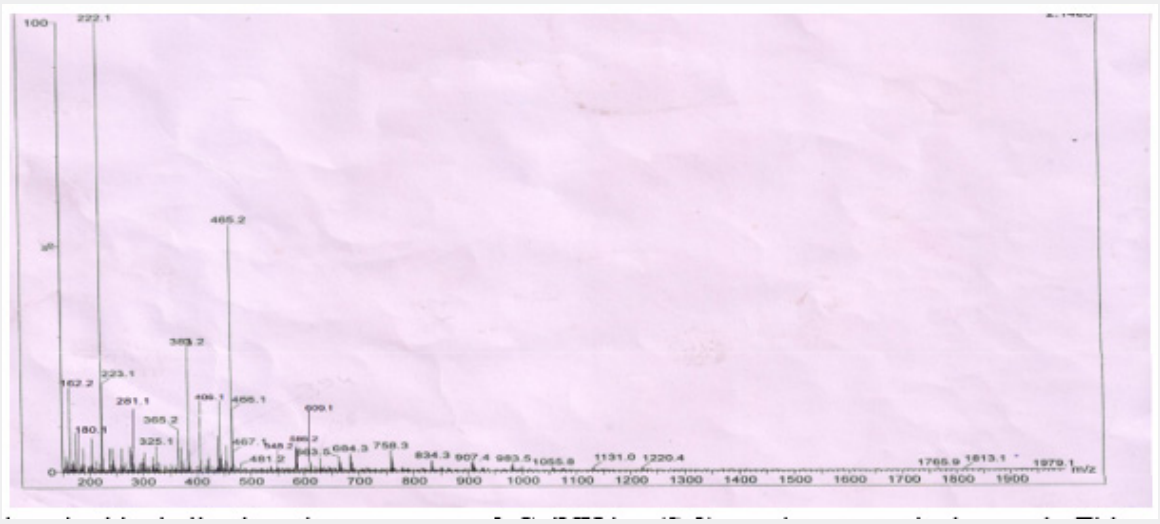
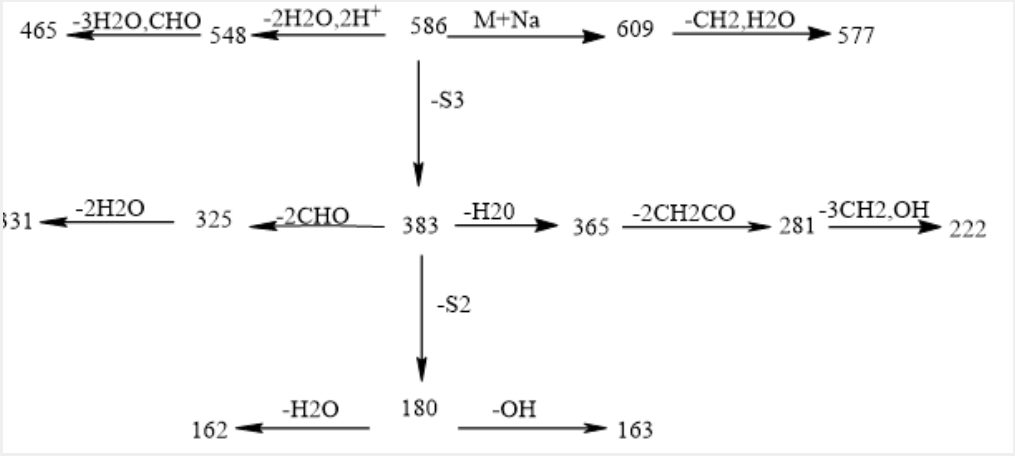


Figure 8: ESI-MS of Friuose

180 (III), which arose due to the loss of β-GalNHAc (S-2) moiety from disaccharide. The other fragmentation pathway in ESI-MSspectrum of Compound A, m/z 586 showed mass ion peak at 609$[M+Na]^+$, 586$[M]^+$, 577[609-CH_2H_2O], 548[586-$2H_2O$,$2H^+$], 465[548-$3H_2O$,CHO], 406[465-$3CH_2OH$], 383[586-S3], 365[383-H_2O], 325[383-2CHO], 281[365-$2CH_2CO$], 222[281-$3CH_2OH$], 180[383-S3], 162[180-H_2O]



Scheme 2: Mass Fragmentation of Friuose.

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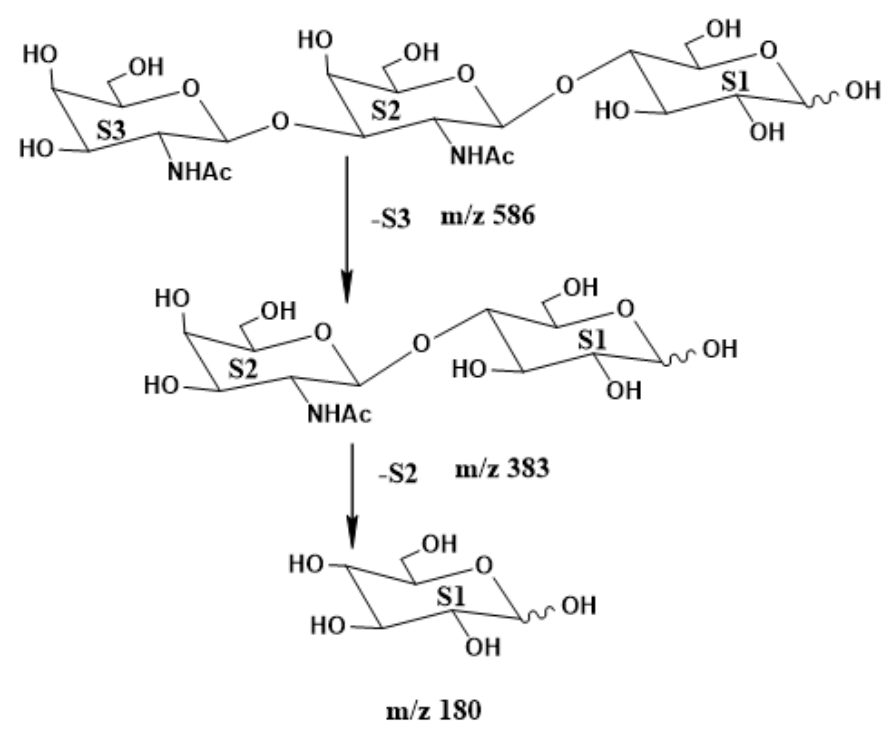

Scheme 3: Mass fragmentation of Friuose.

Conclusion

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

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References

1. 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.
2. Shukla M, Chauhan DDAPS, Deepak D (2024) Isolation, Structure Elucidation and Biological Activity of Milk Oligosaccharides with Special Reference to Indian Cow Breeds-A Review. Trends Carbohydr Res 16(4): 1-157.
3. Mana D, Kozhiyott Mohanan A, Venkatesha RN (2021) Milk and Milk Products in Ayurveda: A Review. Biol Life Sci Forum 6(1): 13.
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. Gronberg G, Lipniunas P, Lundgren T, Lindh F, Nilsson B (1990) Isolation and Structure Analysis of three New Disialylated Oligosaccharide from Human Milk. Arch Biochem Biophys 278(2): 297-311.
6. 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.
7. 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.
8. Schoefberger W, Schlagnitweit J, Muller N (2011) Recent Development in Hetronuclear Multiple Bond Correlation Experiments. Annual Rep NMR Spectrosc 72: 1-60.
9. Shukla M, Yadav S, Deepak D (2023) Isolation and Structure Elucidation of a Novel Oligosaccharide Bacuose. Trends Carbohyd Res 15(3): 66-82.
10. Partridge SM (1949) Aniline Hydrogen Phthalate as a Spraying Reagent for Chromatography of Sugars. Nature 164(4167): 443-443.
11. Feigl F (1975) Spot Tests in Organic Analysis. Elsevier.
12. Warren L (1960) Thiobarbituric Acid Spray Reagent for Deoxy Sugars and Sialic Acids. Nature 186(4720): 237-237.
13. Bush CA (1988) High Resolution NMR in the determination of structure in complex carbohydrates. Bull Magn Reson 10(3/4): 73-78.
14. Kumar K, Deepak D (2016) Mass Spectroscopy Techniques and Library of Monosaccharie Derivatives, Oligosaccharide and Glycosides: A Review 35(2): 928-995.

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