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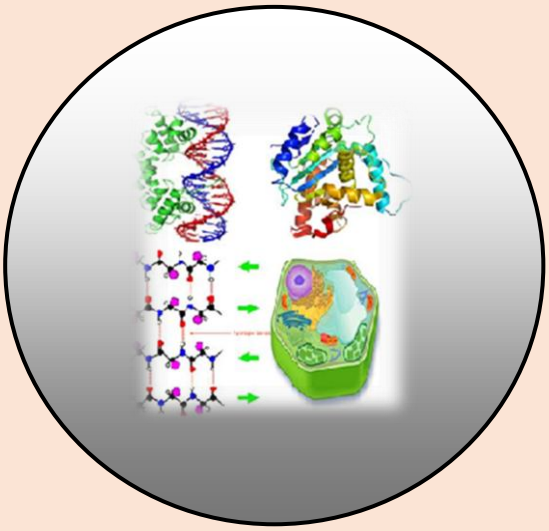

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RESEARCH PAPER

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Stereoscopic Structural Assignment and DFT Studies of Novel Tetrasaccharide 'Taliose' from Gaddi Sheep Milk with rare 1→2 Glycosidic Linkage at Reducing end by 2D NMR and Mass Spectrometry

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ABSTRACT

Carbohydrates are integral constituent of all living organisms and are associated with variety of vital functions, which sustain life. Oligosaccharide are amongst the most biologically diverse and important carbohydrate in biological system. The milk oligosaccharides inhibit the adherence of pathogens to target cells, hence oligosaccharides and their derivatives are used as therapeutic agents and form the basis for the development of anti-tumor vaccines and act as effective drugs in the therapy of pathogenic diseases. Sheep milk oligosaccharides contains fucose which is beneficial for skin diseases and cosmetic purposes., In the present study, to find biologically active novel milk oligoasaccharide, Sheep milk was processed by the method of Kobata and Ginsberg followed by gel filtration HPLC and column chromatography which resulted in the isolation of a novel milk oligosaccharide named Taliose. The structure of isolated and purified milk oligosaccharide was elucidated with the help of chemical degradation, chemical transformation, spectroscopy technique like NMR (^{1}H, ^{13}C, and 2D NMR) and mass spectrometry. The novelty of this novel compound was that it contained Gal(1→2) β-Glc at its reducing end. It is the first report of any milk oligosaccharide with 1→2 glycosidic linkage at the reducing end. The structure of isolated novel oligosaccharide 'Taliose' was interpreted as tetrasaccharide having the following structure which is as under-

β-Gal(1→3) β-GalNHAc(1→3) β -Gal(1→2) β-Glc

Taliose

Keywords: Carbohydrate, Sheep milk, Taliose and 2D NMR.

INTRODUCTION

Milk has processed a range of biologically active oligosaccharides that guard neonates and effects their physiological and pathological functions i.e., molecular reorganisation, signal transaction and exhibit varied biological activities such as anti-tumour (Shahani et al, 1983), anticancer (Rodríguez-Alcalá et al, 2017), immunostimulant (Saksena et al, 1999) and immunological (Fang et al, 1985). Oligosaccharides are an important constituent of milk, responsible for their biological activities. Amongst the various milk, the biological activity of sheep milk is well defined in Ayurveda and other ancient medicine literature. The constituents present in sheep milk have their affect in cardiovascular, nervus and immune system (Ranjan et al, 2015). Gaddi sheep's milk has highest amount of calcium and phosphate which potentially moisturises and nourishes the skin thus it is used to produce skin bars (Coni et al, 1996). Sheep milk protein is an important source of bioactive inhibitory and hypertensive defence and control of microbial infection. It also contains high levels of vitamins, minerals and amino acids, which are essential for the healing process and show homeostasis (Farag et al, 2020). The sheep milk aggravates hiccups and dyspnoea (Sushrut Sanhita). It elevates pitta and kapha. It also decreases the fat. In recent time, sheep milk has been used for enhancing the blood platelet count decreased during Dengue fever (Mahendru et al, 2011).

In view of the above fact, we have continued our studies on sheep milk for this purpose a rare species 'Gaddi Sheep' found at high altitude of Himalayan region was selected and its milk was collected in bulk and was processed by the modified method of Kobata and Ginsberg (Ranjan et al, 2015) involving deproteination, filtration, lyophilization followed by gel filtration, HPLC and column chromatography. During our course of investigation we have described the isolation of Oviose (Ranjan et al, 2015), Osiose (Singh et al, 2016), Ovinose (Ranjan et al, 2017), Ovisose (Sahu et al, 2017), Rieose (Shahi et al, 2017), Ariesose (Shahi et al, 2017) and Aliose (Jamal et al, 2023) and further isolated a novel tetrasaccharide, Taliose having a rare 1→2 glycosidic linkage. Here, we are describing the structure elucidation of the novel Gaddi sheep milk tetrasaccharide, Taliose along with its Density Functional Theory (DFT) studies.

The quantum chemical calculations have been performed on B3LYP functional and 6-31G (d, p) basis set employing DFT. The geometry of compound Taliose was first optimized and the presence of positive wave number values for all the optimized geometry indicated the stability of the compound. All computations were performed using the Gaussian 09 program package.

MATERIALS AND METHODS

General Procedure

General procedure was same as in our previous articles (Jamal et al 2019).

Isolation of Gaddi Sheep milk Oligosaccharide by Modified Method of Kobata and Ginsberg (Jamal et al, 2023)

Sephadex G-25 gel Chromatography of Gaddi Sheep Milk Oligosaccharides (Jamal et al, 2023)

Acetylation of Sheep milk Oligosaccharides (Jamal et al, 2023)

12.8 gm of Oligosaccharides mixture was acetylated with pyridine (12.8 ml) and acetic anhydride (12.8 ml) at 60^{o}C and solution was stirred overnight.

The mixture was evaporated under reduced pressure and viscous residue was taken in CHCl3 (250 ml) and washed twice by ice cold water which was further evaporated to dryness yielding the acetylated mixture (13.0 gm). The acetylation converted the free sugars into their non-polar acetyl derivatives which were resolved nicely on TLC [Cai, 2014], giving 8 spots i.e., a, b, c, d, e, f, g, and h from which compound Taliose acetate was finally separated by column chromatography over silica gel using varying proportions of hexane, chloroform and methanol as eluants.

Purification of Acetylated Oligosaccharide Mixture by Column Chromatography

Acetylated Sheep's milk oligosaccharides mixture (12.0 g) gave eight spots a, b, c, d, e, f, g and h, on TLC which on repeated column chromatography by various proportion of $CHCl_3$ and $CHCl_3$: MeOH resulted into isolation of compound (Taliose acetate) in pure form.

Deacetylation of Compound Taliose acetate (Kumar et al, 2019)

Tetrasaccharide Taliose acetate (30 mg) obtained from fractions 10 – 13 of column chromatography 3 of acetylated oligosaccharide mixture was dissolved in acetone (2 ml) and 2 ml of NH_3 was added and left overnight in a stoppered hydrolysis flask. After 24 h ammonia was removed under reduced pressure and the compound was washed with $CHCl_3$ and the water layer was finally freeze dried obtaining the deacetylated natural tetrasaccharide (14 mg).

Methylglycosidation/ Acid Hydrolysis of Compound Taliose (Agnihotri et al, 2019)

Compound Taliose (5mg) was refluxed 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 Taliose in 1,4-dioxane(1ml), 0.1 N H_2SO_4 (1 ml) was added and the solution was warmed for 30 minutes at 500^{o}Cand 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-methylgalactosides along with the Glc, Gal and GalNHAc. Their identification was confirmed by comparison with authentic samples (TLC, PC).

Kiliani Hydrolysis of Compound Taliose (Ranjan et al, 2023)

Compound Taliose (4 mg) was dissolved in 2 ml Kiliani mixture (AcOH-H_2O-HCl, 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 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 and GalNHAc on comparison with authentic samples of glucose, galactose and GalNHAc.

Description of Compound Taliose

Substance (30 mg) obtained from fractions 10-13 of column chromatography 3. On deacetylation of this acetylated tetrasaccharide with NH_3/Acetone it afforded compound TALIOSE (14 mg) as a viscous mass.

For experimental analysis, this compound was dried over P_2O_5 at 1000 C and 0.1 mm pressure for 10 hr. It gave positive Phenol-sulphuric acid test and Morgon-Elson test.

$C_{26}H_{45}O_{21}N$	**%C**	**%H**	**%N**
Calculated	44.13	6.36	1.98
Found	44.15	6.36	1.98

^{1}H NMR of Taliose acetate: δ in $CDCl_3$ at 300 MHz-

δ 6.22 [d, 1H,α-Glc (S-1), H-1)],δ 5.4 [d, 1H, β-Glc (S-1), H-1)], 3.903 [m, 1H, β-Glc (S-1) H-2] , 4.51 [d, 1H, J=6.0 Hz, β-Gal (S-2) H-1], 3.80 [m, 1H, β-Gal (S-2) H-3] , 4.53 [d, 1H, J=6.0 Hz, β-GalNHAc (S-3), H-1], 3.90 [m, 1H, β-GalNHAc (S-3) H-3], 4.49 [d, 1H, J=9.0 β-Gal (S-4), H-1)].

^{13}C NMR of Taliose Acetate: δ in $CDCl_3$ at 300 MHz

δ 90.14 [1C,β-Glc (S-1), C-1], δ 101.01 [1C, β-Gal (S-2), C-1], δ 101.09 [1C, β-GalNHAc (S-3)], C-1], δ 101.01 [1C, β-Gal (S-4), C-1].

ES mass of Taliose

747[M+K+H]$^+$,730[M+Na]$^+$,708[M+H]+,707[M]$^+$, 710[M+3H]$^+$, 650[M+H+$NHCOCH_3$], 617[M-HOCHCHOH-HCHO], 610[650- $2H_3O^+$- 2H], 575[617 CH_2CO], 571[650-CH_3OH-H_2O-CHO], 545[M-S4], 533[617-2CH_2CO] OR [575-CH_2CO], 491[533-617-CH_2CO] OR [617-3CH_2CO], 418[491-CH_3CO-CHO], 342[M-S4-S3], 331[418-CH_3CO-CH_2CO], 315 [331-CH_3-H$^+$], 289[331-CH_2CO] OR [418-CH_3CHO- 2CH_2CO], 267[331-CH_3CHO-H_3O+H], 247[331-2CH_2CO] OR [289-CH_2CO], 231[247-CH_3-H+], 214[231-OH], 188 [231-CH_3CO], 170[188-H_2O].

RESULTS AND DISCUSSION

Compound 'B', Taliose with molecular formula $C_{26}H_{45}O_{21}N$ was isolated from gaddi sheep milk into its acetylated form and designated as 'b' and gave positive phenol-sulphuric acid test test (Dubois et al, 1956), Feigl test (Figel et al, 1975) and Morgon-Elson test (Partridge et al, 1949) showing the presence of normal and amino sugar(s) in the oligosaccharide. The HSQC spectrum of acetylated Taliose in $CDCl_3$ at 300 MHz showed the presence of five cross peaks of anomeric protons and carbons in the respective region at □ 6.26 x 90.0, □ 5.45 x 90.14, □ 4.52 x 101.01, □ 4.50 x 101.09 and □ 4.50 x 101.01 suggesting the presence of five anomeric protons and carbons in it. The presence of five anomeric protons were further confirmed by the presence of five anomeric proton doublets at δ 6.26 (1H), δ 5.45 (1H), δ 4.52 (1H),and δ 4.50 (2H) in the ^{1}H NMR spectrum of acetylated Taliose in $CDCl_3$ at 300 MHz. The ^{13}C NMR spectrum of Taliose acetate in $CDCl_3$ at 300 MHz also confirmed the presence of five anomeric carbons at δ 90.0(1C), 90.14(1C), 101.01(2C) and 101.09(1C). The chemical shifts of α and β anomeric protons/carbons signals respectively at δ 6.26, δ 5.45 and □ 90.0, □ 90.14 confirmed that the reducing nature of oligosaccharide. The chemical shift value of α and β anomeric proton was having resemblance with the literature value of β-Glc thus the monosaccharide (S-1) was confirmed as β-Glc (Khan et al, 2017). The reducing nature of compound Taliose was further confirmed by its methylglycosidation by MeOH / H$^+$ followed by its acid hydrolysis which led to the isolation of □ and □-methyl glucoside along with Gal and GalNHAc leading to the presence of glucose at the reducing end in the oligosaccharide.

Table 1. Assignment of anomeric protons and carbons of Taliose acetate by HSQC spectrum.

SUGAR	^{13}C	^{1}H
S1	90.14	5.45
S2	101.01	4.52
S3	101.09	4.50
S4	101.01	4.50

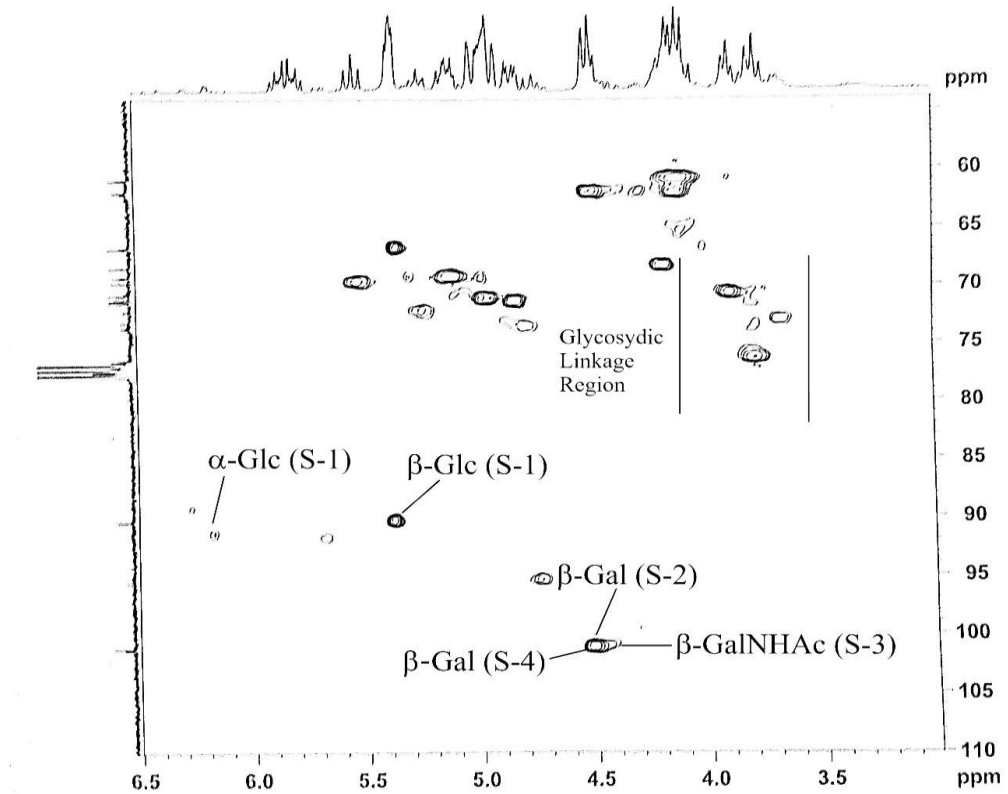
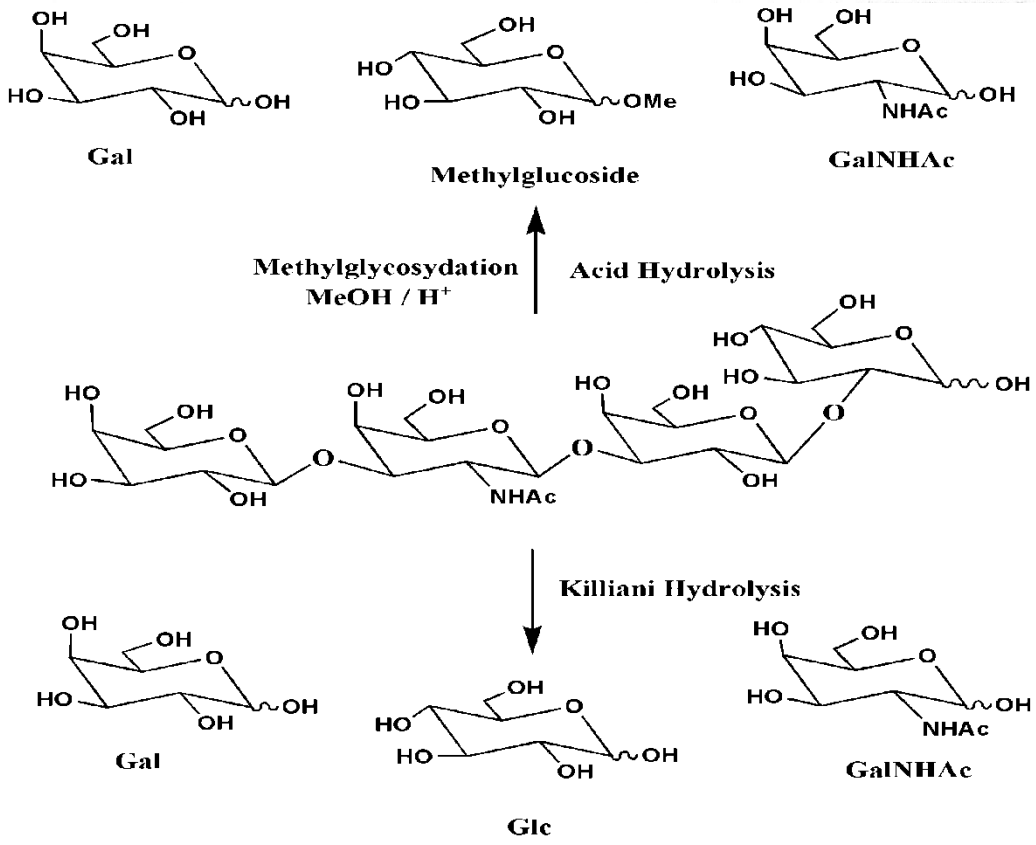


Fig : HSQC Spectrum of Taliose Acetate in CDCl3 at 300 MHz



Methylglycosidation / Acid Hydrolysis and Killiani Hydrolysis of Taliose

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. The monosaccharide constituents in Taliose were also confirmed by its Killiani hydrolysis (Killiani et al, 1930) under strong acidic condition followed by paper chromatography and TLC. In this hydrolysis three spots were found identical with the authentic samples of Glc, Gal and GalNAc by co-chromatography (PC, TLC), which confirmed that Taliose contained three types of monosaccharide units i.e., Glc, Gal and GalNAc. The ^{1}H and ^{13}C NMR spectra of Taliose justify the five anomeric signals for tetrasaccharide with total integral intensity of four anomeric protons and carbons. Further the mass ion peak at 707 $[M]^{+}$ present in ES-MS of Taliose was in agreement with molecular formula of Taliose **$C_{26}H_{45}O_{21}N$**.

The anomeric proton signal present at δ5.45 in ^{1}H NMR Spectrum of Taliose acetate assigned to β-Glc (S-1) (Jamal et al, 2023) gave three cross peaks at δ5.45x3.90, δ5.45x4.90 and δ5.45x5.5 in TOCSY Spectrum of Taliose acetate which was later identified as H-2, H-3 and H-4 of reducing Glc respectively by COSY spectrum of Taliose acetate. The chemical shift of the cross peak at δ5.45x3.90 suggested that in glucose S-1, H-2 position was available for glycosidic linkage by next monosaccharide unit. Further the ^{1}H signal present at δ3.90 assigned to H-2 of reducing Glc (S-1) gave a cross peak at δ3.90x101.01 in HMBC spectrum of Taliose acetate which was between H-2 of reducing Glc and C-1 of S-2, confirmed the (1→2) linkage between Glc (S-1) and S-2. The anomeric carbon of S-2 at δ101.01 gave its complimentary anomeric proton signal at δ4.52 (6.0 Hz) in the HSQC spectrum of Taliose acetate. The chemical shift values of anomeric carbon at δ101.01 and anomeric proton at δ4.52 were having resemblance with literature value of anomeric chemical shift value of Gal hence S-2 was confirmed as Gal (Jamal et al, 2023). The large coupling constant J=6.0 Hz of □□Gal (S-2) confirmed the □-configuration of the linkage between β-Glc (S- 1) and β-Gal (S-2).

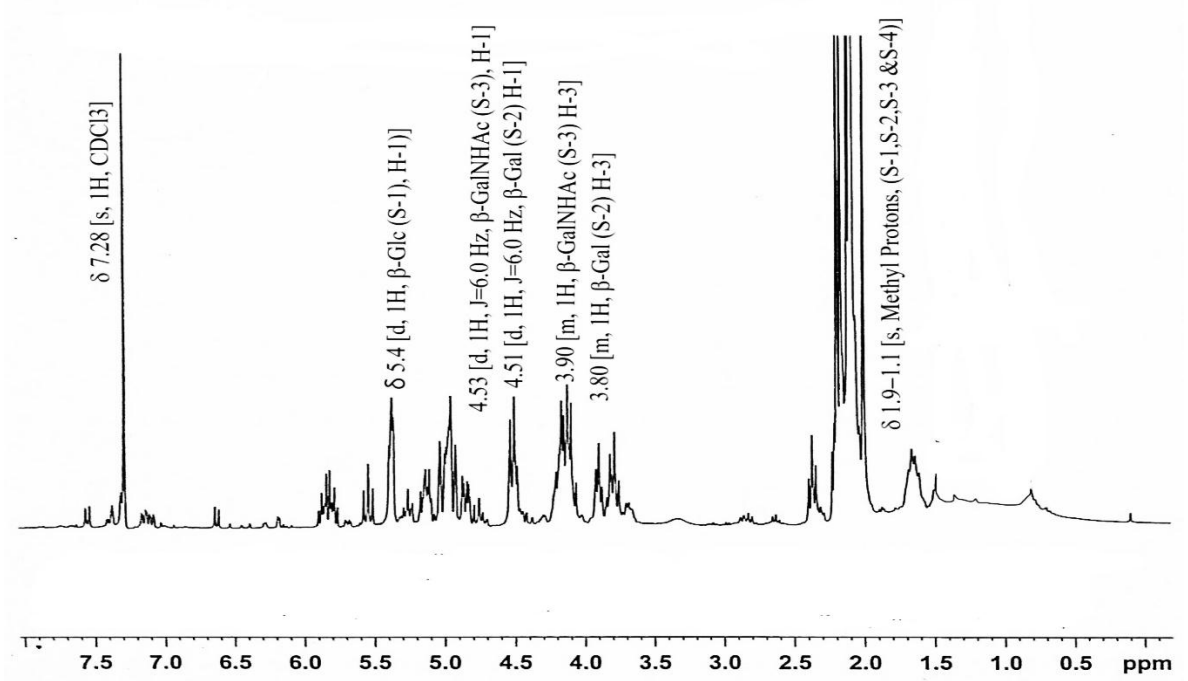


Fig : ^{1}H NMR Spectra of Taliose Acetate at 300 MHz in $CDCl_3$

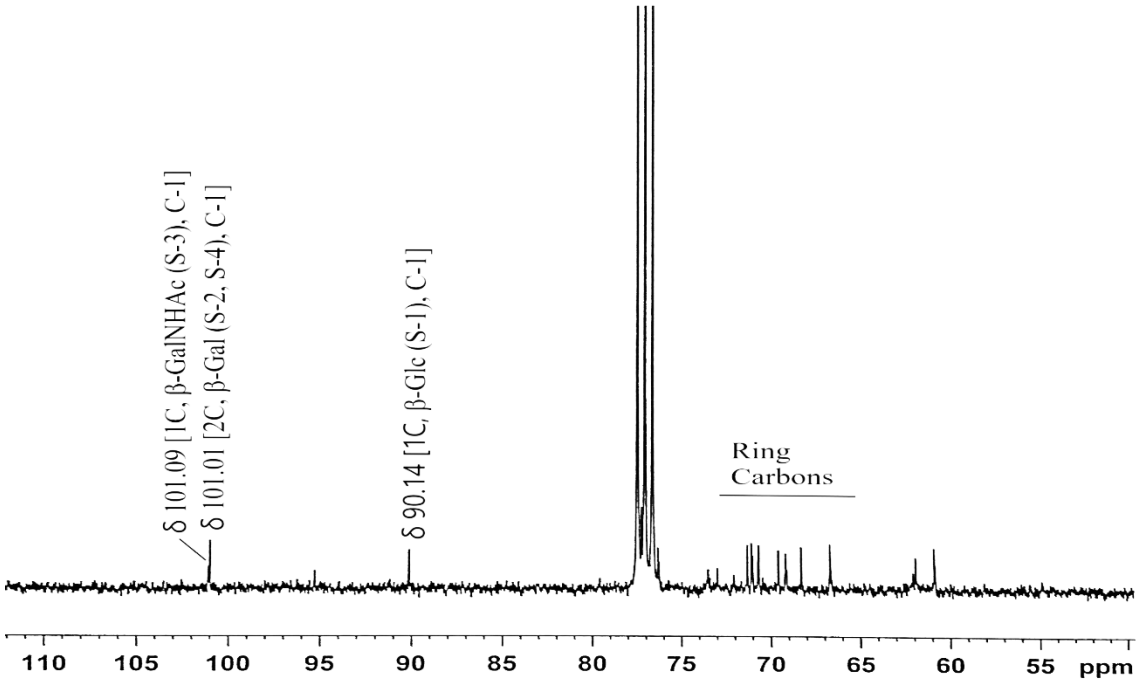


Fig : ^{13}C NMR Spectrum of Taliose Acetate at 300 MHz in $CDCl_3$

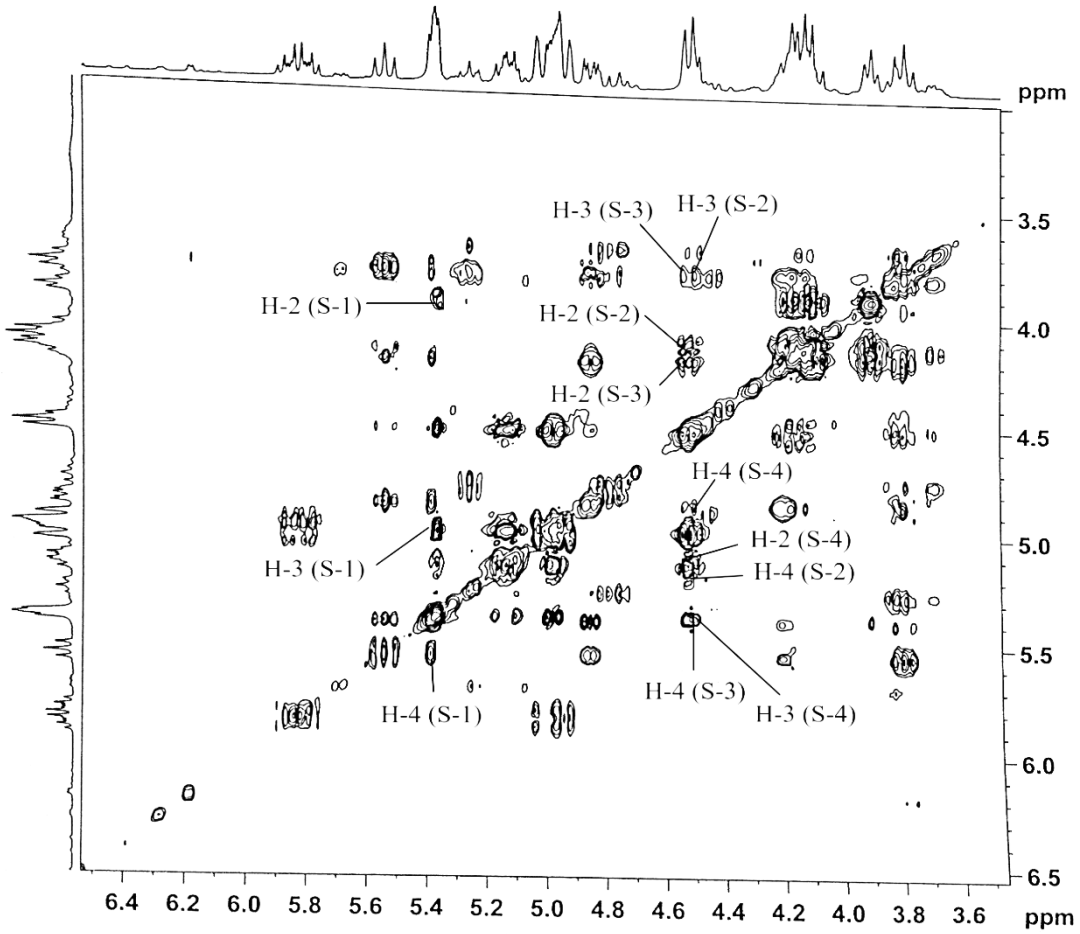


Fig : TOCSY Spectrum of Taliose Acetate at 300 MHz in $CDCl_3$

The next anomeric proton signal present at δ4.52 in ^{1}H NMR Spectrum of Taliose acetate assigned to β-Gal (S-2) gave three cross peaks at δ4.52x3.80, δ4.52x4.20 and δ4.52x5.21 in TOCSY Spectrum of Taliose acetate, which were later identified as H-3, H-2 and H-4 respectively with COSY spectrum of Taliose acetate. Out of these signals one proton signal at δ3.80 corresponded to H-3 position of β-Gal (S-2) suggested that H-3 was available for glycosidic linkage by the next monosaccharide unit. Further ^{1}H signal present at δ3.80 assigned to H-3 of Gal (S-2) gave a cross peak at δ3.80x101.09 in HMBC spectrum of Taliose acetate which was between H-3 of Gal (S-2) and C-1 of S-3, confirmed the (1→3) linkage between Gal (S-2) and S-3. The anomeric carbon of S-3 at δ101.09 gave its complimentary anomeric proton signal at δ4.50 (6.0 Hz) in the HSQC spectrum of Taliose acetate. The chemical shift values of anomeric carbon at δ101.09 and anomeric proton at δ4.50 were having resemblance with literature value of anomeric chemical shift value of GalNHAc hence S-3 was confirmed as GalNHAc (Singh et al, 2016). The large coupling constant J=6.0 Hz of □□GalNHAc (S-3) confirmed the □-configuration of the linkage between β-Gal (S-2) and β-GalNHAc (S-3).

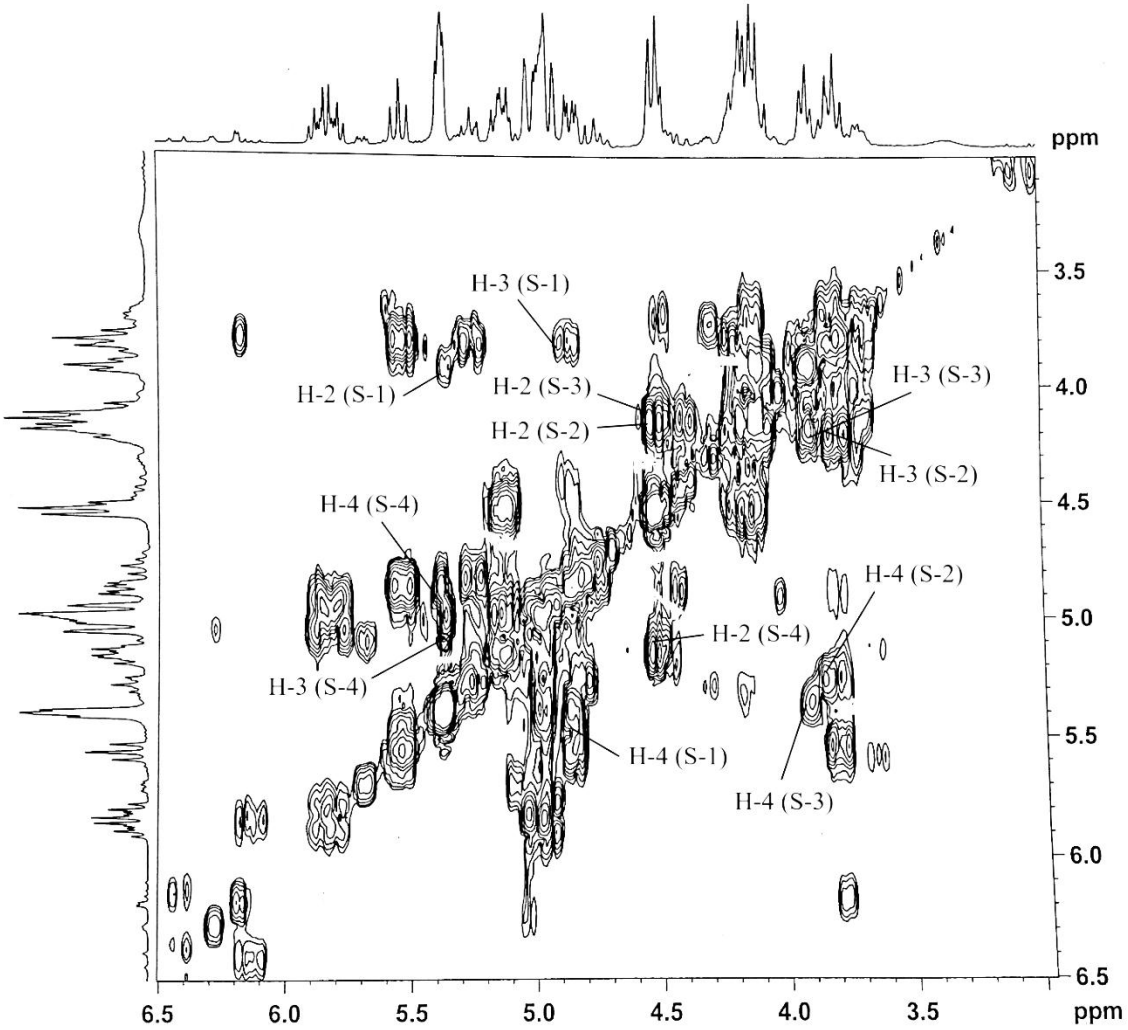


Fig : COSY Spectrum of Taliose Acetate at 300 MHz in $CDCl_3$

Further the anomeric proton signal present at δ4.50 in ^{1}H NMR Spectrum of Taliose acetate assigned to β-GalNHAc (S-3) gave three cross peaks at δ4.50x3.90, δ4.50x4.10 and δ4.50x5.40 in TOCSY Spectrum of Taliose acetate, which were later identified as H-3, H-2 and H-4 respectively with COSY spectrum of Taliose acetate. Out of these signals one proton signal at δ3.90 corresponded to H-3 position of β-GalNHAc (S-3) suggested that H-3 was available for glycosidic linkage by the next monosaccharide unit. ^{1}H signal present at δ3.90 assigned to H-3 of GalNHAc (S-3) gave a cross peak at δ3.90x101.01 in HMBC spectrum of Taliose acetate which was between H-3 of GalNHAc(S-3) and C-1 of S-4, confirmed the (1→3) linkage between GalNHAc (S-3) and S-4. The anomeric carbon of S-4 at δ101.01 gave itscomplimentary anomeric proton signal at δ4.50 (6.0 Hz) in the HSQC spectrum of Taliose acetate. The chemical shift values of anomeric carbon at δ101.01 and anomeric proton at δ4.50 were having resemblance with literature value of anomeric chemical shift value of Gal hence S-4 was confirmed as Gal (Yoon et al, 2003). The large coupling constant J=6.0 Hz of β-Gal (S- 4) confirmed the β-configuration of the linkage between β-GalNHAc (S-3) and β-Gal (S- 4).

Further the next anomeric proton signal present at δ4.50 in ^{1}H NMR Spectrum of Taliose acetate assigned to β-Gal (S-4) gave three cross peaks at δ4.50x4.90, δ4.50x5.10 and δ4.50x5.40 in TOCSY Spectrum of Taliose acetate, which were later identified as H-4, H- 2 and H-3 respectively with COSY spectrum of Taliose acetate. Since in the TOCSY spectrum of Taliose acetate does not show any complementary methine signals of □- Gal (S4) in glycosidic linkage region i.e., □ 3-4 showed that none of their OH groups were involved in glycosidic linkages suggested that □-Gal(S-4) was present at the non- reducing end. All the ^{1}H NMR assignments for ring proton of monosaccharide units of Taliose were confirmed by COSY and TOCSY experiments.

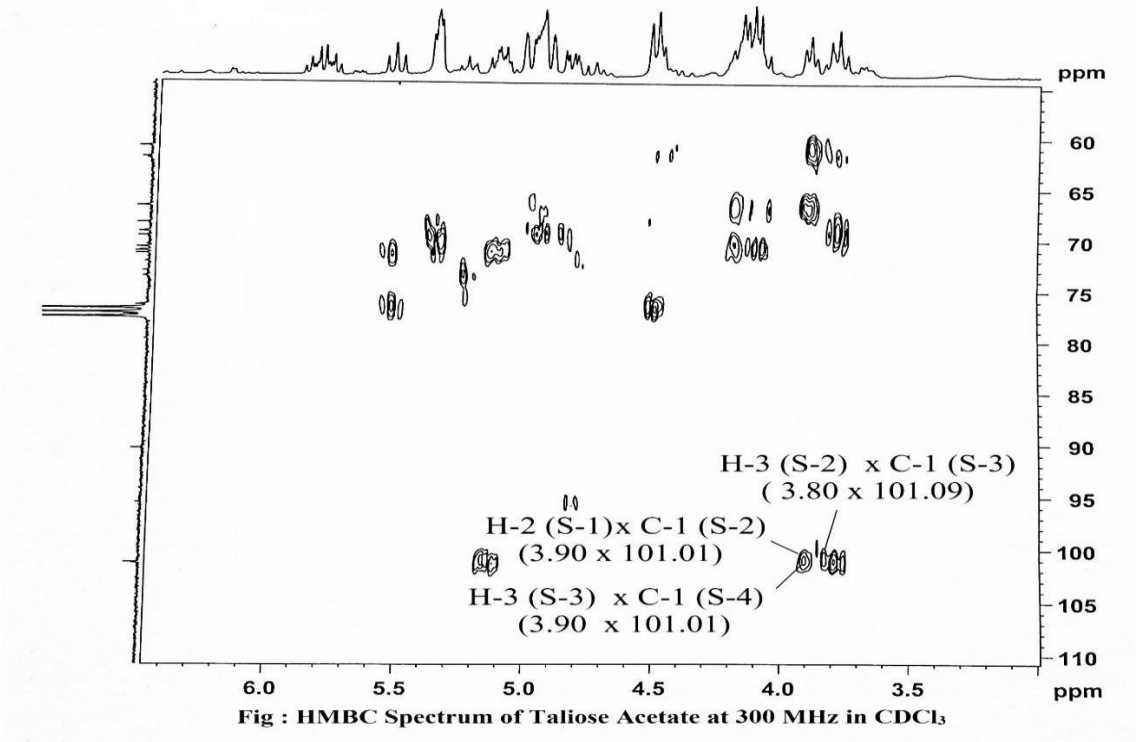


Fig : HMBC Spectrum of Taliose Acetate at 300 MHz in $CDCl_3$

Table 2. Order of ring protons in monosaccharides assigned by TOCSY spectrum.

ORDER OF S1	ORDER OF S2	ORDER OF S3	ORDER OF S4
5.45	4.52	4.50	4.50
3.90	3.80	3.90	4.90
4.90	4.20	4.10	5.10
5.5	5.21	5.40	5.40

Table 3. Assignment of ring proton correlation by COSY spectrum of Taliose acetate.

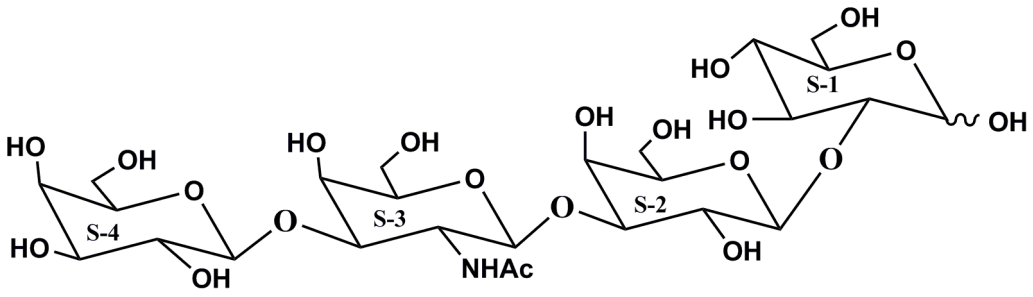
NO. OF PROTON	ORDER OF S1	ORDER OF S2	ORDER OF S3	ORDER OF S4
H1	5.45	4.52	4.50	4.50
H2	3.903	4.20	4.10	5.1
H3	4.90	3.80	3.90	5.4
H4	5.5	5.21	5.40	4.90

Table 4. Assignment of glycosidic linkages by HMBC spectrum of Taliose acetate.

Monosaccharide Linkages	HMBC cross peaks	TYPE OF LINKAGE
S1-S2	δ 3.90 × 101.01	1→2 (β-Gal→β-Glc S1)
S2-S3	δ 3.80 × 101.09	1→3 (β-GalNHAc→ β-Gal)
S3-S4	δ 3.90 × 101.01	1→3 (β-Gal→β-GalNHAc)

The heteronuclear single quantum coherence (HSQC) spectrum of acetylated compound Taliose confirmed linkages in ^{1}H and ^{13}C NMR spectra by showing cross peaks of Glc(S1) H-2 and C-2 at (3.903 x 71.06) showed (12) linkage of S2 and S1 i.e., its 2- position of Glc (S1) were involved in linkage, Gal (S2) H-3 and C-3 (3.80 x 76.61) showed (13) linkage of S3 S2. GalNHAc (S3) H-3 and C-3 at (3.90 x 71.28) showed (13) linkage of S4 and S3 showing in the same chemical region in acetylated and deacetylated spectra. It was further confirmed by the presence of same peaks in COSY and TOCSY spectrum. Thus, based on the pattern of chemical shifts of ^{1}H, ^{13}C, HOMOCOSY , and TOCSY, HSQC NMR experiments, it was interpreted that the compound Taliose was a tetrasaccharide consist of one Glc, two Gal and one GalNHAc moieties in it and having the following structure:

β-Gal(1→3) β-GalNHAc(1→3) β -Gal(1→2) β-Glc



TALIOS

MASS FRAGMENTATION

The Electrospray Mass Spectroscopy data of compound not only confirmed the derived structure but also supported the sequence of monosaccharide in Taliose. The highest mass ion peak was recorded at m/Z 731 and 747 which was due to $[M+Na+H]^+$ and $[M+K+H]^+$ respectively. The other mass ion peak recorded at m/Z 708 which was due to $[M+H]^+$ confirming the molecular weight of Taliose as 707 and was in agreement with its molecular formula $C_{26}H_{45}O_{21}N$. Further the mass fragments were formed by repeated H transfer in the tetrasaccharide and was accompanied by the elimination of terminal sugar less H_2O. The tetrasaccharide m/Z 707 (I) fragmented to give mass ion at m/Z 545 (II) [707-162 (S4)], this fragment was arised due to loss of β-Gal (S_4) moiety from tetrasaccharide .The fragment (II) at m/Z 545 was arised due to the loss of β-Gal (S_4) which further fragmented to give mass ion peak at m/z 342 (III) [545- 203(S_3)] which was a disaccharide (III), was arised due to loss of β-GalNAc (S_3) moiety from trisaccharide (II).The disaccharide (III) again fragmented to give mass ion peak at m/z 180 (IV) [342-162 ($S_{2)}$], which was due to loss of Gal (S_2) moiety from disaccharide (III) .These three mass ion peak II, III, IV were appeared due to the consequent loss of S_4, S_3 and S_2 from original molecule. The other fragmentation pathway in ES-MASS spectrum of Taliose, m/z 707 shows the mass ion peaks at 726 $[M+H_3O^+]$, 710 $[M+3H]^+$, 696 $[710\text{-}CH_3^+]$, 684 $[717\text{-}H_2O\text{-}CH_3^+\text{-}H]$, 675 $[707\text{-}CH_3OH]$, 668 $[710\text{-}CH_2CO]$, 660 [707-HCHO-OH] and [654 $[668\text{-}CH_3^+]$.

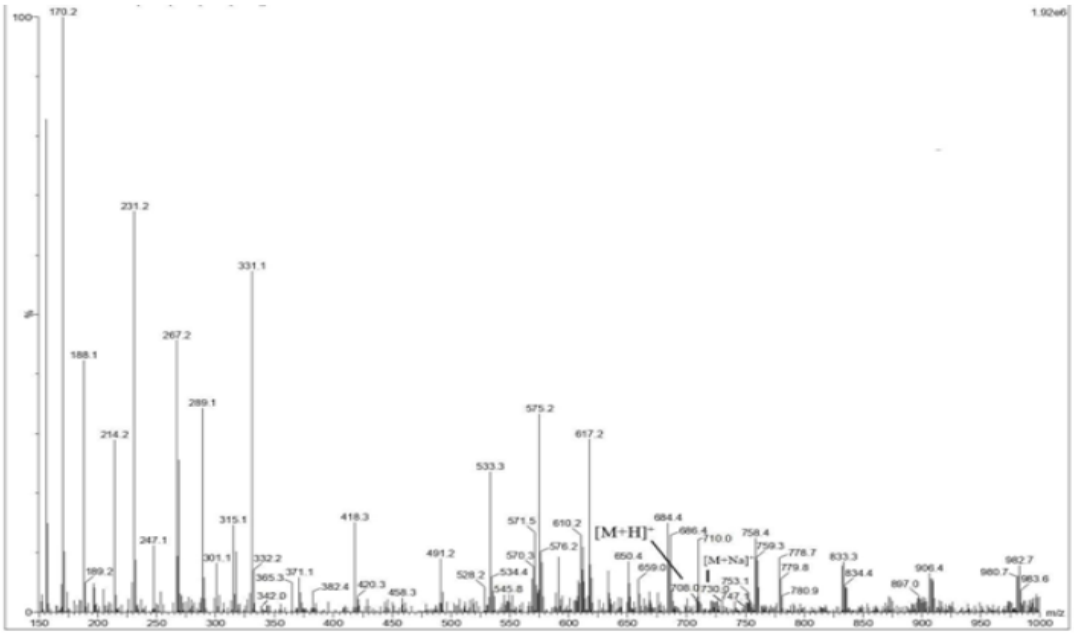
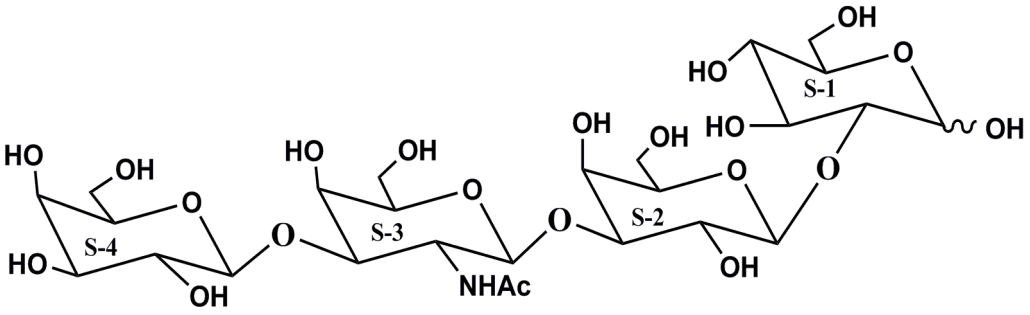
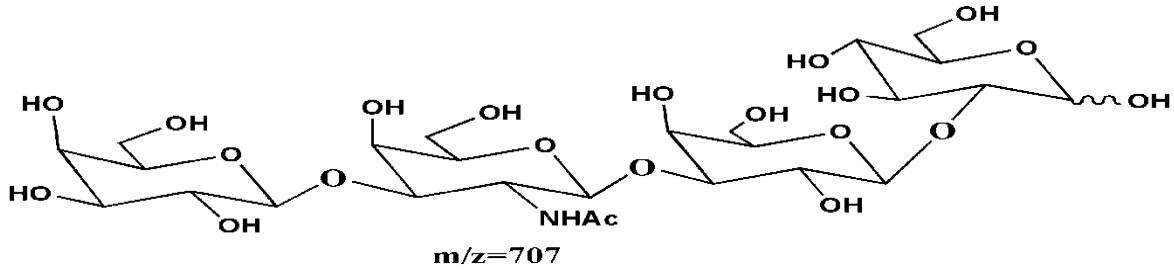


Figure: ES-Mass spectrum of compound Taliose

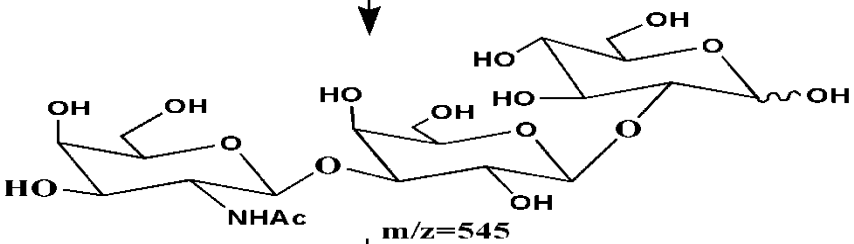
Based on results obtained from chemical degradation/acid hydrolysis, Chemical transformation, Electro spray mass spectrometry and ^1H, ^{13}C NMR and HOMOCOSY, TOCSY and HSQC 2-D NMR techniques the structure and sequence of isolated sheep milk oligosaccharide molecule Taliose was deduced as -



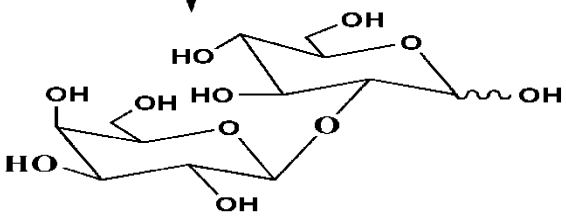
TALIOSE



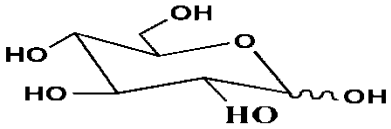
m/z=707



m/z=545



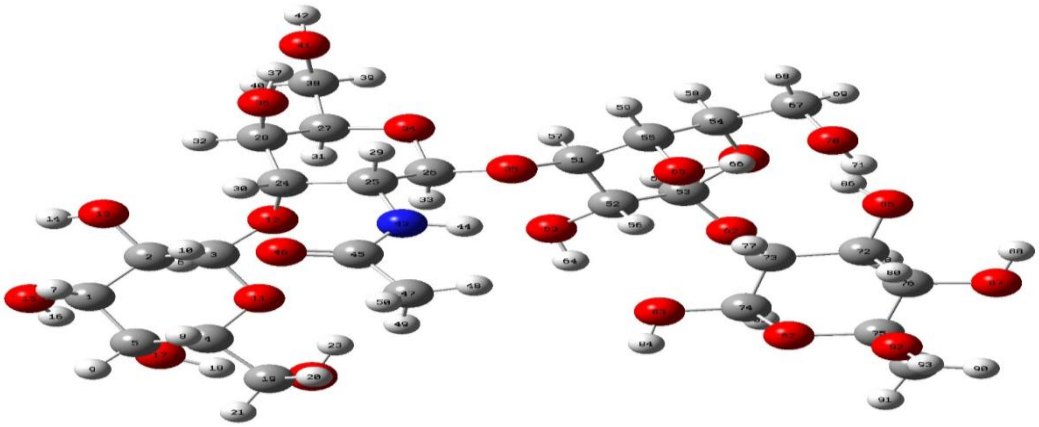
m/z=342



m/z=180

MASS-FRAGMENTATION OF COMPOUND TALIOSE

OPTIMISED STRUCTURE OF TALIOSE

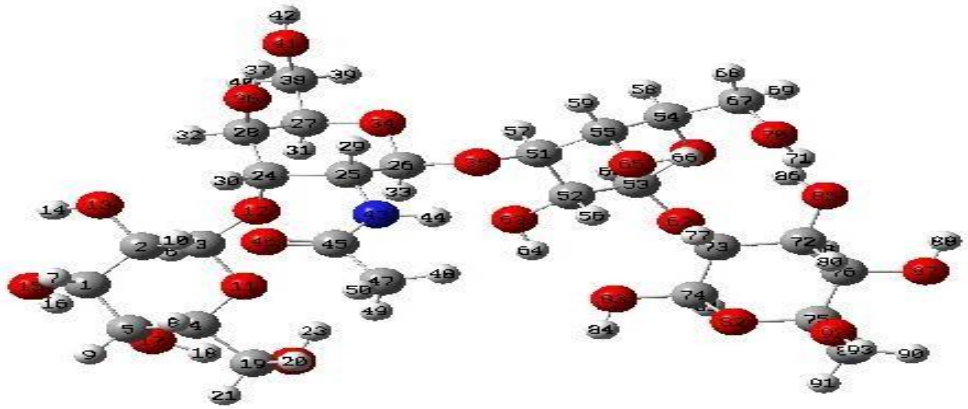


COMPUTAIONAL ANALYSIS OF ISOLATED OLIGOSACCHARIDE TALIOSE

The computational studies were performed on Taliose using Density Functional Theory (DFT) of Gaussian 09 W programme. For this, structure of Taliose was drawn on Gauss View 5.0 and was further optimized. The optimized structure was utilized for the calculations of molecular orbitals, i.e., HOMO and LUMO. Other evaluations like bond lengths, bond angles, molecular electrostatic potential and mulliken charges were also performed from the optimized geometry.

OPTIMISED STRUCTURE OF TALIOSE (Tiwari et al, 2023)

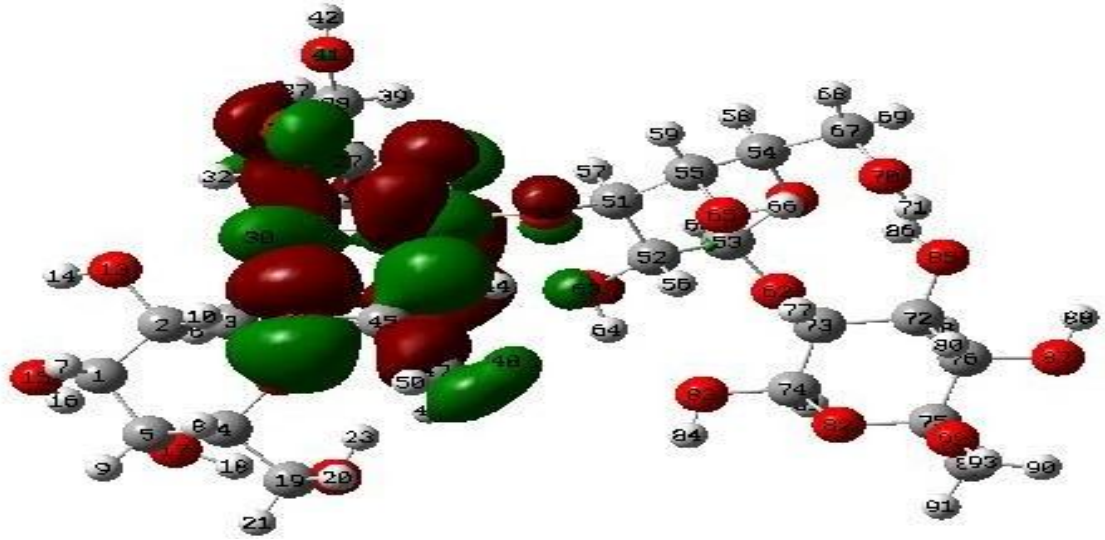
The structural geometry was optimized by minimizing its energies compared to all geometrical variables without forcing any molecular symmetry restrictions. The molecular structures of the optimized compounds were drawn by Gauss View 5.0.



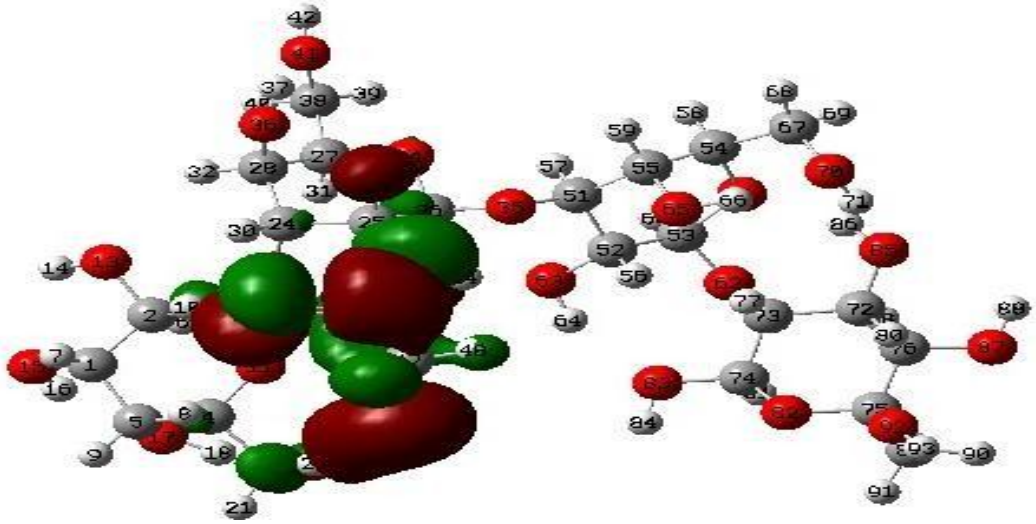
FRONTIER MOLECULAR ORBITALS

Frontier molecular orbitals (FMOs) are the highest occupied molecular orbital (HOMO) with electrons, so it is an electron donor and the lowest unoccupied molecular orbital (LUMO) that has a space to accept electrons, so it is an electron acceptor. These orbitals control the mode of the interaction of the compounds with the receptors. Moreover, HOMO and LUMO are very important quantum chemical parameters to determine the reactivity of the molecules and are used to calculate many important parameters such as the chemical reactivity descriptors.

Structure of HOMO of Taliose

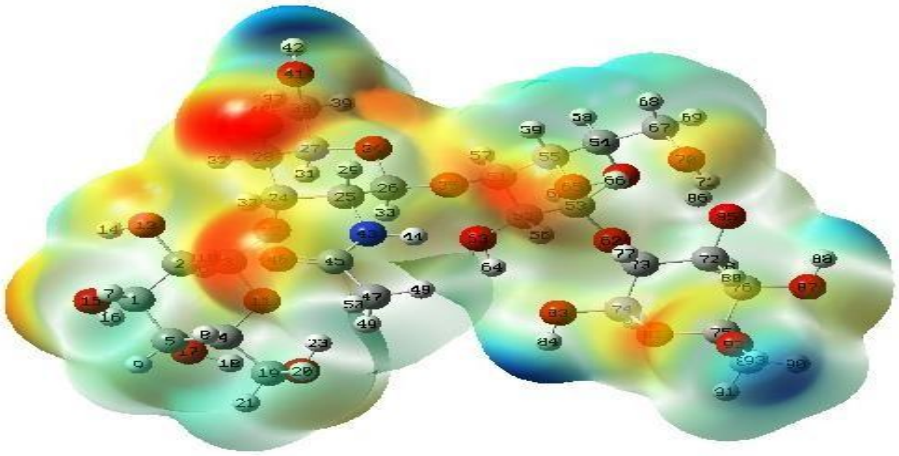


Structure of LUMO of Taliose



MOLECULAR ELECTROSTATIC POTENTIAL (Ghous et al, 2023)

The MEP gives an indication about the molecular size and shape of the positive, negative as well as the neutral electrostatic potential. In the MEP, the maximum negative region is the preferred sites for electrophilic attack, indicated as red color. So, an attacking electrophile will be attracted by the negatively charged sites, and the opposite situation for the blue regions. It is obvious that the molecular size and the shape as well as the orientation of the negative, positive, and the neutral electrostatic potential varied according to the compound because of the type of the atoms and its electronic nature. The structure shows the total electron density is as under:



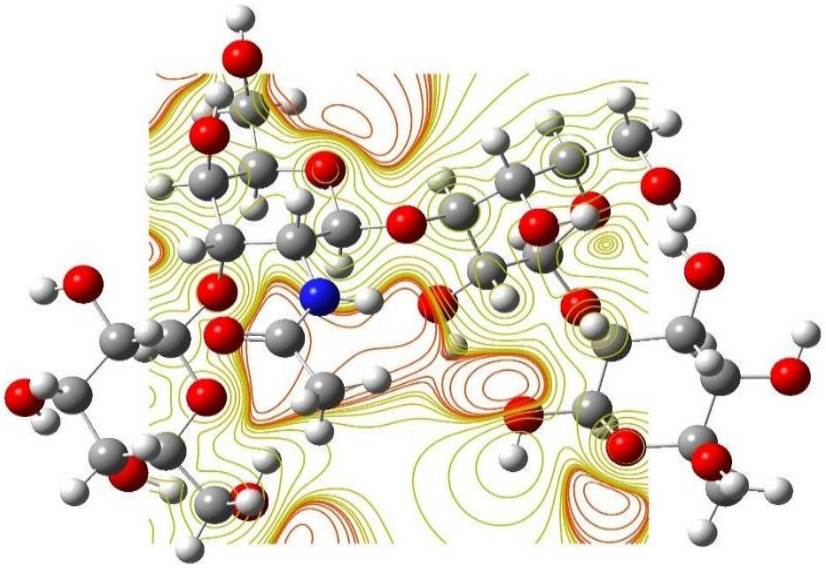
GLOBAL REACTIVITY DESCRIPTORS

The reactivity descriptors such as electronegativity (χ) = –1/2(εLUMO + εHOMO), chemical potential (μ) = 1/2 (εLUMO + εHOMO), global hardness (η) = 1/2 (εLUMO – εHOMO), global softness (S) = 1/2η and electrophilicity index (ω) = μ2 /2η are good approach to predict global reactivity trends in the compound. Electronegativity, chemical potential (μ), global hardness (η), global softness (S), and electrophilicity index (ω) had been calculated and listed in the following table:

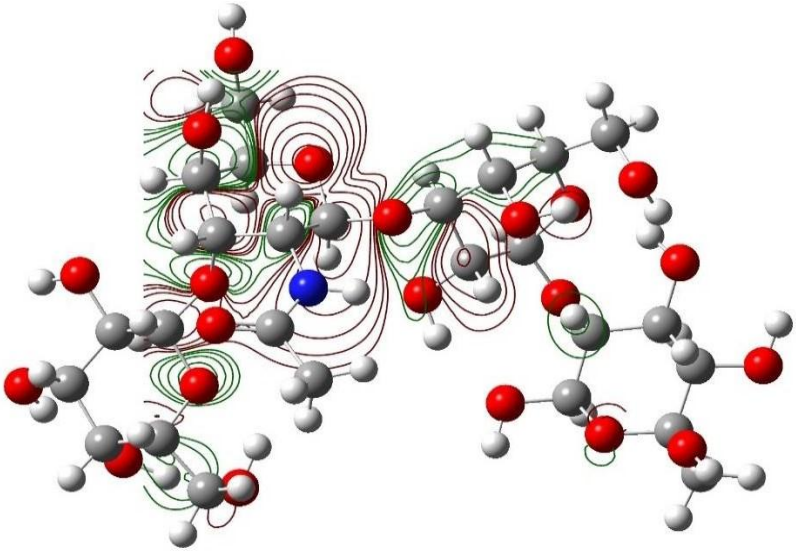
Sugar	HOMO	LUMO	E	χ	η	S	ω	μ
Taliose	-8.04	-1.649	7.09	5.70	3.44	0.08	1.440	5.34

COUNTOUR SURFACES – Various contour surfaces such as electrostatic potential contour surfaces, HOMO contour surfaces and LUMO contour surfaces of the isolated trisaccharide enTaliose were obtained by DFT calculations using Gauss 09W programme and B3LYP basis set and mentioned below-

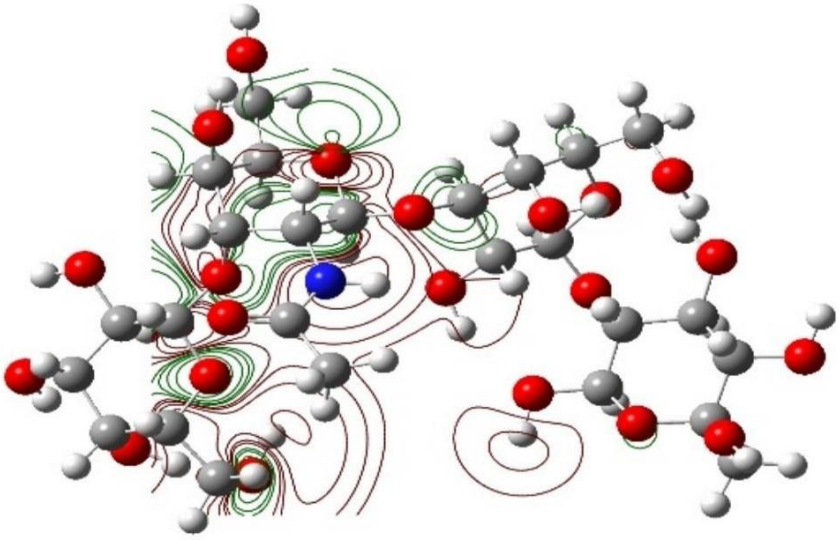
1. Electrostatic Potential Contour Surface of Taliose



2. HOMO Contour Surfaces of Taliose



3. LUMO Contour Surfaces of Taliose



MULLIKEN CHARGES (Singh et al, 2016)

The Mulliken atomic charges of the estimated compounds were calculated by the DFT using B3LYP basis set, the data for compound is arranged in the following Table. It showed that the average charge on carbon atoms is approx. 0.22 and average charge on oxygen atoms is about -0.51. Compound Taliose has amino sugar therefore nitrogen atom is also present in the compound having -0.315544 mulliken charge on it. All oxygenshave negative charges and all carbons have positive charges only in Compound Taliose.

The positively charged centers are the most susceptible sites for nucleophilic attacks. However, the most negatively charged centers are the most susceptible sites for electrophilic one. Sum of mulliken charges is -0.0000.

The mulliken charges on the atoms of compound Taliose are mentioned in the following table:

S. NO.	ATOM NO.	ATOM	CHARGES	S. NO.	ATOM NO.	ATOM	CHARGES
1	1	C	0.223831	18	34	O	-0.526689
2	2	C	0.266857	19	35	O	-0.550873
3	3	C	0.516888	20	36	O	-0.235530
4	4	C	0.198176	21	38	C	0.273518
5	5	C	0.255048	22	41	O	-0.257881
6	11	O	-0.536382	23	43	N	-0.315544
7	12	O	-0.538458	24	45	C	0.556999
8	13	O	-0.244287	25	46	O	-0.514378
9	15	O	-0.233946	26	47	C	0.002894
10	17	O	-0.272493	27	51	C	0.286349
11	19	C	0.282691	28	61	O	-0.587504
12	22	O	-0.223922	29	62	O	-0.518941
13	24	C	0.266299	30	63	O	-0.232123
14	25	C	0.298122	31	65	O	-0.241754
15	26	C	0.504283	32	67	C	0.275614
16	27	C	0.234446	33	75	C	0.245515
17	28	C	0.280173	34	76	C	0.294568

BOND LENGTH

The theoretical calculation done by DFT using Gauss 09W programme, the C-C, C-H and C-O bond lengths were obtained and arranged in the following table. The data shows that the bond length for C-C single bond is approx. 1.5299 A^O while bond length between C- H is approx. 1.1021.The bond length for O-H is 0.9688 while C-O bond length is 1.427. Taliose has amino-sugar therefore it has C-N and N-H bond lengths also. C25-N43 bond length is 1.4663, N43-C45 bond length is 1.3609 and N43-H44 bond length is 1.0125 A^O.

PARAMETER	BOND LENGTH	PARAMETER	BOND LENGTH	PARAMETER	BOND LENGTH
C1-C2	1.5337	C26-O35	1.4320	C54-C67	1.5326
C1-C5	1.5320	C27-C28	1.5407	C55-H59	1.0989
C1-H7	1.0952	C27-H31	1.1001	C55-O65	1.4486
C1-O15	1.4528	C27-O34	1.4697	O62-C73	1.4649
C2-C3	1.5208	C27-C38	1.5185	O63-H64	0.9844
C2-H6	1.0970	C28-H32	1.0987	O65-H66	0.9916
C2-O13	1.4485	C28-O36	1.4468	C67-H68	1.0928
C3-H10	1.0954	O35-C51	1.4530	C67-H69	1.0964
C3-O11	1.4689	O36-H37	0.9860	C67-O70	1.4583
C3-O12	1.4109	C38-H39	1.0963	O70-H71	0.9911
C4-C5	1.5402	C38-H40	1.0984	C72-C73	1.5264

C4-H8	1.0998	C38-O41	1.4631	C72-C76	1.5251
C4-O11	1.4654	O41-H42	0.9745	C72-H78	1.0985
C4-C19	1.5278	N43-H44	1.0125	C72-O85	1.4602
C5-H9	1.0995	N43-C45	1.3609	C73-C74	1.5188
C5-O17	1.4576	C45-O46	1.2585	C73-H77	1.0927
O12-C24	1.4690	C45-C47	1.5205	C74-H81	1.1014
O13-H14	0.9813	C47-H48	1.0942	C74-O82	1.4470
O15-H16	0.9853	C47-H49	1.0967	C74-O83	1.4306
O17-H18	0.9899	C47-H50	1.0921	C75-C76	1.5350
C19-H20	1.0949	C51-C52	1.5245	C75-H79	1.1012
C19-H21	1.0915	C51-C55	1.5366	C75-O82	1.4720
C19-O22	1.4671	C51-H57	1.0968	C75-C89	1.5166
O22-H23	0.9846	C52-C53	1.5392	C76-H80	1.0952
C24-C25	1.5366	C52-H56	1.0896	C76-O87	1.4508
C24-C28	1.5383	C52-O63	1.4458	O83-H84	0.9787
C24-H30	1.0886	C53-H60	1.0932	O85-H86	0.9929
C25-C26	1.5327	C53-O61	1.4662	O87-H88	0.9787
C25-H29	1.0968	C53-O62	1.4300	C89-H90	1.0969
C25-N43	1.4663	C54-C55	1.5401	C89-H91	1.0978
C26-H33	1.0943	C54-H58	1.1001	C89-O92	1.4520
C26-O34	1.4553	C54-O61	1.4711	O92-H93	0.9765

BOND ANGLE

Bond angle is a parameter which helps in the formation of molecular structure. In this study, different bond angles were calculated in the isolated compound Taliose. Values of calculated bond angles among different three atoms inside the molecule are mentioned in the following table. Average bond angle is approx. 110.55 whereas maximum bond angle is about (C25-N43-C45) 128.3106. Those bond angles in which nitrogen atom is involved are have higher values then other bond angles such as - C25-N43-H44, C25-N43-C45, H44-N43-C45, N43-C45-O46 and N43-C45-C47.

PARAMETER	BOND ANGLE	PARAMETER	BOND ANGLE	PARAMETER	BOND ANGLE
C2-C1-C5	111.6964	O34-C26-O35	108.2675	C51-C55-C54	109.3454
C2-C1-H7	108.7533	C28-C27-H31	107.2269	C51-C5-H59	108.6105
C2-C1-O15	109.0230	C28-C27-O34	110.2139	C51-C55-O65	107.6900
C5-C1-H7	110.5961	C28-C27-C38	115.5175	C54-C55-H59	108.6267
C5-C1-O15	110.5827	H31-C27-O34	108.7513	C54-C55-O65	111.8742
H7-C1-O15	106.0058	H31-C27-C38	107.6256	H59-C55-O65	110.6344
C1-C2-C3	110.3197	O34-C27-C38	107.3245	C53-O61-C54	120.4323
C1-C2-H6	109.3996	C24-C28-C27	109.9726	C53-O62-C73	116.6143
C1-C2-O13	109.2539	C24-C28-H32	107.8457	C52-O63-H64	106.9226
C3-C2-H6	108.2611	C24-C28-O36	105.5550	C55-O65-H66	108.9892

C3-C2-O13	108.5587	C27-C28-H32	108.6926	C54-C67-H68	109.9531
H6-C2-O13	111.0378	C27-C28-O36	113.1666	C54-C67-H69	109.3891
C2-C3-H10	112.1354	H32-C28-O36	111.4512	C54-C67-O70	112.2216
C2-C3-O11	107.1749	C26-O34-C27	111.8059	H68-C67-H69	108.6016
C2-C3-O12	111.3647	C26-O35-C51	117.1174	H68-C67-O70	106.0241
H10-C3-O11	108.7610	C28-O36-H37	108.4648	H69-C67-O70	110.5492
H10-C3-O12	110.9014	C27-C38-H39	108.6661	C67-O70-H71	111.0056
O11-C3-O12	106.2220	C27-C38-H40	109.0013	C73-C72-C76	110.9263
C5-C4-H8	108.7556	C27-C38-O41	109.5801	C73-C72-H78	109.4412
C5-C4-O11	111.5551	H39-C38-H40	109.2697	C73-C72-O85	110.7091
C5-C4-C19	113.5273	H39-C38-O41	110.3822	C76-C72-H78	109.0824
H8-C4-O11	109.3147	H40-C38-O41	109.9121	C76-C72-O85	107.9465
H8-C4-C19	110.2904	C38-O41-H42	111.8114	H78-C72-O85	108.6825
O11-C4-C19C1- C5-C4	103.2683 110.9127	C25-N43-H44 C25-N43-C45	112.6278 128.3106	O62-C73-C72O62- C73-C74	109.0024 107.7766
C1-C5-H9	109.5936	H44-N43-C45	118.6818	O62-C73-H77	111.3044
C1-C5-O17	105.3756	N43-C45-O46	124.4336	C72-C73-C74	111.0317
C4-C5-H9	108.4882	N43-C45-C47	114.8191	C72-C73-H77	108.9930
C4-C5-O17	113.7850	O46-C45-C47	120.7286	C74-C73-H77	108.7393
H9-C5-O17 C3-O11-C4	108.5979 114.0282	C45-C47-H48 C45-C47-H49	113.1747 109.1903	C73-C74-H81 C73-C74-O82	110.7732 109.6810
C3-O12-C24 C2-O13-H14	118.2007 107.1944	C45-C47-H50 H48-C47-H49	108.8399 108.3130	C73-C74-O83 H81-C74-O82	108.4029 109.7889
C1-O15-H16 C5-O17-H18	106.1031 110.2742	H48-C47-H50 H49-C47-H50	109.3025 107.8884	H81-C74-O83 O82-C74-O83	110.7385 107.3818
C4-C19-H20 C4-C19-H21	110.1730 111.9612	O35-C51-C52 O35-C51-C55	112.4150 107.0289	C76-C75-H79 C76-C75-O82	108.8007 109.6430
C4-C19-O22	108.9663	O35-C51-H57	108.4973	C76-C75-C89	113.1238
H20-C19-H21	109.4087	C52-C51-C55	107.1136	H79-C75-O82	109.1450

DIHEDRAL ANGLES

A dihedral angle is defined as the angle between two planes both of which pass through the same bond. There is much weaker preference for particular values of the dihedral angle around single bonds. Usually, the value of 0^o (eclipsed) is avoided, and values of around 60^o (staggered) to 90^o are somewhat preferred, depending on the number of lone pairs on the termini. 179.9017 is the maximum dihedral angle among H19-C4-O11-C3 atoms in the oligosaccharide. The values of some important dihedral angles in the isolated tetrasaccharide Taliose are arranged in the following table:

Parameter	D. Angle	Parameter	D. Angle	Parameter	D. Angle
C5-C1-C2-C3	-56.17240	O12-C24-C25-N43	58.04980	H19-C4-O11-C3	179.9017
C5-C1-C2-H6	62.81750	C28-C24-C25-C26	-178.9162	C5-C4-H19-H20	-171.5068
C5-C1-C2-O13	-175.4373	C28-C24-C25-H29	-177.8290	C5-C4-H19-H21	-49.51030

H7-C1-C2-C3	66.16560	C28-C24-C25-N43	-62.87750	C5-C4-H19-O22	67.72110
H7-C1-C2-H6	-174.8446	H30-C24-C25-C26	60.15650	H8-C4-H19-H20	-49.17270
H7-C1-C2-O13	-53.09930	H30-C24-C25-H29	-63.99370	H8-C4-H19-H21	72.82380
H29-C25-C26-O35	62.60730	H30-C24-C25-N43	54.38120	C67-C54-C55-H59	70.42730
N43-C25-C26-H33	64.76790	O12-C24-C28-C27	173.6177	C67-C54-C55-O65	-51.98810
N43-C25-C26-O34	-173.1095	O12-C24-C28-H32	55.21500	C55-C54-O61-C53	38.77040
N43-C25-C26-O35	-56.18390	O12-C24-C28-O36	173.5899	H58-C54-O61-C53	-80.97450
C24-C25-N43-H44	156.8181	C72-C73-C74-O83	172.8517	C67-C54-O61-C53	162.9191
C24-C25-N43-C45	-30.44050	H77-C73-C74-H81	174.6329	C55-C54-C67-H68	-59.67820
C26-C25-N43-H44	34.22670	N43-C45-C47-H48	22.30730	C55-C54-C67-H69	-178.8566
C26-C25-N43-C45	-153.0319	N43-C45-C47-H49	-98.41580	C76-C72-O85-H86	-165.0386
H29-C25-N43-H44	-82.43350	N43-C45-C47-H50	144.0420	C76-C72-C73-O62	-172.7464
H29-C25-N43-C45	90.30790	O46-C45-C47-H48	-159.1980	C76-C72-C73-C74	-54.16800
C25-C26-O34-C27	-62.24490	O46-C45-C47-H49	80.07900	C76-C72-C73-H77	65.59090
H33-C26-O34-C27	61.35100	O46-C45-C47-H50	-37.46320	H78-C72-C73-O62	-52.33880
C25-N43-C45-O46	2.349100	O35-C51-C52-C53	-179.7195	H78-C72-C73-C74	66.23960
C25-N43-C45-C47	-179.2198	O35-C51-C52-H56	-59.50030	H78-C72-C73-H77	-174.0015
H44-N43-C45-O46	174.7100	O35-C51-C52-O63	60.89810	O85-C72-C73-O62	67.43620
H44-N43-C45-C47	-6.858900	C55-C51-C52-C53	-62.43140	O85-C72-C73-C74	-173.9854
N43-C45-C47-H48	22.30730	C55-C51-C52-H56	57.78780	O85-C72-C73-H77	-54.22650

DIPOLE MOMENT

Dipole moment is arises in any system in which there is a seoaration of cahrge. In compound Taliose carbon, nitogen, oxygen and hydrogen atoms are present and among them carbon and hydrogens are positively charged whereas oxygen and nitrogen are negatively charged. Due to this charge sepration, dipole moment is generated in the molecule. Dipole moment (field-independent basis, Debye) for the isolatedtetrasaccharide Taliose was also calculated or obtained by DFT calculations and mentioned below-

X= 6.2815 Y= -0.5244 Z= -3.8363 Tot=7.3790

CONCLUSION OF DFT STUDIES

After complete study, an oligosaccharide 'Taliose' from gaddi sheep's (*Ovis aries orientalis*) milk was isolated and its structure was elucidated with the help of 1-D and 2-D NMR spectroscopic techniques. Further DFT calculations were performed using Gauss09W software and B3LYP/6-31+G (d.p.) basis set which indicates that compound was found to be stable and different electrophilic and nucleophilic centres were present in the compound which indicates the reactive regions of the compound. The data of bond lengths, bond angle, dihedral angles and mulliken charges of the compound were also studied.

CONCLUSION

A Novel Tetrasaccharide Taliose was isolated and its stereoscopic structure was elucidated with the help of 2D NMR and Mass spectrometry. It was found that Taliose contained β-Gal

(1→2) Glc instead of β-Gal (1→4) Glc (Lactose) present at the reducing end which is found in most of the milk oligosaccharides. It also showed its rarity by having 1→2 glycosidic linkages into its structure. It is the first repot of this rare oligosaccharide from any natural or synthetic source.

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