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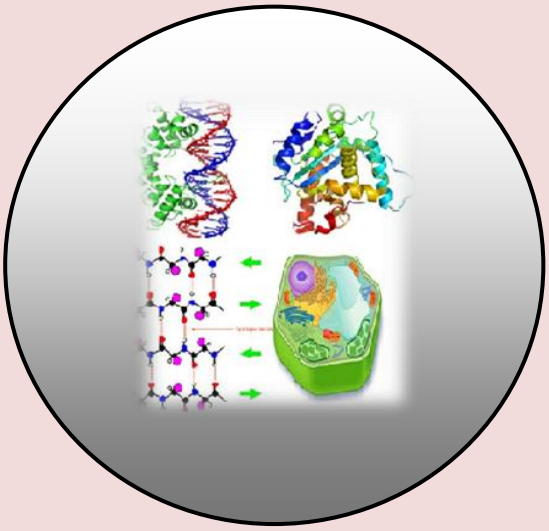

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RESEARCH PAPER

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Isolation, Purification and 2-D NMR Studies of a Novel Hexasaccharide (Eutheriose) from Buffalo Milk

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ABSTRACT

Oligosaccharides present in milk either in form of free molecules or conjugated with other compounds are beneficial for new born babies as well as adults. In all mammals buffalo is the major milk cattle of north India. Buffalo milk oligosaccharides induce significant stimulation of antibody, delayed type hypersensitivity response to Sheep RBC in BALB/c mice. In continuation to our previous work on Buffalo milk, another novel hexasaccharide Eutheriose was isolated from buffalo milk and its structure was elucidated. In the present process buffalo milk was collected and processed by Kobata and Ginsburg method followed by gel filtration, HPLC and CC technique. Structure of the isolated oligosaccharide was elucidated by chemical transformation, chemical degradation, NMR (like ^{1}H NMR, ^{13}C NMR), 2D-NMR (COSY, TOCSY and HSQC) techniques, and mass spectrometry. The structure of novel isolated oligosaccharide was deduced as under-

α-GalNAc(1→4)α-Gal(1→3)β-GlcNAc(1→3)β-Gal(1→4)β-Glc
|
(1→2)β-Gal

Key Words- Oligosaccharides, NMR, Kobata and Ginsburg, Milk and Eutheriose.

INTRODUCTION

Milk oligosaccharides confer unique health benefits to the neonate (*Diana L Oliveira et. al,. 2015*). Various factors present in milk are known to modulate the developing microbiota within the infant gastrointestinal tract (GIT), including immunoglobulins, lactoferrin, lysozyme, bioactive lipids, leucocytes and various milk glycans (glycolipids, glycoproteins and free oligosaccharides) of the functional ingredients, oligosaccharides are arguably the most important, as they function as prebiotics *(Newburg D S et. al.,2005).* Milk oligosaccharides participate in several protective and physiological roles, including immunoregulation and inhibition of pathogen adhesion in the gastrointestinal tract of infant (*Gangwar L. et al., 2018).* Amongst all the mammals Buffalo milk, is second largest global source for milk, differs from its closely related ruminant species with higher proportions of proteins, carbohydrates and fat. A recent study indicated that subjects with cow milk allergies are capable of tolerating buffalo milk, thus adding to the nutritional benefits of buffalo milk *(Sheehan WJ et al., 2009, Mohamed H. e.t al., 2011).* A processed oligosaccharide mixture of buffalo milk induces significant stimulation of antibody, delayed-type hypersensitivity response to sheep red blood cells in BALB/c mice.

This also stimulated non-specific immune response of the animals measured in terms of macrophage migration index *(Rina Saksena et. al., 1999).* In continuation to our previous work on buffalo milk oligosaccharides we have isolated another novel oligosaccharide from buffalo's milk by the method of Kobata and Ginsburg followed by chemical degradation, chemical transformation and its structure elucidation was done by the various one and two dimensional NMR spectroscopy and Mass spectrometric analytical methods.

EXPERIMENTAL

General procedure

General procedure was the same as described in our previous articles ***(L Gangwar et. al., 2017).***

Isolation of buffalo milk oligosaccharides by the method of Kobata and Ginsburg-

Isolation of oligosaccharides from buffalo's milk was done by the method of Kobata and Ginsburg *(L Gangwar et. al., 2017, 8 A. Kobata et. al., 1970),* which yielded 215 g of oligosaccharide mixture.

Acetylation of oligosaccharide mixture-

For acetylation 10.5 g of oligosaccharide mixture which gave positive phenol-sulphuric acid test *(M. Dubois et. al., 1956)* was acetylated by standard method of acetylation by acetic anhydride and pyridine, which was yielded 12.5 gm acetylated mixture of oligosaccharides *(L Gangwar et. al., 2017).*

Purification of compounds on Silica Gel Chromatography-

Separation or purification of acetylated oligosaccharides (10.5 gm) was carried over silica gel (500gm) using varied proportion of Hex: CHCl3, $CHCl_3$ and $CHCl_3$: MeOH as eluents, collecting fractions of 300ml each. All these fractions were checked on TLC and those showing similar spots were taken together for further investigations. So nine fractions namely I(3.28gm), II (736mg), III (2.19gm), IV (458mg), V (250mg) ,VI(2.007gm), VII(1.06gm), VIII (765mg) and IX (309mg) respectively were obtained. Substance Eutheriose (60.5mg) was isolated by repeated column chromatography from fraction III.

Deacetylation of Eutheriose acetate

Compound **Eutheriose acetate** (47 mg) was obtained from column chromatography 3 of acetylated oligosaccharide mixture. 35 mg of compound 'a' was dissolved in acetone (3 ml) and 3.5 ml of NH_4OH was added in it and was left overnight in a stopper hydrolysis flask. After 24 h ammonia was removed under reduced pressure and the compound was washed with (3 x 5 ml) $CHCl_3$ (to remove acetamide) and the water layer was finally freeze dried giving the natural oligosaccharide Eutheriose (29 mg).

Methyl glycosidation/Acid hydrolysis of Eutheriose

Compound **Eutheriose** (5 mg) was ref1uxed with absolute MeOH (2 ml) at 70°C for 18 h in the presence of cation exchange IR-l20 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. In the reaction mixture, 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 h. The hydrolysate was neutralized with freshly prepared $BaCO_3$ filtered and concentrated under reduced pressure to afford α-and β-methylglucosides along with the Glc, GalNAc and GlcNAc. Their identification was confirmed by comparison with authentic samples (TLC, PC) of Glc, GalNAc and GlcNAc.

Kiliani hydrolysis of Eutheriose

Compound Eutheriose (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 $CHCl_3$ *(Killiani H. et. al., 1930)*. The aqueous residual solution was made neutral by addition of 1-2 drops of 2N NaOH and was evaporated under reduced pressure to afford glucose, GalNAc and GlcNAc on comparison with authentic samples of glucose, GalNAc and GlcNAc.

DESCRIPTION OF COMPOUND-

Compound Eutheriose acetate (60.5 mg) was obtained from fraction 41-63 of column chromatography 7. Deacetylation of its 35 mg was done by using NH_4OH/ acetone and it gave approx 29 mg of Eutheriose as a viscous mass, $[\alpha]_D$ **+** 1.39. For experimental analysis, this compound was dried over P_2O_5 at 100o C and 0.1 mm pressure for 8 hrs.

$C_{40}H_{68}O_{31}N_2$	%C	%H	%N
Calculated	44.77	6.34	2.61
Found	44.76	6.33	2.61

It gave positive Phenol sulphuric acid test *(M. Dubois et. al., 1956)*, Feigl test *(Fiegl, F. et. al., 1975)* and Morgan Elson test *(Gey, M.H. et. al., 1996).*

δ in $CDCl_3$: 1H NMR (Acetylated)

δ 6.17 [d, 1H, J=4Hz, α-Glc (S_1)**],** δ 5.68 [d, 1H, J=8Hz, β-Glc (S_1)**],** δ 5.40 [d, 1H, J=4Hz, α-Gal (S_4)**],** δ 5.37 [d, 1H, J=4Hz, α-GalNAc (S_5)**],** δ 4.74 [d, 1H, J=8Hz, β-GlcNAc (S_3)**],** δ 4.60 [d, 1H, J=8Hz, β -Gal (S_6)**],** δ 4.53 [d, 1H, J=8Hz, β -Gal (S_2)**],** δ 4.12 [m, 1H, J=8Hz, β -GlcNAc (S_3)**,** H-3**],** δ 3.90 [m, 1H, J=4Hz, α-Gal (S_4)**,** H-4**],** δ 3.75 [m, 1H, J=8Hz, β -Glc (S_1)**,** H-4**],** δ 3.69 [m, 1H, J=8Hz, β -Gal (S_2)**,** H-2**],** δ 3.50 [m, 1H, J=8Hz, β -Gal (S_2)**,** H-3**].**

δ in $CDCl_3$: 13C NMR (Acetylated)

δ 101.73 [1C, β-Gal (S_6), C-1], δ 100.68 [1C, β-Gal (S_2), C-1], δ 95.00 [1C, β -GlcNAc (S_3), C-1], δ 90.50 [2C, α-Glc (S_1), β -Glc (S_1), C-1], δ 90.0 [2C, α-Gal (S_4), α-GalNAc (S_5), C-1].

δ in D_2O: 1H NMR (Deacetylated)

δ 5.44 [d, 1H, J=4Hz, α-Glc (S_1)**],** δ 5.21 [d, 1H, J=8Hz, β -Glc (S_1)**],** δ 5.10 [d, 2H, J=4Hz, α-GalNAc (S_5)**,** α-Gal (S_4)**],** δ 4.55 [d, 1H, J=8Hz, β -GlcNAc (S_3)**],** δ 4.40 [d, 1H, J=8Hz, β -Gal (S_6)**],** δ 4.33 [d, 1H, J=8Hz, β -Gal (S_2)**],** δ 3.18 [t, 1H, J=8Hz, β -Glc (S_1)**,** H-2**],** δ 1.88 [s, 3H, α -GalNAc (S_5)**,** NHCOC$\underline{H}_3$**],** δ 1.78 [s, 3H, β -GlcNAc (S_3)**,** NHCOC$\underline{H}_3$**].**

δ in D_2O: 13C NMR (Deacetylated)

δ 104.0 [1C, β-Gal (S_2), C-1], δ 103.0 [2C, β-Gal (S_6), β-GlcNAc (S_3), C-1], δ 96.0 [2C, α-GalNAc (S_5), α-Gal (S_4), C-1], δ 92.5 [2C, β-Glc (S_1), α-Glc (S_1), C-1].

ES-mass

m/z 1134 [M+Na+K]+, m/z 1095 [M+Na]+, m/z 1072 [M]+, m/z 1041, m/z 1024, m/z 1006, m/z 972, m/z 946, m/z 943, m/z 929, m/z 907, m/z 873, m/z 869, m/z 837, m/z 833, m/z 779, m/z 775, m/z 762, m/z 757, m/z 728, m/z 717, m/z 707, m/z 692, m/z 683, m/z 671, m/z 666, m/z 663, m/z 640, m/z 637, m/z 601, m/z 583, m/z 582, m/z 566, m/z 565, m/z 547, m/z 545, m/z 485, m/z 451, m/z 427, m/z 422, m/z 388, m/z 359, m/z 342, m/z 180.

RESULT AND DISCUSSION

Compound Eutheriose, $C_{40}H_{68}O_{31}N_2$, $[\alpha]_D$ $+1.39^0$, gave positive Phenol-sulphuric acid test[9], Fiegl test *(Fiegl, F. et. al., 1975)* and Morgon-Elson test *(Gey, M.H. et. al., 1996)* showing the presence of normal and amino sugars moietie(s) in the compound A. The HSQC spectrum of acetylated Eutheriose showed the presence of seven cross peaks of anomeric protons and carbons in their respective region at δ 6.17 x 90.5, δ 5.68 x 90.5, δ 5.40 x 90.0, δ 5.37 x 90.0, δ 4.74 x 95.00, δ 4.60 x 101.73, δ 4.53 x 100.68, suggesting the presence of seven anomeric protons and carbons in it. The presence of seven anomeric protons were confirmed by the presence of seven anomeric doublets at δ 6.17 (1 H), δ 5.68 (1H), δ 5.40 (1H), δ 5.37 (1H), δ 4.74 (1 H), δ 4.60 (1H) and δ 4.53 (1H) in the 400 MHz ^{1}H NMR of acetylated Eutheriose. The presence of seven anomeric carbons were confirmed by seven anomeric carbon signals at δ 101.73 (1C), δ 100.68 (1C), δ 95.0 (1C), δ 90.5 (2C), and 90.0 (2C) in the ^{13}C NMR spectrum of acetylated Eutheriose at 400 MHz. Since all these spectrums contained downfield shifted α and β anomeric protons and carbons suggested that compound Eutheriose may be a hexasaccharide in its reducing form. The hexaasaccharide nature of Eutheriose was further supported by seven anomeric proton signals for seven anomeric protons as doublets at δ 5.44(1H), δ 5.21(1H), δ 5.10(2H), δ 4.55(1H), δ 4.40(1H) and δ 4.33(1H) in the ^{1}H NMR spectrum of Eutheriose in D_2O at 400 MHz. The hexasaccharide nature of Eutheriose was also confirmed by the appearance of seven anomeric carbons signals at δ 104.0 (1C), δ 103 (2C), δ 96.0 (2C) and δ 92.5 (2C) in the ^{13}C NMR spectrum of Eutheriose at 400 MHz.

The reducing nature of compound Eutheriose was further confirmed by methylglycosylation of compound Eutheriose by $MeOH/H^+$ followed by its acid hydrolysis which led to the isolation of α and β-methyl glucosides, suggesting the presence of glucose at the reducing end. For convenience all six monosaccharide present in compound 'A' were designated as S-1, S-2, S-3, S-4, S-5 and S-6 respectively starting from glucose (S-1) at the reducing end. The monosaccharide constituents in compound A were confirmed by its Killiani hydrolysis *(Killiani H. et. al., 1930)* under strong acidic conditions, followed by Paper chromatography and TLC. In this hydrolysis four spots were found on TLC and PC which were found identical with the authentic samples of Glucose, Galactose, GlcNAc and GalNAc by co-chromatography. Thus, the hexasaccharide contained four types of monosaccharide units i.e., Glc, Gal, GalNAc and GlcNAc in it. Further the presence of two anomeric proton signals at δ 5.44(J=4.0 Hz) and δ 5.21 (J=8.0 Hz) in the ^{1}HNMR spectrum of Eutheriose in D_2O at 400 MHz were assigned for α and β anomers of glucose (S-1) confirming the presence of Glc (S-1) at the reducing end *(Dorland, F. et. al., 1977, Prasoon Chaturvedi et. al., 1990)* in compound Eutheriose. In the TOCSY spectrum of acetylated Eutheriose, the anomeric signal of β-Glc (S-1) at δ 5.68 gave a cross peak in linkage region at δ 3.75, which was further assigned as H-4 of β-Glc (S-1) by the COSY spectrum of acetylated Eutheriose, showing that β-Glc (S-1) was 1→4 linked with the next monosaccharide unit. Further the presence of another anomeric proton doublet at δ 4.33(J=8.0 Hz) in the ^{1}H NMR spectrum of Eutheriose in D_2O showed the presence of β-Gal (S-2) residue as the next monosaccharide, which was linked with β-Glc (S-1) by (1→4) linkage. The 1→4 linkage between S-1 and S-2 was further confirmed by the appearance of β-Glc (S-1) H-2 signal as triplet at δ 3.18 (SRG) *(Gunnar Gronberg et. al., 1990, VK Dua et. al., 1983)* and hence the presence of lactosyl moiety *(Uemura, Y. et. al., 2006)* was confirmed at the reducing. The coupling constant of anomeric signal β-Gal (S-2) with J value of 8.0 Hz confirmed the β configuration of the β-Gal (S-2) moiety and hence β 1→4 glycosidic linkage between S-2 and S-1. Further the anomeric proton signal of β-Gal (S-2) at δ 4.53 in the ^{1}H NMR of Eutheriose acetate in $CDCl_3$ showed two consequent complementary signals in the linkage region at δ 3.69 and δ 3.50 in TOCSY spectrum of Eutheriose acetate showing that the two hydroxyl groups of Gal (S-2) were available for glycosidic linkage. These signals were identified for H-2 and H-3 respectively of β-Gal(S-2) by the COSY spectrum of Eutheriose acetate suggesting that H-2 and H-3 of β-Gal(S-2) were available for glycosidic linkages by the next monosaccharide units. The next anomeric proton signal which appeared at δ 4.55 (J=8.0 Hz) along with a singlet of amide methyl (-$NHCOCH_3$) at δ 1.78 in the ^{1}H NMR of Eutheriose in D_2O was due to the presence of β-GlcNAc(S-3) *(Urashima, T. et. al., 2004 and VK Dua, et. al., 1985)* moiety. The downfield shifted H-4 proton of β-Gal (S-2) at δ 4.19 confirmed that β-Gal (S-2) was glycosidically linked at C-3 position by β-GlcNAc (S-3) moiety (SRG) *(P. Chaturvedi et. al., 1988 and 967, Tarrago, M.T. et. al., 1988)*. The 1→3 linkage between S-3 and S-2 was further supported by the ^{1}HNMR spectrum of acetylated Eutheriose in which the signal for H-3 of S-2 appeared at δ 3.50 which was confirmed by COSY, TOCSY and HSQC spectrum of acetylated Eutheriose. The coupling constant of anomeric signal of (S-3) with J value 8.0 Hz confirmed the β-configuration of the β-GlcNAc(S-3) moiety. Therefore the glycosidic linkage between S-3 and S-2 was confirmed as β1→3. Another anomeric proton signal which appeared as a doublet at δ 4.4 (J=8.0 Hz) in ^{1}HNMR spectrum of Eutheriose in D_2O was due to assigned β-Gal(S-6). Since, the positions H-2 and H-3 were available for glycosidic linkages and position H-3 of β-Gal (S-2) was already linked with β-GlcNAc(S-3), the leftover H-2 position of β-Gal (S-2) must be linked by β-Gal(S-6) which was further confirmed by the appearance of H-2 signal of S-2 at δ 3.69 in 1H NMR of Eutheriose acetate and also confirmed by COSY and TOCSY spectrum of Eutheriose acetate at 400 MHz in $CDCl_3$. The large coupling constant of β-Gal(S-6) of J=8.0 Hz confirmed the β- glycosidic linkage between β-Gal(S-6) and β-Gal(S-2). Since, it was ascertained by COSY and TOCSY spectrum of Eutheriose acetate that none of its methine proton was coming in linkage region hence it must be linked at reducing end. The next monosaccharide unit in Eutheriose was identified as α-Gal (S-4) by the presence of anomeric proton doublet at δ 5.1 (J=4.0 Hz) in the ^{1}HNMR of Eutheriose in D_2O. Since in the TOCSY spectrum of Eutheriose acetate the anomeric proton of β-GlcNAc (S-3) at δ 4.53 showed a cross peak at δ 4.12 showing that the one position of β-GlcNAc (S-3) was available for glycosidic linkage.

Later this signal of δ 4.12 was ascertained as H-3 of β-GlcNAc (S-3) by COSY spectrum of Eutheriose acetate showing that H-3 of S-3 was glycosidically linked to H-1 of α-Gal (S-4), hence, 1→3 linkage between S-3 and S-4 was confirmed. It was further confirmed by H-1 of α-Gal (S-4) at δ 5.40 and H-5 at δ 4.15 suggested that α-Gal is linked to 3 position of β-GlcNAc. The small coupling constant of α-Gal (S-4) J=4.0 Hz confirmed α- glycosidic linkage between α-Gal (S-4) and GlcNAc (S-3), hence α 1→3 glycosidic linkage between S-4 and S-3. Since in the TOCSY spectrum of Eutheriose acetate, the anomeric signal at δ 5.40 in $CDCl_3$ at 400 MHz showed a cross peak at δ 3.90 suggested that one position of S-4 was available for glycosidic linkage by next monosaccharide unit which was later identified as H-4 of S-4 confirming that H-4 of Gal (S-4) was available for glycosidic linkage. The next anomeric proton which was present at δ 5.10 along with a singlet for amide methyl at δ 1.88 in D_2O in the ^1H NMR of Eutheriose at 400 MHz confirmed the presence of GalNAc which was glycosidically linked to Gal S-4. The small coupling constant J=4.0 Hz confirmed the α glycosidic linkage between S-5 and S-4 and hence α 1→4 glycosidic linkage between S-5 and S-4. Since the anomeric signal of α-GalNAc (S-5) at δ 5.37 present in the TOCSY spectrum of Eutheriose acetate does not contain any methine protons in glycosidic linkage region at δ 3-4 ppm showed that none of OH group of α-GalNAc (S-5) was involved in glycosidic linkage hence, confirmed that α-GalNAc (S-5) was present at non-reducing end and is linked with H-4 of α-Gal (S-4), which was confirmed by the TOCSY and COSY spectrum. All the ^1H NMR assignments for ring protons of monosaccharide units of Eutheriose were confirmed by HOMOCOSY *(Gronburg, G. et. al., 1989 and Gronburg, G. et. al., 1992)* and TOCSY *(Kover K.E. et. al., 2000)* experiments. The positions of glycosidation in the oligosaccharide were confirmed by position of anomeric signals, structure reporter groups and comparing the signals in ^1H and ^{13}C NMR of acetylated Eutheriose and Eutheriose.

The glycosidic linkages in Eutheriose were assigned by the cross peaks for glycosidically linked carbons with their protons in the HSQC *(Strecker, G. et. al., 1992 and Bodenhausen, G. et. al., 1971)* spectrum of acetylated Eutheriose. The values of these cross peaks appeared as β-Glc(S-1) H-4 and C-4 at δ 3.75 x 76 showed (1→4)linkage between S-2 and S-1, β-Gal(S-2) H-3 and C-3 at δ 3.50 x 73 showed (1→3) linkage between S-3 and S-2, β-GlcNAc(S-3) H-3 and C-3 at δ 4.12 x 71 showed (1→3) linkage between S-4 and S-3, β-Gal(S-2) H-2 and C-2 at δ 3.69 x 73 showed (1→2) linkage between S-6 and S-2, α –Gal (S-4) H-4 and C-4 at δ 3.90 x 70.5 showed (1→4)linkage between S-5 and S-4. All signals obtained in ^1H and ^{13}C NMR of compound Eutheriose were in conformity by 2D ^1H-^1H COSY, TOCSY and HSQC experiments. Thus, based on the pattern of chemical shifts of ^1H, ^{13}C, COSY, TOCSY and HSQC NMR experiments it was interpreted that the compound 'A' was a hexasaccharide having structure as-

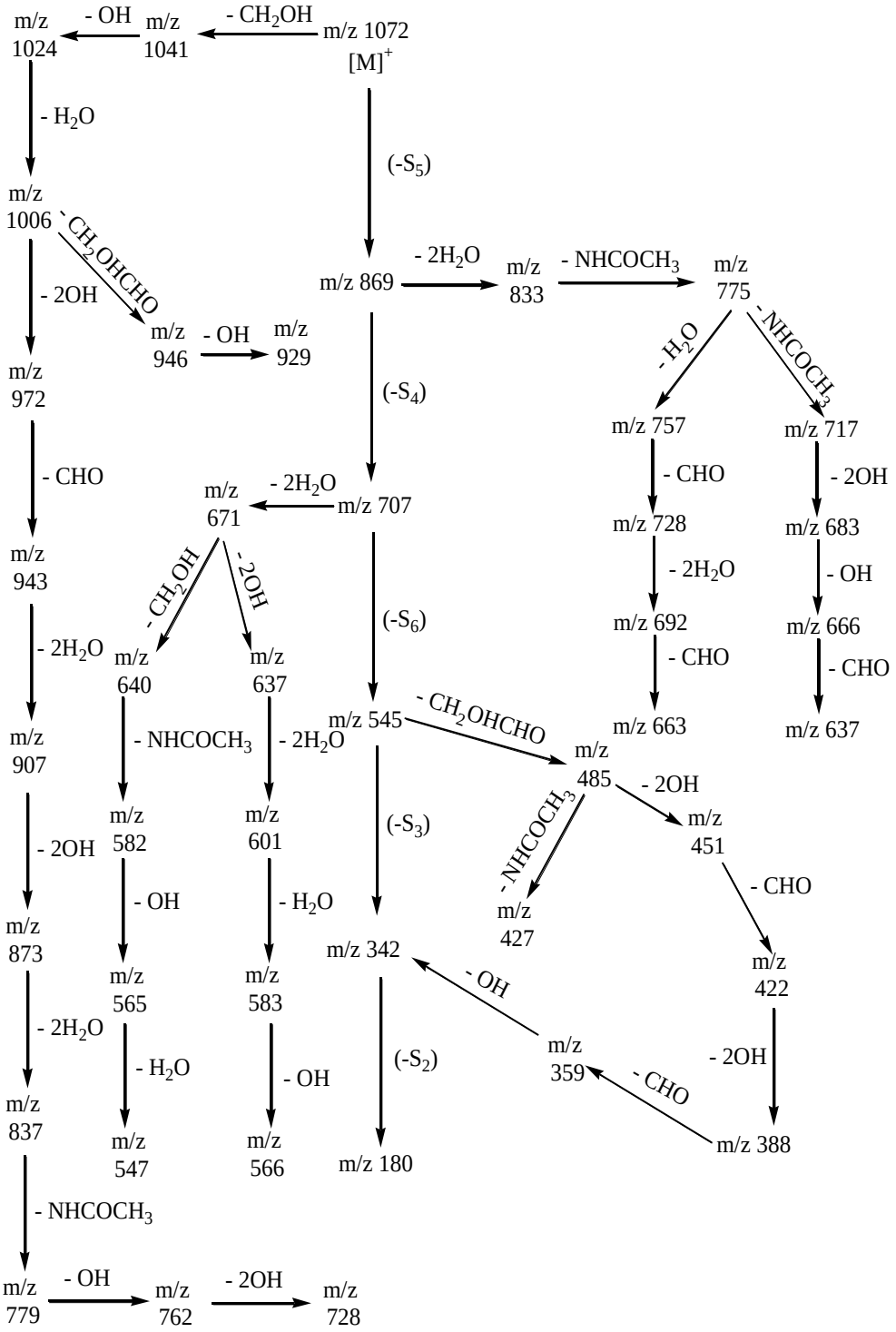
α-GalNAc(1→4)α-Gal(1→3)β-GlcNAc(1→3)β-Gal(1→4)β-Glc
|
(1→2)β-Gal

EUTHERIOSE

The Electronspray Mass Spectrometry data of Eutheriose not only confirmed the derived structure but also supported the sequence of monosaccharide in Eutheriose. The highest mass ion peaks were recorded at m/z 1134 assigned to $[M+Na+K]^+$ and m/z 1095 assigned to $[M+Na]^+$, it also contain the molecular ion peak at m/z 1072 confirming the molecular weight as 1072 which was in agreement with its molecular formula $C_{40}H_{68}O_{31}N_2$. The mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water.

The hexaasaccharide m/z 1072 (I) fragmented to give mass ion at m/z 869 (II) [1072-(S-5)], this fragment was arised due to the loss of α -GalNAc (S-5) moiety from hexaasaccharide. After this, it fragmented to give mass ion peak at m/z 707(III) [869-(S-4)] which was due to the loss of α-Gal (S-4) moiety from pentaasaccharide. This pentaasaccharide of m/z 707 is then fragmented to give mass ion peak at m/z 545 (IV) [707-(S-6)] which was a tetrasaccharide(IV), due to loss of β-Gal (S-6) moiety. This trisaccharide unit fragmented to give mass ion peak at m/z 342 (V) [545-(S-3)], which was due to loss of β-GlcNAc (S-3) moiety from trisaccharide, which on further fragmentation resulted in m/z 180 (VI) [342- (S-2)] of β-Glc (S-1). These five mass ion peak II, III, IV, V and VI were appeared due to the consequent loss of S-5, S-4, S-3, S-6 and S-2 from original molecule.

The other fragmentation pathway in ES Mass spectrum of compound A m/z 1072 shows the mass ion peak at 907 [1072-CH_2OH (31), OH (17), H_2O (18), 2OH (34), CHO (29), $2H_2O$ (36)], 837 [907-2OH (34), $2H_2O$ (36)], 728 [837- $NHCOCH_3$ (58), OH (17), 2OH (34)], 637 [869- $2H_2O$ (36), $NHCOCH_3$ (58), $NHCOCH_3$ (58), 2OH (34), OH (17), CHO (29)], 637 [707-$2H_2O$ (36), 2OH (34)], 566 [637- $2H_2O$ (36), H_2O (18), OH (17)], 565 [707-$2H_2O$(36), CH_2OH (31), $NHCOCH_3$ (58), OH (17)], 547 [565- H_2O (18)], 422 [545- CH_2OHCHO (60), 2OH (34), CHO (29)], 388 [422- 2OH (34)], 180 [342-S2].

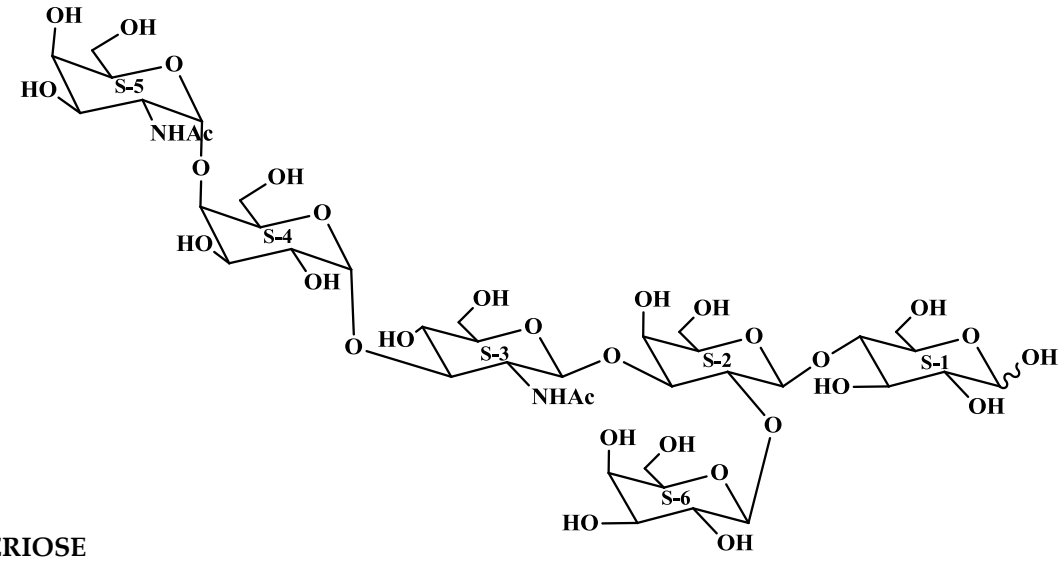


ES-MS FRAGMENTS OF COMPOUND EUTHERIOSE

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

α-GalNAc(1→4)α-Gal(1→3)β-GlcNAc(1→3)β-Gal(1→4)β-Glc

(1→2)β-Gal



EUTHERIOSE

CONCLUSION

From the above informations, we have concluded the structure of isolated buffalo milk oligosaccharide, Eutheriose. This oligosaccharide was reported for the first time from any natural source or any milk and structure of compound was elucidated with the help of spectroscopic technique like ^{1}H, ^{13}C, 2-DNMR (COSY, TOCSY and HSQC) spectroscopy and mass spectroscopy.

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