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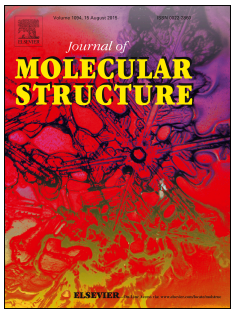

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Structure Elucidation of a Novel Oligosaccharide (Medalose) from Camel Milk

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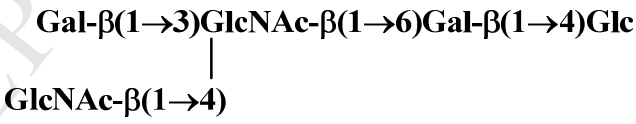
Abstract-

Free oligosaccharides are the third most abundant solid component in milk after lactose and lipids. The study of milk oligosaccharides indicate that nutrients are not only benefits the infant's gut but also perform a number of other functions which include stimulation of growth, receptor analogues to inhibit binding of pathogens and substances that promote postnatal brain development. Surveys reveal that camel milk oligosaccharides possess varied biological activities that help in the treatment of diabetes, asthma, anaemia, piles and also a food supplement to milking mothers. In this research, camel milk was selected for its oligosaccharide contents, which was then processed by Kobata and Ginsburg method followed by the HPLC and CC techniques. Structure elucidation of isolated compound was done by the chemical degradation, chemical transformation and comparison of chemical shift of NMR data of natural and acetylated oligosaccharide structure reporter group theory, the ^{1}H, ^{13}C NMR, 2D-NMR (COSY, TOCSY and HSQC) techniques, and mass spectrometry. The structure was elucidated as under:-

MEDALOSE



Key words- Milk Oligosaccharides, 2D NMR and Medalose.

1. Introduction

Milk is a source of infant growth, development and contains proteins, fatty acids, minerals, vitamins and carbohydrates especially, lactose and large number of oligosaccharides. Oligosaccharides in milk exert various bioactivities and modulate the immune system [**1**]. Milk oligosaccharides participate in several protective and physiological roles including immunoregulation and inhibition of pathogen adhesion in the gastrointestinal tract of infant. Human milk oligosaccharides (HMOs) perform a number of functions including serving as prebiotics to stimulate the growth of beneficial intestinal bacteria, as receptor analogues to inhibit binding of pathogens, and as substances that promote postnatal brain development. Human breast milk play a very important role in gut colonization and modulation of the infants gut **[2].** Sheep milk aggravates hiccup and dyspnoea. It eliminates pitta, kapha and fat. It also contains fucose in its oligosaccharides which causes various biological activities **[3].** Ancient literature of Ayurveda has declared the importance of cow milk as immuno-stimulant and natural aphrodisiac, it improves intelligence, cures heart diseases and Leucoderma, also cures bleeding disorders in intestine [**4**]. In Middle East and Mongolia camel milk is consumed as a nutrient provider for infants' development. Since the time of Chinggis Khaan (Genghis Khan), Mongolians have used camel milk as a health-promoting product. Mongolians literature says that fermented camel milk has been used for treatment of Edema in pregnant women and as an antiscorbutic agent [**5**]. In India camel milk is used therapeutically against dropsy, jaundice, problems of the spleen, tuberculosis, asthma, anaemia, and piles [**6**]. Camel milk has proven to be beneficial for lung ailments and for the treatment of tuberculosis [**7**]. Patients with chronic hepatitis had improved liver function after being treated with camel milk [**8**].

In the present study we have described the structure elucidation of novel camel milk oligosaccharide isolated from camel milk by the method of Kobata and ginsburg followed by chemical degradation, chemical transformation and various one and two dimensional NMR spectroscopy and Mass spectrometric analytical methods.

2. Experimental-

2.1 General procedure

Same as described in our previous articles **[9].**

2.2 Isolation of camel milk oligosaccharides by the method of Kobata and Ginsburg-

Isolation of oligosaccharides from camel's milk was done by the method of Kobata and Ginsburg [**10**], which yielded 315gm of oligosaccharide mixture.

2.3 Acetylation of oligosaccharide mixture-

For acetylation 12 g of oligosaccharide mixture which gave positive phenol-sulphuric acid test **[11]** was acetylated by standard method of acetylation by acetic anhydride and pyridine, which yielded 13.5 gm acetylated mixture mixture of oligosaccharides **[9].**

2.4 Purification of compounds by Silica Gel Chromatography-

Separation or purification of acetylated oligosaccharides (13.5 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 ten fractions namely I(4.28gm), II (736mg)**,** III (3.29gm), IV (468mg), V (380mg) ,VI(2.007gm), VII(1.06gm), VIII (767mg), IX (319mg) and X (137mg) were obtained. Substance B (95mg) was isolated from fraction II by repeated column chromatography. The $[\alpha]_D$ value of medalose acetate (B) was +46.

2.5 Deacetylation of compounds Medalose Acetate

Medalose acetate (45mg) obtained from the column chromatography of acetylated oligosaccharide mixture was dissolved individually in acetone (3 ml) and NH_4OH (3.5ml) was added and this was left overnight in a stoppered 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 deacetylated oligosaccharide i.e. Medalose (37mg).

Methylglycosidation/ Acid Hydrolysis of Medalose

Medalose (15mg) was ref1uxed with absolute MeOH (2 ml) at 70°C for 18 h in the presence of cation exchange !R-l20 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To a solution of methylglycoside of Medalose in 1,4-dioxane (I ml), 0.1 N H_2S0_4 (1 ml) was added and the solution was warmed for 30 minutes at 50^0C. The hydrolysis was complete after 22 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 GIc, Gal and GlcNAc. Their identification was confirmed by comparison with authentic samples (TLC, PC).

2.6 Killiani Hydrolysis of Medalose

Medalose (15mg) was dissolved in 2 ml of Killiani mixture (AcOH-H_2O-HCI, 7:11:2)[**12]** separately and heated at 100^0C for 1 h followed by evaporation under reduced pressure. It was dissolved in 2 ml of H_20 and extracted twice with 3 ml $CHCl_3$. 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 GlcNAc on comparison with authentic samples of glucose, galactose and GlcNAc.

2.7 Description of Compound-

Compound Medalose-

Compound b Medalose $C_{34}H_{58}N_2O_{26}$ (37mg) viscous syrup, $[\alpha]_D$ $+52.8^0$(c,4,H_2O). Elemental analysis: Calculated. %C 44.83, %H 6.37, %N 3.08 and found -%C 44.79, %H 6.29, %N 3.06. The molecular formula of compound Medalose was $C_{34}H_{58}N_2O_{26.}$ For experimental analysis, this compound was dried over P_2O_5 at 100^0 C and 0.1 mm pressure for 8 hr.

The presence of sugar units in compound Medalose have been confirmed by NMR and Mass spectrometry.

^{1}H NMR values of Medalose in D_2O:

2.00 ppm[s, 3H, $NHCOCH_3$, β-GlcNAc (S-3)], 3.28 ppm [t, 1H, J=7.0 Hz, β-Glc(S-1), H-2], 3.93 ppm [, β-Gal (S-2), H-4],4.06 ppm [t, 1H, β-GlcNAc(S-3), H-3],4.47 ppm [d,2H, J=7.5 Hz, β-Gal (S-2 and S-4), H-1] ,4.53 ppm [d, 1H, J=7.8 Hz, β-GlcNAc (S-3), H-1],4.56 ppm [d, 1H, J=9.0 Hz, β-GlcNAc (S-5), H-1], 4.67 ppm [d , 1H ,J=8.7 Hz, β-Glc (S-1), H-1], 5.23 ppm [d,1H, J=3.8 Hz, α-Glc (S-1),H-1].

^{13}C NMR values of Medalose in D_2O:

21.8 ppm [s,$NHCOCH_3$]., 24.9 ppm [s,$NHCOCH_3$], 91.8 ppm [α-Glc (S-1),C-1], 96.5 ppm [β-Glc (S-1),C-1], 102.3 ppm [β-GlcNAc (S-3), C-1], 102.8 ppm [β-Gal (S-2 and S-4),& β-GlcNAc (S-5), C-1], 173.6 ppm [s,$NHCOCH_3$] .

^{1}H NMR values of Acetylated Medalose in $CDCl_3$:

4.48 ppm [d, 1H, J=8.4 Hz, β-GlcNAc (S-3), H-1], 4.49 ppm [d,2H, J=8.3 Hz, β-Gal (S-2 and S-4), H-1], 4.51 ppm [d, 1H, J=9.0 Hz, β-GlcNAc (S-5), H-1], 5.68 ppm [d, 1H ,J=8.7 Hz, β-Glc (S-1), H-1], 6.25 ppm [d,1H, J=3.6 Hz, α-Glc (S-1),H-1].

^{13}C NMR values of Acetylated Medalose in $CDCl_3$:

89.1 ppm [α-Glc (S-1), C-1], 91.7 ppm [β-Glc (S-1),C-1], 101.1 ppm [β-GlcNAc (S-3& S-5), C-1]., 101.3 ppm [β-Gal (S-2 & S-4), C-1]

ES Mass of Medalose:

933[M+Na]$^+$, 910 [M$^+$], 908 (95% in exp), 892, 861, 848(68% in exp), 844, 837, 819, 819, 789 (60 % in exp), 771, 758, 748 (33% in exp), 730, 703 (40% in exp), 699, 681 (20% in exp), 659, 652 (10% in exp), 621, 611, 590, 579, 576, 558(5%), 545, 527, 496, 479, 465(100%), 406(18%), 402, 384(6%), 375, 357, 342, 325, 293, 275, 266, 260(28%), 239, 223(35%), 200, 190, 187(10%).

3. Result and Discussion-

Medalose, $C_{34}H_{58}N_2O_{26}$ $[\alpha]_D$ +52.8 gave positive Phenol- sulphuric acid test[**11**], Feigl test[**13**], Morgon-Elson test[**14**] showed the presence of normal and amino sugar(s) in the compound. The HSQC spectrum of acetylated medalose acetate in $CDCl_3$ showed the presence of five cross peaks of six anomeric protons and carbons in the respective region at δ 6.25 ppm x 89.1 ppm (1C), δ 5.69 ppm x 91.7 ppm (1C), δ 4.48 ppm x 101.1 ppm (1C), δ 4.51 ppm x 101.1 ppm (1C) and δ 4.49 ppm x 101.3 ppm (2C) suggesting the presence of six anomeric protons and carbons in it. Further ^{1}H NMR spectrum of medalose acetate at 300 MHz in $CDCl_3$ showed five signals for six anomeric proton at δ 6.25 ppm (1H), δ 5.69 ppm (1H), δ 4.48 ppm (1H), δ 4.51 ppm (1H), and δ 4.49 ppm (2H) indicating that the Medalose acetate may be pentasaccharide in its reducing form. It was further supported by appearance of five signals for the six anomeric carbons at δ 89.1 ppm (1C), δ 91.7 ppm (1C), δ 101.1 ppm (1C), δ 101.1 ppm (1C) and δ 101.3 ppm (2C) in the ^{13}C NMR spectrum of acetylated Medalose in $CDCl_3$. The ^{1}H NMR spectrum of Medalose at 300 MHz in D_2O exhibited five doublets for six anomeric proton signals at δ 5.23 ppm (1H), 4.67 ppm (1H), 4.56 ppm (1H), 4.53 ppm (1H) and 4.47 ppm (2H) indicating that the compound may be a pentasaccharide in its reducing form giving signals for α and β anomers of glucose at its reducing end. Methylglycosidation of medalose by MeOH/H$^+$ followed by its acid hydrolysis led to isolation of α and β- methyl glucosides, which suggested the presence of glucose at the reducing end of the oligosaccharide. It was also confirmed by the presence of two anomeric proton signals at δ 5.23 ppm and δ 4.67 ppm for α and β-Glc. The pentasaccharide nature of medalose was further confirmed by the presence of four doublets for six anomeric carbon at δ 91.8 ppm (1C), δ 96.5 ppm (1C),δ 102.3 ppm (1C) and δ 102.8 ppm (3C) in ^{13}C NMR of medalose in D_2O. The five monosaccharides present in Medalose have been designated as S_1, S_2, S_3, S_4, and S_5 for convenience starting from reducing end. To confirm the monosaccharide

constituents in Medalose, it was hydrolysed under strong acidic conditions. In Killiani hydrolysis under strong acid condition, it gave three monosaccharides i.e. glucose, galactose and N-acetyl-glucosamine, confirming that the pentasaccharide consists of three types of monosaccharide units. The ^{1}HNMR of Medalose acetate contains two anomeric proton signals at δ6.25 ppm and δ5.69 ppm showed the presence of α and β-glucose (S_1) present at the reducing end. The anomeric proton present at δ5.69 ppm contain three cross peaks at δ5.02 ppm, δ5.23 ppm and δ3.69 ppm in the TOCSY spectrum of Medalose acetate, out of which the cross peak present at δ3.69 ppm was assigned for glycosidic linkage which was later identified for H-4 of the reducing glucose by the COSY spectrum of Medalose acetate confirming that H-4 of reducing glucose was available for glycosidic linkage by the next monosaccharide. Further another anomeric proton doublet which was present at δ4.61 ppm was due to presence of galactose which was linked to H-4 of reducing glucose, showing presence of Lactosyl moiety at the reducing end in Medalose. Since the presence of glucose was also confirmed at its reducing end of Medalose by methyl glycosidation/acid hydrolysis was also supported by ^{1}H NMR of Medalose which contains two anomeric proton signals for α- and β-Glc at δ 5.23 ppm (J=3.8 Hz) and at δ 4.67 ppm (J=8.7 Hz)[**15,16**]. It also contains another anomeric proton doublet at δ 4.47 ppm (J=7.5 Hz) which was due to presence of β-Gal (S_2) moiety [**17**] in the Medalose. The large coupling constant of anomeric proton signal of Gal(S_2) δ 4.47 ppm (J=7.5 Hz) confirmed the β glycosidic linkage between S_2 and S_1. Presence of reducing glucose along with β-Gal moiety present in Medalose suggested the presence of a lactosyl moiety i.e. Gal-β-(1→4) Glc in Medalose which was further confirmed by β-Glc (S_1) H-2 signal (a structure reporter group) [**18**] which appeared as a triplet at δ 3.29 ppm confirmed the presence of lactosyl moiety in Medalose. Further the anomeric proton of β-galactose present at δ 4.48 ppm showed three cross peaks at δ5.15 ppm, 4.53 ppm and δ3.73 ppm in the TOCSY spectrum of Medalose acetate, out of which the cross peak present at δ3.73 ppm showed availability for glycosidic linkage by the next monosaccharide unit which was later identified for H-6 of the β-galactose by the COSY spectrum of Medalose acetate confirming that H-6 of β-galactose (S_2) was available for glycosidic linkage by the next monosaccharide unit. The presence of another anomeric proton signal present at δ4.51 in the ^{1}HNMR of Medalose acetate was due to presence of GlcNAc showed that the GlcNAc was present as the third monosaccharide unit linked to Gal S_2 at H-6. Further the presence of another anomeric proton doublet at δ 4.53 ppm (J=7.8Hz) along with signal of amide methyl

group at δ 2.00 ppm ^{1}HNMR of Medalose in D_2O, was due to the presence of β-GlcNAc (S_3) moiety [**19**]. The large coupling constant of anomeric proton signal of GlcNAc (S_3) δ4.51 ppm (J=7.5 Hz) confirmed the β glycosidic linkage between S_3 and S_2. The H-4 proton resonance of β Gal (S_2), which appeared at δ 3.93 ppm implies that the β-GlcNAc (S_3) may be 1→6 linked to β-Gal (S_2)(SRG)[**20,21**]. This was confirmed on the basis of presence of β-Gal (S_2), H-6 at δ3.84 ppm and C-6 at δ73.8 ppm and chemical shift analogies of β-GlcNAc (1→6) Gal-β as given by Dua et.al (S_3) **[22].** Further the anomeric proton of β-GlcNAc showed three cross peaks at δ4.12 ppm, δ3.62 ppm and δ3.80 ppm in TOCSY spectrum of Medalose acetate. Further the COSY spectrum of Medalose acetate confirmed that signal at chemical shift at δ4.12 ppm was due to H-2 methene proton of GlcNAc (S_3), while the signal at δ3.80 ppm and δ3.62 ppm was due to H-3 and H-4 protons of S_3 respectively, out of which the positions of H-3 and H-4 protons of β- GlcNAc (S_3) at δ 3.80 ppm and δ 3.62 ppm respectively which imply that H-3 and H-4 of β-GlcNAc (S_3) were involved in the glycosydation with the next monosaccharide unit. The presence of another anomeric proton signal present at δ4.49 ppm was due to the presence galactose in the spectrum of Medalose acetate. Since H-4 showed a signal at δ3.62 ppm confirmed that Gal S_4 was glycosydically linked to H-3 of S_3. The large coupling constant of anomeric proton signal of Gal (S_4) δ4.51 ppm (J=7.5 Hz) confirmed the β glycosidic linkage between S_4 and S_3. Further the presence of another anomeric proton signal appeared at δ 4.47 ppm (J=7.5 Hz) which was due to presence of a β-GlcNAc moiety (S_5) confirmed that S_5 (GlcNAc) was linked to H-4 of β-GlcNAc (S_3) in spectrum of Medalose in D_2O. The large coupling constant of anomeric proton signal of GlcNAc (S_5) δ4.51 ppm (J=7.5 Hz) confirmed the β glycosidic linkage between S_5 and S_3. The linkage between S_3 and S_4 was established on the basis of presence of downfield shifted H-3 proton of GlcNAc (S_3) at δ 4.06 ppm as triplet. It was further confirmed by anomeric proton chemical shift value of this β-Gal moiety (S_4), which was identical with the anomeric proton chemical shift value of β-Gal (S_2) of lactosyl moiety, which is a structure reporter group for S_4 and S_3 linkage [**23**]. Another anomeric signals appeared at δ 4.56 ppm (J=9.0 Hz) along with signal of methyl group at δ 2.00 ppm was due to presence of β-GlcNAc moiety (S_5) which was linked to H-4 of β-GlcNAc (S_3).

^{1}H and ^{13}C NMR values in D_2O (TABLE 1)

	^{1}H NMR (in ppm)	^{13}C NMR (in ppm)	Coupling Constt.(J)(in Hz)
α-Glc	5.23	91.8	3.8
β-Glc	4.67	96.5	8.7
β-Gal	4.47	102.8	7.5
β-GlcNAc	4.53	102.3	7.8
β-Gal	4.47	102.8	7.5
β-GlcNAc	4.56	102.8	9.0

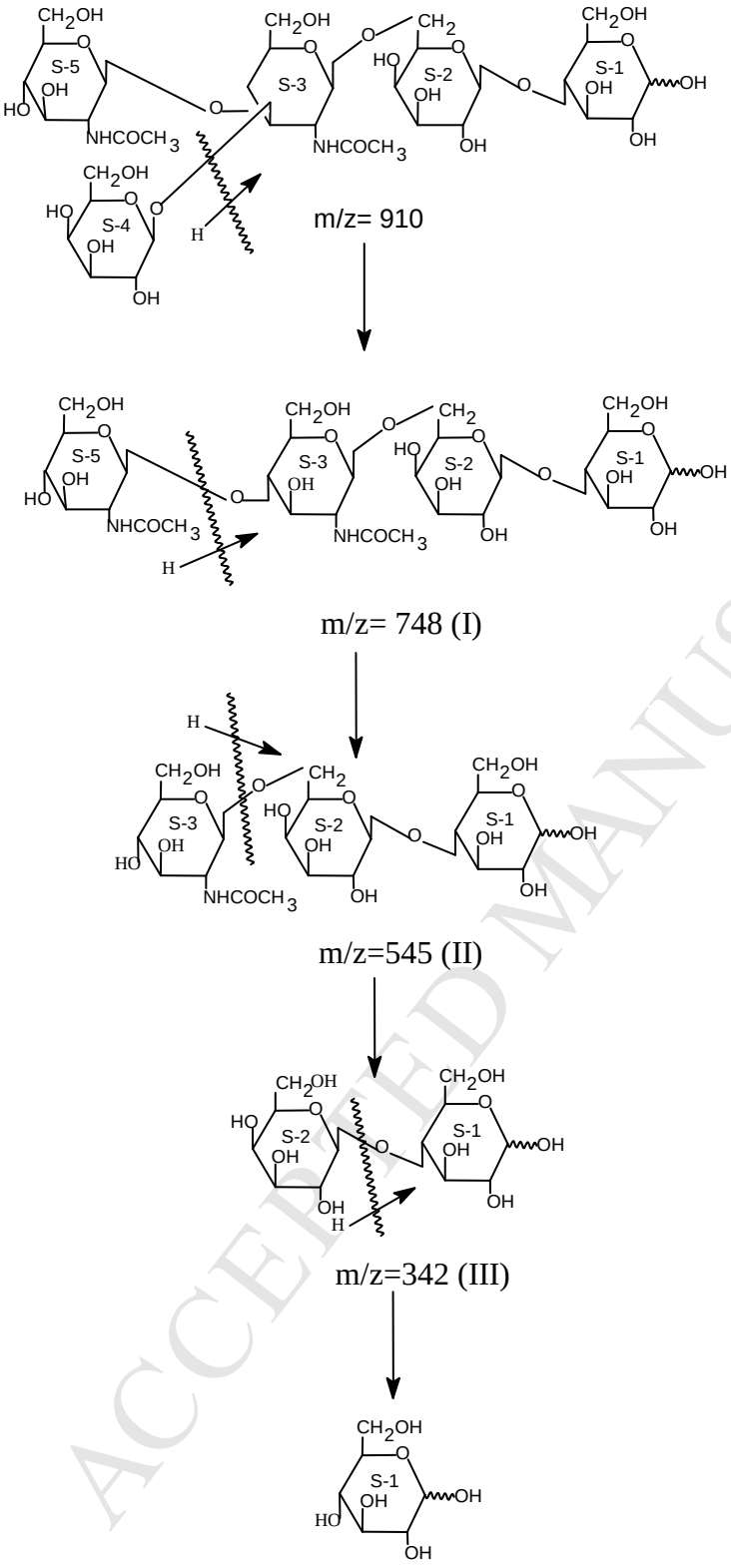
The ^{13}C NMR values of anomeric carbons and ring carbons of Medalose are given in table 2. The various values of ring carbons are in accordance with ^{13}C value of their respective monosaccharides, which also supports the derived structure.

^{13}C NMR values (in ppm) of Medalose (TABLE 2)

	C-1	C-2	C-3	C-4	C-5	C-6	CO	CH_3
α-Glc	91.8	73.6	71.65	78.01	70.9	60.44		
β-Glc	96.5	74.9	75.2	77.59	74.9	62.0		
β-Gal	102.8	70.9	73.5	70.7	70.8	73.8		
β-GlcNAc	102.3	60.9	78.5	78.01	74.9	62.0	173.6	21.8
β-Gal	102.8	71.0	72.4	70.9	76.18	60.9		
β-GlcNAc	102.8	61.9	74.9	72.4	75.29	62.0	173.6	24.9

The pentasaccharide nature of medalose was further confirmed by the spectral studies of acetylated product of compound B. These studies are made on the basis of HOMOCOSY, TOCSY and HSQC connectivities. The glycosidic linkages were assigned by the cross peaks for glycosidically linked carbons with their protons in HSQC spectrum of medalose acetate.

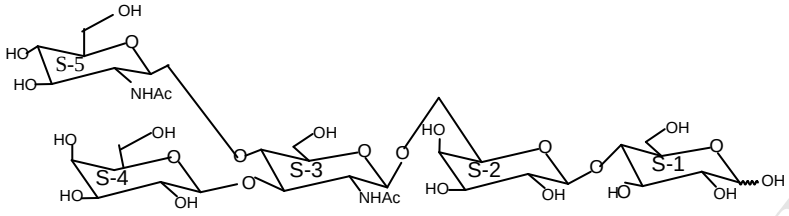
The values of these cross peaks are as- β-Glc (S_1) H-4 and C-4 at δ3.79 ppm x 75.94 ppm shows (1→4) linkage, β-Gal (S_2 & S_4) H-6 and C-6 at δ 3.82 ppm x 73.67 ppm shows (1→6) linkage, β-GlcNAc(S_3) H-3 and C-3 at δ 3.88 ppm x 75.94 ppm shows (1→3) linkage and also it's H-4 and C-4 at δ 3.8 ppm 2 x 75.81 ppm shows (1→4) linkage respectively. The ES MASS spectrum of medalose not only confirmed the derived structure but also confirmed the sequences of the monosaccharides in medalose. The highest mass ion peak were recorded m/z 933 which was due to $[M+Na]^+$. It also showed the M^+ 910 which confirmed that the molecular weight of compound was 910. Further the mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water. The fragmentation pathway confirmed the sequence of monosaccharide units in the pentasaccharide (scheme 1).



SCHEME 1:- ES MASS FRAGMENTS OF COMPOUND MEDALOSE

The pentasaccharide on fragmentation gave a mass ion peak at m/z 748(I), corresponding to tetrasaccharide unit, which was due to loss of S-4 sugar unit i.e. Gal (S-4) sugar unit linked to the S-3 of pentasaccharide. It was supported by its respective fragment at m/z 180, which confirmed the presence of Gal (S-4) at non reducing end. The tetrasaccharide on fragmentation gave a mass ion peak at m/z 545(II), which was due to loss S-5 sugar unit i.e. GlcNAc (S-5) sugar unit linked to the S-3 of tetrasaccharide unit.. It was supported by m/z 527 (545-H_2O). The trisaccharide on fragmentation gave mass ion peak at m/z 342(III), which was due to loss of S-3 sugar unit i.e. GlcNAc (S-3) sugar unit linked to the S-2 of trisaccharide unit. This disaccharide on further fragmentation gave a mass ion peak at m/z 180(IV), which was due to loss of S-2 sugar unit i.e. Gal (S-2) sugar unit linked to the S-1 of disaccharide. The other mass fragments obtained at m/z892(910-H_2O), m/z 844(892-CH_2OH), m/z771(844-CH_2OH), m/z 848(910-2CH_2OH), m/z 789(848-CH_2CO-OH), m/z 758(789- CH_2OH), m/z861(910- CH_2OH-H_2O), m/z 819(861- CH_2CO), m/z 771(819-CH_2OH-OH), m/z837(910-CH_2OH- CH_2CO) and m/z819(837-H_2O). The pentasaccharide m/z 910 on fragmentation gave tetrasaccharide m/z748 (M-S_4), which was further confirmed by its other fragment ions at m/z 730(748-H_2O), m/z 699(730-CH_2OH), m/z 681(699-H_2O), m/z 621(681-CH_2OHCHO), m/z 590(621-CH_2OH), m/z 688(748-CH_2OHCHO), m/z 652(688-2H_2O), m/z 621(652-CH_2OH), m/z 579(621-CH_2CO), m/z 659(748-$NHCOCH_3$-CH_2OH), m/z 611(659-CH_2OH-OH), m/z 593(611-H_2O), m/z 576(593-OH) and m/z 558(576-H_2O). The tetrasaccharide m/z 748 on fragmentation gave trisaccharide m/z527 due to (M-S_5- H_2O), which was further confirmed by its other fragment ions at m/z 465(527-2CH_2OH), m/z 406(465- CH_2CO-OH), m/z 375(406- CH_2OH), m/z 357(375- H_2O), m/z 496(545- CH_2OH- H_2O), m/z479(496-CH_2OH- H_2O), m/z462(479-OH), m/z402(462-CH_2OHCHO), m/z 384(402- H_2O) and m/z 342(384- CH_2CO). The trisaccharide m/z 545 on fragmentation gave disaccharide m/z342, which was further confirmed by its other fragment ions at m/z 293(342- CH_2OH- H_2O), m/z 275(293- H_2O), m/z 257(275- H_2O), m/z 239(257-H_2O), m/z190(239- CH_2OH- H_2O), m/z 325(342-OH),m/z 266(325- CH_2CO- OH), m/z 218(266- CH_2CO- OH) and m/z 200(218- H_2O).The disaccharide m/z 342 on fragmentation gave monosaccharide m/z 180. Based on the results obtained from chemical degradation and chemical transformation, mass spectrometry and ^{1}H, ^{13}C, HOMOCOSY, TOCSY, HSQC NMR, the structure of the isolated pentasaccharide is deduced as

Gal-β(1→3)GlcNAcβ(1→6)Gal-β(1→4)Glc
4
↓
1GlcNAc β



MEDALOSE

4. Conclusion

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

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Highlights

1. Isolation and structure elucidation of a novel oligosaccharide from camel milk.
2. Detailed structural analysis by ^{1}H, ^{13}C, 2D-NMR and Mass Spectrometry.
3. Traditional methods were also been used.
4. Biological activities of camel's milk.