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By

Manisha Shukla, Mayank Sharma, Rinku Singh and Desh Deepak

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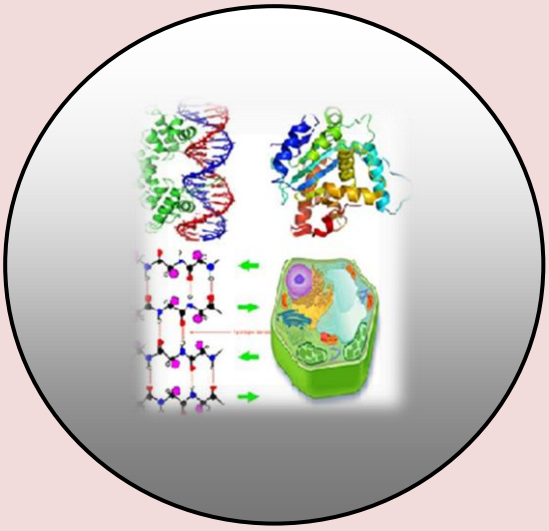

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Dr. Manisha Shukla
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RESEARCH PAPER

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Structural Characterization of Novel Milk Hexasaccharide 'Omedose' from Camel Milk

Manisha Shukla, Mayank Sharma, Rinku Singh and Desh Deepak
Department of Chemistry, University of Lucknow, Lucknow-226007, U.P., India

ABSTRACT

Camel milk is called white gold of the desert and is a rich source of biologically significant oligosaccharides containing sialylic acid, which plays a pivotal role in the brain development of a neonate. Camel milk has special medicinal properties against dropsy, jaundice, and other diseases affecting lungs, spleen. It enhances the human immune defence system. In continuation to our previous studies on camel milk, a novel milk hexasaccharide 'Omedose' has been isolated from camel milk oligosaccharide mixture by employing Kobata and Ginsberg method involving deproteination, centrifugation, gel chromatography and column chromatography of acetylated oligosaccharide mixture. Its structure has been elucidated with the help of the results obtained from the spectroscopic experiments such as 1D NMR (^{1}H and ^{13}C), 2D NMR (HSQC, COSY and TOCSY) and Mass spectrometry in combination with structure reporter group theory. The structure of the novel oligosaccharide 'Omedose' was established as under:

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

Omedose

Keywords: Camel milk, Kobata and Ginsberg method, Omedose, NMR and Mass spectrometry.

INTRODUCTION

Milk is a complex biofluid that delivers bioactive components for the growth and development of neonates (Smilowitz et al., 2014). Carbohydrates as oligosaccharides, proteins and fats are important components of milk. Milk oligosaccharide (MOs) protect infants by reducing the number of pathogenic infections and promote colonization of the gut micro biota (Li et al., 2021, Newburg, 2013, Kunz et al. 2006).

Milk oligosachharides also play a key role in various physiological, pathological and biological activities. It shows anti-complementary, anticoagulant, anti-inflammatory, antiviral and immunological activities (Singh et al. 2016, Schwonzen et al., 1992, Abe et al., 1983, Srivastava et al., 1989). A study (Deepak et.al, 1998) established that the Donkey milk oligosaccharides have ability to stimulate non-specific and specific immunological resistance. Furthermore, donkey milk oligosaccharides may be used for prevention of atherosclerosis (Tafaro et al., 2007). The Buffalo milk oligosaccharides have ability to stimulate non-immunological resistance of the host against parasitic infections (Saksena et al., 1999). Camel milk is called white gold of the desert (Jilo et al., 2016) which contains low cholesterol, low sugar, oligosaccharides, high minerals, high vitamin C, protective proteins like lactoferrin, lactoperoxidase, immunoglobulins and lysozyme etc. (Yadav et al., 2015). It is closest to human mother's milk and safe for children (Yadav et al., 2015, Hosseini et al., 2017). Research studies confirmed that camel milk has better nutritional values, as it exhibits many unique and amazing health-promoting properties in comparison to cow milk (Kumar et al., 2016). Recent studies confirmed that camel milk has shown different biological activities such as antioxidant, anti-hepatitis, hypoallergenic (Katz et al., 2008), antihypertensive (Salami et al., 2009), hypoglycemic, anticancer, antithrombotic (Korashy et al., 2012, Al-Juboori et al., 2013, Sharma et al., 2014), antibacterial and antiviral activities (Alavi et al., 2017) etc. Camel milk has more oligosaccharide contents in comparison to bovine milk (Mohammadian et al., 2017). The milk oligosaccharides isolated from camel milk showed potent ant-tubercular activity (Ilse et al., 2004). Recent study showed that camel milk is a good source of biologically significant sialyl oligosaccharides (Fukuda et al., 2010).They are involved in numerous functions such as anti-adhesive effects against certain pathogens (Hakkarainen et al., 2005, Matrosovich et al., 2003, Wang et al., 2007) and provide important nutrients for brain development. Hence in continuation to our previous studies on camel milk (Gangwar et al., 2018, Gunjan et al., 2018) one more novel hexasaccharide was isolated from camel milk. Its structure was elucidated on the basis of the evidences obtained from chemical degradation, chemical transformation, structure reporter group (SRG) theory and spectroscopic method like 1D NMR, 2D NMR (COSY, TOCSY and HSQC) as well as ESI-MS.

EXPERIMENTAL

General Procedure

^{1}H, ^{13}C and 2 D NMR spectra were recorded on a Bruker AM 300 MHz FT NMR spectrometer at 25°C.NMR experiments were performed in $CDCl_3$ and D_2O and TMS was used as an internal indicator. ESI-MS spectra were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. For Optical rotation, sample was analyzed with a PERKIN-ELMER 241 automatic polarimeter in 1cm tube. The C, H and N analysis were recorded on CARLO-ELBA 1108 an elemental analyzer. CC and TLC analysis were carried out onsilica gel (SRL, 60-120 mesh) and silica gel 60 F254 (SRL) respectively. Paper Chromatography (PC) was performed on Whatmann No.1 filter paper using solvent system toluene: butanol (4:1) saturated with H_2O. Oligosaccharides were visualized by spray of 50% aqueous H_2SO_4 reagent on TLC plates and with acetyl acetone and p-dimethyl amino benzaldehyde reagents on PC. Sephadex G–25 (PHARMACIA) was used in gel permeation chromatography. Freeze drying of the compound was done with the help of CT 60e (HETO) lyophylizer and centrifuged by a cooling centrifuged Remi instruments C-23 JJRCI 763.

Reverse phase HPLC, equipped with Perkin Elmer 250 solvent delivering system, 235 diode array detector and G.P. 100 printer plotter was used to analyze the homogeneity of the compound. Authentic samples of glucosamine, galactosamine, galactose and glucose were purchased from Aldrich Chemicals.

Isolation of camel milk oligosaccharide by Kobata and Ginsberg method (Saksena et al., 1999).

12 liter camel milk was collected from a single domestic camel and stored at -20°C, equal amount of alcohol i.e. 12 liter was added to it and stored overnight. Milk was centrifuged for 30 min at 5000 rpm at -4°C. After the removal of solidified lipid layer through glass wool column in cold, ethanol was added to clear filtrate to a final concentration of 68% and the resulting solution was left over night at 0°C. The white precipitate formed, mainly of lactose and protein was removed by centrifugation (30 min at 5000 rpm at -4°C) and washed twice with 68 % ethanol at 0°C. The supernatant and washings were combined and filtered through a microfilter and lyophilized affording crude oligosaccharide mixture. The lyophilized material responded positively to Morgan-Elson test (Patridge et al., 1948) and thiobarbituric acid assay (Bryant et al., 1953) suggesting the presence of N-acetyl sugars and sialic acid in oligosaccharide mixture. This lyophilized material (mixture of oligosaccharides) was further purified by fractionating it on Sephadex G-25 chromatography using glass triple distilled water as eluent at a flow rate of 3 ml/min. each fraction was analyzed by phenol sulphuric acid reagent (Duboiset al., 1956) [for the presence of neutral sugars].

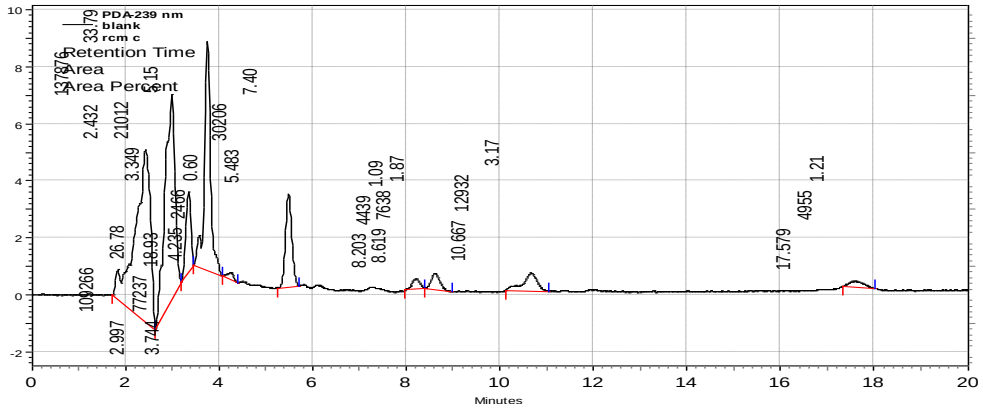


Figure 1. Reverse phase HPLC of crude oligosaccharide fraction. Elution was monitored by U.V absorbance at 215 nm.

Confirmation of homogeneity of camel milk oligosaccharide by reverse phase HPLC

Homogeneity of oligosaccharide mixture was qualitatively analyzed by reverse phase HPLC. The HPLC system was equipped with Perkin Elmer 250 solvent delivering system, 235 diode array detector and G.P. 100 printer plotter. The solvents used for the analytical HPLC was a binary gradient system of acetonitrile: 0.5% trifluro-acetic acid (5:95) in triple distilled water (TDW) to CH_3CN: 0.5 % TFA (60:40) within 25 min at a flow rate of 1 ml/min. The eluents were detected at 215 nm. Ten peaks were observed in the sample at the varied retention times from 2.41 min. to 17.63min, for convenience the peaks were numbered in their increasing order of retention time i.e. [2.432 min(R_1), 2.997 min(R_2), 3.349 min(R_3), 3.744 min(R_4), 4.235 min(R_5), 5.483 min(R_6), 8.203 min(R_7), 8.619 min(R_8), 10.667 min(R_9), and 17.579 min(R_{10})] (fig 1).

Acetylation of Oligosaccharide Mixture

Oligosaccharide Mixture (12 gm) was acetylated with acetic anhydride(12ml) in pyridine (12ml) at 60°C with continuous stirring for 12 hrs affording acetylated oligosaccharide mixture (12.8gm). The acetylation converted the free sugars into their nonpolar acetyl derivatives which were resolved nicely on TLC, giving ten spots on TLC i.e. a, b, c, d, e, f, g, h, i and j of which acetylated compound "h" was finally separated by column chromatography over silica gel using various proportions of chloroform and $CHCl_3$:MeOH as eluents.

Purification of acetylated milk oligosaccharide on silica gel column

Isolation of acetylated oligosaccharide mixture (12gm)was carried over silica gel (600g) column using varying proportion of Hex: $CHCl_3$, $CHCl_3$ and $CHCl_3$: MeOH as eluents, collecting fractions of 300ml each. All these fractions were checked on TLC, hence ten fractions namely I(3.28 g), II(736mg), III (3.29g), IV (468mg), V (380 mg),VI(1.007g), VII(1.06g), VIII (767mg), IX (319mg), and X (137mg) respectively were obtained. Repeated column chromatography of fraction VII lead to the isolation of novel oligosaccharide i.e. Omedose acetate (h)(139mg).

Deacetylation of Compound h, Omedose acetate

30 mg of Omedose acetate was taken in acetone (2 ml) and NH_3(3 ml) was added and left overnight in a stoppered hydrolysis flask and usual work-up (Gunjan et al., 2018) afforded deacetylated oligosaccharide i.e. Omedose (H)(24 mg).

DESCRIPTION OF THE COMPOUND

Compound H, Omedose

Compound H, Omedose was isolated as viscous syrup, $[\alpha]_D$+60.61° (c, 2, H_2O) and was assigned the molecular formula $C_{40}H_{68}N_2O_{31}$, as shown by its ESI-MS data. For experimental analysis, this compound was dried over P_2O_5 at 100°C and 0.1 mm pressure for 8 hr.
Elemental analysis:

Calculated	**%C**	**%H**	**%N**
	44.78	6.34	2.61
Found	44.76	6.34	2.61

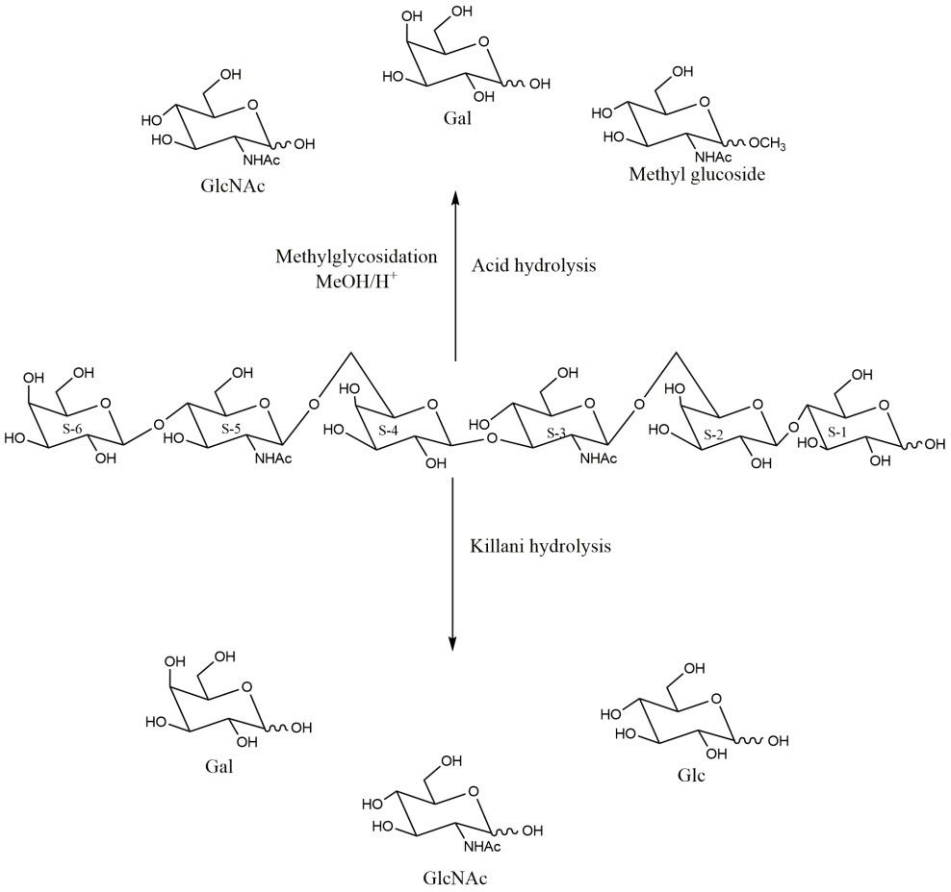
It gave positive test for sugars (Phenol-sulphuric acid test and Feigl test (Fiegl et al., 1975) and amino sugar (Morgan-Elson test).

Methylglycosidation/Acid Hydrolysis

Omedose (8mg) was refluxed with absolute MeOH (2 ml) at 70°C for 18 hr in the presence of cation exchange IR-120 (H^+)resin. The reaction mixture was filtered while hot and filtrate was concentrated. In the solution of methylglycoside of H, 1, 4-dioxane (1 ml), and 0.1N H_2S0_4 (1 ml) was added, solution was warmed for 30 minutes at 50°C. The hydrolysis was completed after 24 hrs. The hydrolysate was neutralized with freshly prepared $BaCO_3$, filtered and concentrated under reduced pressure to afford α-and β-methylglucosides along with glucose (Glc), galactose (Gal) and glucosamine (GlcNAc) (Scheme 1). Their identification was confirmed by comparison with authentic samples (TLC and PC).

Kiliani Hydrolysis of Omedose H

Omedose (5mg) was dissolved in 2 ml Killiani mixture (AcOH-H_20-HCl, 7:11:2), and heated at l00°C for 1 hr. Usual workup as reported earlier(Gunjan et al., 2018) afforded glucose, galactose and GlcNAc on comparison with authentic samples of glucose, galactose and GlcNAc (Scheme 1).



Scheme 1. Methylglycosidation/Acid hydrolysis and Killiani hydrolysis of Omedose.

Mannich-Siewert hydrolysis of Omedose H

Omedose (5mg) was dissolved in 2 ml acetone and conc. HCl (0.02 ml) was added (Mannich et al., 1942). The solution was kept under carbon dioxide in dark room at room temperature. After two days paper chromatogram showed three spots, the faster moving spot was identical in mobility with authentic sample of Gal and the spot with lowest mobility was identical with unreacted compound Omedose (I), further the compound with the intermediate mobility may be the pentasaccharide (II). Further, after four days two new spots were observed of which one was identical in mobility with authentic sample of GlcNAc and other, which was faster in mobility, then pentasaccharide, may be tetrasaccharide (III). It was again checked after six days and a new spot was observed which was having faster mobility than tetrasaccharide, so it may be trisaccharide (IV), which was formed by the loss of another Gal moiety. Further after eight days a new spot was observed, which was faster in mobility than the trisaccharide and having same mobility as the authentic sample of lactose (V) and it was formed by the loss of GlcNAc moiety.

The hydrolysis was partially completed in ten days and showed a new spot, which were found identical with authentic sample of Glc on TLC and PC. Thus the hydrolysis was completed in ten days showing three spots on TLC which were found identical with authentic sample of GlcNAc, Gal and Glc (TLC, PC).

^{1}H NMR value of Omedose H in D_2O (δ value) (for convenience the six sugar units of compound Omedose are designated as S-1, S-2, S-3, S-4, S-5 and S-6)

δ1.92[s, 3H, $NHCOCH_3$, β-GlcNAc (S-3)], δ2.02[s, 3H, $NHCOCH_3$, β-GlcNAc (S-5)], δ3.29[t, 1H, J=8.4Hz, β-Glc(S-1), H-2], δ3.94[d, 2H, β-Gal (S-2 & S-4), H-4], δ4.06[t, 1H, β-GlcNAc(S-3 & S-5), H-3], δ4.46[d, 2H, J=7.5 Hz, β-Gal (S-2 and S-4), H-1], δ4.53[d, 2H, J=7.8 Hz, β-Glc (S-1)& Gal(S-6) , H-1], δ4.59[d, 1H, J=8.7 Hz, β-GlcNAc (S-3), H-1], δ4.67[d, 1H, J=7.8 Hz, β-GlcNAc (S-5), H-1], δ5.23[d,1H, J=3.6 Hz, α-Glc (S-1),H-1].

^{13}C NMR value of Omedose H in D_2O (δ value)

δ68.5[β-Gal (S-2),C-6], δ68.8[β-Gal (S-4),C-6], δ77.6[α&β-Glc (S-1), C-4], δ77.8[β-GlcNAc(S-5),C-4], δ78.1[β-GlcNAc(S-3),C-3], δ91.1 [α-Glc (S-1), C-1], δ95.4 [β-Glc (S-1), C-1], 101.7 [β-GlcNAc(S-3),C-1], δ102.3[β-Gal (S-2), C-1] δ102.4[β-Gal (S-4 & S-6), β-GlcNAc (S-5),C-1],δ173.1[$NH\underline{CO}CH_3$].

^{1}H NMR value of Omedose acetate h in $CDCl_3$ (δ value)

δ3.70[β-Gal(S-2&S-4),H-6], δ3.83[β-Glc(S-1)&β-GlcNAc(S-5),H-4], δ3.85[β-GlcNAc(S-3),H-3], δ3.93[α-Glc(S-1),H-4], δ4.43[d,2H,J=6.6,β-Gal(S-2 & S-4),H-1], δ4.40[d,1H,J=7.2,β-Gal (S-6) H-1], δ4.62[d, 2H, J=8.4,β-GlcNAc(S-3 & S-5), H-1], δ5.7[d, 1H, J=8.1,β-Glc (S-1),H-1],δ6.27[d, 1H, J=3.6, α-Glc(S-1),H-1].

^{13}C NMR value of Omedose acetate h in $CDCl_3$ (δ value)

δ68.37[β-Gal(S-2 & S-4),C-6], δ76.8 [α-Glc(S-1),C-4], δ77.2[β-Glc(S-1),C-4], δ77.7[β-GlcNAc(S-3)C-3 & (S-5)C-4], δ89.3[α-Glc(S-1),C-1], δ91.7[β-Glc(S-1),C-1], δ101.2[β-Gal(S-2 & S-4),C-1], δ103.7[β-GlcNAc(S-3 & S-5),β-Gal(S-6),C-1].

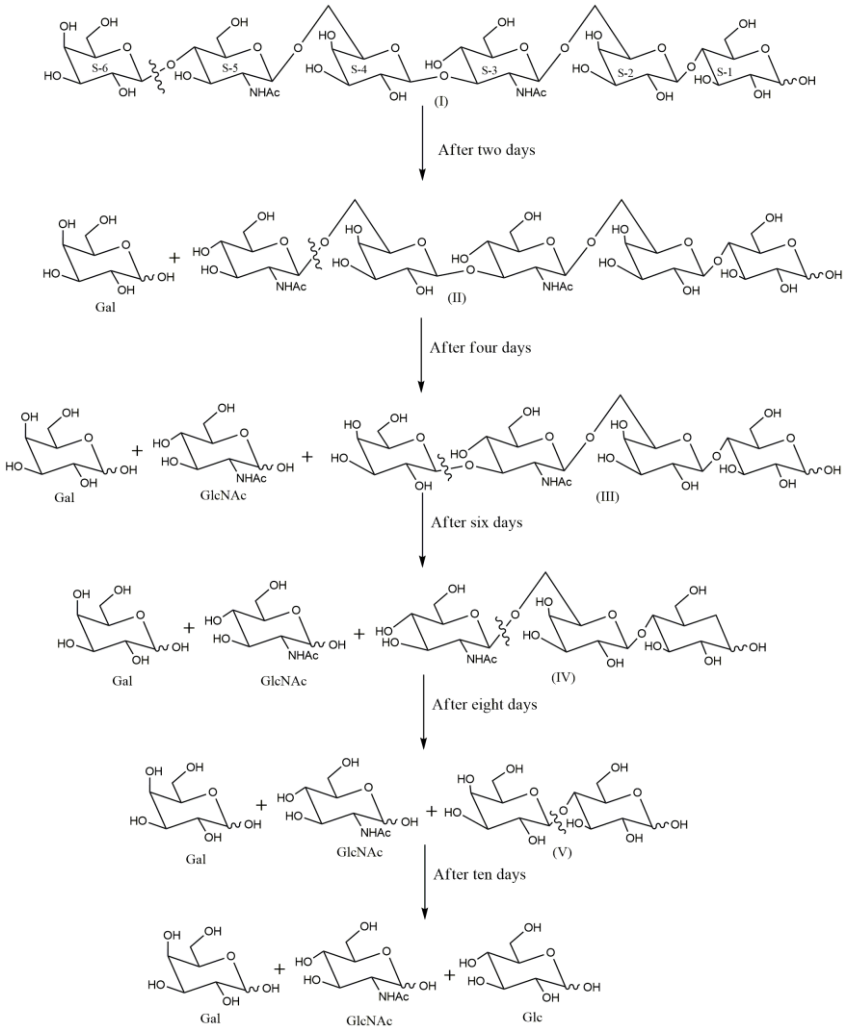
ES-MS of compound Omedose H

1095$[M+Na]^+$, 1072$[M]^+$, 910, 875, 841, 778, 743, 677, 623, 545, 487, 460, 458, 427, 415, 410, 342, 304, 260, 241, 218, 214, 180.

RESULTS AND DISCUSSION

Compound H, Omedose was isolated as viscous syrup, $[\alpha]_D$+60.61^0(c, 2, H_2O) and was assigned the molecular formula $C_{40}H_{68}N_2O_{31}$, as evident by its ESI-MS at m/z 1095 ($[M+Na]^+$). It gave positive Phenol-sulphuric acid test, Feigl test, and Morgan-Elson test showing the presence of normal and amino sugar in the compound Omedose. The ^{1}H NMR spectrum of Omedose exhibited five doublets for seven anomeric proton(s) at δ5.23 (1H), δ4.67 (1H), δ4.59 (1H), δ4.53 (2H) and δ4.46 (2H) indicating that the Omedose may be a hexasaccharide in its reducing form. ^{13}C NMR spectra of Omedose also supported the above results by the presence of seven-anomeric carbons at δ91.1(1C), δ95.4(1C), δ101.7(1C) and δ102.4(4C).

Monosaccharide constituents of Omedose were confirmed by its Killiani hydrolysis under strong acidic condition. Stronger condition gave three monosaccharides i.e. glucose, galactose and N-acetyl-glucosamine, which were found identical with authentic samples of glucose, galactose and N-acetyl-glucosamine, by TLC and PC comparison. Monosaccharide constituents and their sequence in Omedose were further supported by Mannich-Siewert hydrolysis (mild acidic condition) and were monitored on paper chromatography (PC) (Scheme 2).



Scheme 2. Mannich-Siewart hydrolysis of Omedose.

In this hydrolysis after two days, paper chromatogram showed three spots, the faster moving spot was identical in mobility with authentic sample of Gal and the spot with lowest mobility was identical with unreacted compound Omedose (I), the compound with the intermediate mobility may be the pentasaccharide (II). After four days, two new spots were observed, of which one was identical in mobility with authentic sample of GlcNAc and other, which was faster in mobility then pentasaccharide, may be tetrasaccharide (III). It was again checked after six days and a new spot was observed which was having faster mobility than tetrasaccharide, so it may be trisaccharide (IV), which was formed by the loss of another Gal moiety.

Further after eight days a new spot was observed, which was faster in mobility than the trisaccharide and having same mobility as the authentic sample of lactose (V) and it was formed by the loss of GlcNAc moiety. The hydrolysis was partially completed in ten days and showed a new spot, which were found identical with authentic sample of Glc on TLC and PC confirming sequence of monosaccharide in hexasaccharide as Gal-GlcNAc-Gal-GlcNAc-Gal-Glc. The hydrolysates were isolated for these compounds as GlcNAc, Gal and Glc and it was compared with authentic sample of GlcNAc, Gal and Glc on PC and TLC.

Table 1.^{1}H NMR shifts of Omedosein D_2O and $CDCl_3$.

Sugar moiety	^{1}H NMR (δ shifts in D_2O)	Coupling Constant(J)	^{1}H NMR (δ shiifts in $CDCl_3$)	Coupling Constant(J)
α-Glc	5.23	3.6	6.28	3.6
β-Glc	4.53	7.8	5.70	8.1
β-Gal	4.46	7.5	4.43	6.6
β-GlcNAc	4.59	8.7	4.62	8.4
β-Gal	4.46	7.5	4.43	6.6
β-GlcNAc	4.67	7.8	4.62	8.4
β-Gal	4.53	7.8	4.40	7.2

Table 2.^{13}C NMR shifts of Omedose in D_2O.

Sugar moiety	C-1	C-2	C-3	C-4	C-5	C-6	CO	CH_3
α-Glc	91.1	72.07	72.0	77.6	70.8	61.6		
β-Glc	95.4	74.5	74.2	77.6	74.5	61.6		
β-Gal	102.4	70.8	72.07	67.9	74.9	68.8		
β-GlcNAc	101.7	60.58	78.1	72.08	74.5	61.6	173.1	21.4
β-Gal	102.4	71.1	72.07	68.8	74.9	68.5		
β-GlcNAc	102.4	61.6	74.2	77.8	74.9	61.6	173.1	24.6
β-Gal	102.4	74.2	71.1	70.5	74.9	63.5		

Methylglycosidation of Omedose 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 Omedose. The presence of the glucose moiety at the reducing end of Omedose confirmed by the doublets at chemical shifts of δ 5.23 (J= 3.6 Hz)/91.1 for the signals of H-1α/C-1α and δ 4.53(J= 7.8 Hz)/95.4 for the signals of H-1β/C-1β in^{1}H and ^{13}C NMR spectrum of Omedose in D_2O (Kiliani, 1951, Urashima, et al., 2004). Next anomeric proton doublet at δ4.46 (J=7.5 Hz) was due to presence of β-Gal (S-2) moiety in the Omedose. β-Gal moiety present in Omedose suggested the presence of a lactosyl moiety i.e. Gal-β-(1$\rightarrow$4) Glc. This was further confirmed by β-Glc (S_1), H-2 signal which is a structure reporter group for lactosyl moiety (Urashima, et al., 2002), which appeared as a triplet at δ3.29 (J=8.4 Hz). Further the presence of another anomeric proton doublet at δ4.59 (J=8.7Hz) along with signal of amide methyl group at δ2.02, was due to the presence ofβ-GlcNAc(S_3) moiety (Gronberg et al., 1990).

The H-4 proton resonance of β-Gal (S_2), which appeared at δ3.93, implies that the β-GlcNAc (S_3) may be 1→6 linked to β-Gal (S_2)(Haeuw-Fievre et al., 1993, Urashima et al., 1993). This was confirmed on the basis of downfield shifting of H-6 at δ3.69 and C-6 at δ68.8 in ^{1}H and ^{13}C NMR of Omedose and chemical shift analogies of β-GlcNAc (1→6) Gal-β as given (Strecker et al., 1992).

The anomeric proton signal at δ4.46 (J=7.5 Hz) was assigned to β-Gal moiety (S_4) which was linked to H-3 of β-GlcNAc(S-3) The linkage between β-GlcNAc(S-3) and β-Gal (S-4) was established on the basis of presence of downfield shifted H-3 proton of GlcNAc (S_3) at δ4.05 as triplet which indicate a β-Gal-(1→3)-GlcNAc sequence (SRG) (Bush et al., 1985). The signal for H-1 at δ4.67 (J=7.8Hz) and H-4 at δ3.83, along with signal of methyl group at δ1.92, was due to β linked GlcNAc(S-5) moiety (Gronberg et al., 1990). The downfield shift of H-6 and C-6 of β-Gal (S-4) of Omedose at δ3.69 and δ68.8 indicate that β-GlcNAc (S-5) is linked to 6-position of β-Gal (S_4) which was further confirmed by structure reporter group value of H-4 of β-Gal (S_4) at δ3.93 (Haeuw-Fievre et al., 1993, Urashima et al., 1993).

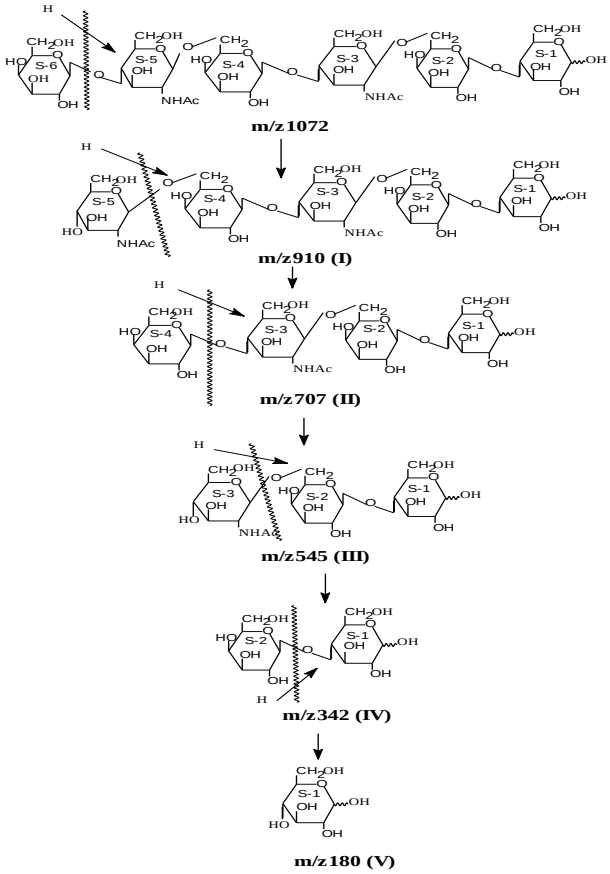


Figure 2. ESI-MS fragmentation of Omedose.

The next anomeric signal appeared at δ4.53 was due to the presence of β-Gal (S-6) moiety. The presence of H-4 and C-4 chemical shift value of β-GlcNAc (S-5) at δ3.83 and δ77.8 suggested that β-Gal (S-6) is linked to the 4thposition of β-GlcNAc (S_5). Since β-Gal (S_6) was present at non-reducing end, H-1 β-Gal was down field shifted (Dua et al., 1983, Chaturvedi et al., 1988).

The β-glycosidic linkage of the six sugar moieties were shown by the larger coupling constant of the anomeric proton signals in the ^{1}H NMR spectrum of Omedose. Further, HSQC experiment of Omedose acetate also confirmed the glycosidic linkage between sugar moieties. HSQC spectra of Omedose acetate showed following correlations between glycosidically linked carbons with their protons. The values of these cross peaks are as α-Glc (S-1) H-4 and C-4 at δ3.93 x 76.78 shows (1→4) linkage of S-2and S-1, β-Glc (S-1) H-4 and C-4 at δ3.83 x 77.2 shows (1→4) linkage of S-2and S-1, β-Gal (S-2& S-4) H-6 and C-6 at δ3.70 x 68.37 for (1→6) linkage of S-3→ S-2and of S-5→ S-4, β-GlcNAc(S-3) H-3 and C-3 at δ3.85 x 78.1 for (1→3) linkage of S-4and S-3 and β-GlcNAc (S-5) H-4 and C-4 at δ3.83 x 77.8 for (1→4) linkage of S-6and S-5 respectively. The COSY spectrum also gave assignments of ring protons involved in glycosidic linkage at δ3.93 (H-4) for α-Glc, δ3.83 (H-4) for β-Glc, δ3.70 (H-6) for β-Gal (S-2) & (S_{-4}), δ3.85 (H-3) for β-GlcNAc (S-4) and δ3.83 (H-4) for β-GlcNAc (S-5) and it was further confirmed by the TOCSY spectrum of Omedose acetate.

The structure of Omedose was further confirmed by the ESI-MS spectrum of Omedose. It contain highest mass ion peak at m/z 1195 which was due to $[M+Na]^{+}$ and the other mass ion peaks are at m/z 1072 due to $[M]^{+}$.The molecular formula of Omedose was derived as $C_{40}H_{68}N_2O_{31}$. 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 monosaccharides in the oligosaccharide (figure 2). The hexasaccharide m/z 1072 on fragmentation gave pentasaccharide at m/z 910 (I), which was due to loss of S-6 sugar unit i.e. Gal (S-6) sugar unit linked to the S-5 of hexasaccharide. It was supported by its respective fragment at m/z 180 that confirmed the presence of Gal (S-6) at non reducing end. The pentasaccharide on fragmentation gave a mass ion peak at m/z707 (II), corresponding to tetrasaccharide unit, which was due to loss of S-5 sugar unit i.e. GlcNAc (S-5) sugar unit linked to the S-4 of pentasaccharide. This tetrasaccharide fragment on fragmentation gave a mass ion peak at m/z 545(III), which was due to loss S-4 sugar unit i.e. Gal (S-4) sugar unit linked to the S-3 of tetrasaccharide unit. The trisaccharide on fragmentation gave mass ion peak at m/z 342(IV), 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(V), 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/z 875(910-H_2O-OH), m/z 841(875-2OH), m/z 778(875-CH_2OH –CHO-$2H_2O$), and m/z 743(778-H_2O-OH). The pentasaccharide m/z 910 on fragmentation gave tetrasaccharide m/z 707 (M-S-5), which was further confirmed by its other fragment ions at m/z 677(707-HCHO), m/z 634(677-CH_2CHO) and m/z 623(707-$2CH_2CO$). The tetrasaccharide m/z 707 on fragmentation gave trisaccharide m/z 545 due to (M-S-4), which was further confirmed by its other fragment ions at m/z 487(545-$NHCOCH_3$), m/z 460(545-CH_2CHO-CH_2CO),m/z 458(545-$NHCOCH_3$-CHO),m/z 427(487-2HCHO), m/z 415(487-CH_2CO-HCHO) and m/z 410(487-HCHO-OH).The trisaccharide m/z 545 on fragmentation gave disaccharide m/z342, which was further confirmed by its other fragment ions at m/z 304(342-$2H_3O^{+}$), m/z 260(342-CH_3OH-H_2O), m/z 241(342-CH_2OHCHO-CHO), m/z 218(260-CH_2CO), and m/z 214(304-3HCHO).The disaccharide m/z 342 on fragmentation gave monosaccharide m/z 180.The result obtained from the ES mass spectrum further substantiated the structure of Omedose which was derived by its ^{1}H and ^{13}C NMR spectra.

In the light of foregoing evidences, the structure of isolated hexasaccharide, Omedose was determined to be:

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

OMEDOSE

CONCLUSION

In summary, we concluded that the novel compound Omedose was reported for the first time from camel milk or any other source and its structure was elucidated with the help of spectroscopic technique like ^{1}H,^{13}C and 2D NMR (TOCSY, COSY and HSQC) along with Mass spectrometry in combination with SRG theory.

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Corresponding author: Dr. Desh Deepak, Department of Chemistry, University of Lucknow, Lucknow-226007, U.P., India

Email: deshdeepakraju@rediffmail.com