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Isolation, Purification and Structure elucidation of novel camel milk oligosaccharides and their DFT studies

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Abstract: Milk provides energy and nutrition to any mammalian neonate; it is also responsible for growth of children and nourishment of adults. Besides the protein and fat, it contains carbohydrates, consisting of lactose and oligosaccharides. The oligosaccharides present in milk have numerous biological activity such as antitumor, anticancer, antiviral, anti-inflammatory, anticoagulant, antioxidant and immunostimulant activities. The milk of camel has shown antioxidative, antibacterial, antiviral, antifungal, antihepatitis, anticancer, antitumor, antihypertensive and antimicrobial activities. Keeping in mind the biological activity of camel milk and oligosaccharide present therein, it was collected in bulk and was processed by Kobata and Ginsberg for obtaining its oligosaccharides constituents. During the process the milk was deproteinated, filtered by microfilter and lyophilized followed by the gel filtration HPLC, column chromatography and thin layer chromatography, which resulted into the isolation of a novel milk oligosaccharides namely Dariose. The stereoscopic structures of this purified compound were elucidated with the help of chemical degradation, chemical transformation and spectroscopic techniques like NMR (^{1}H, ^{13}C, COSY, TOCSY and HSQC) and mass Spectrometry. The optimized geometry of compound Dariose at B3LYP method and 6-311+G basis set on Gaussian 09 program; show that the compound Dariose was stable compound.

Compound D

Keywords: Isolation**,** Oligosaccharides, Camel Milk and Dariose.

1. Introduction

Oligosaccharides are most important class of compounds found as natural constituents of milk, fruits, vegetables, blood, bacteria and fungus etc. Milk oligosaccharide has shown various physiological functions such as the improvement of intestinal microflora, stimulation of mineral absorption, anticariogenicity and the improvement of plasma cholesterol and blood glucose level [1-3]. The milk is an important source of these bioactive oligosaccharides with varied biological activities depending on the mammal it belongs. The oligosaccharides present in various milks shown antitumor, anticancer, antiviral, anti-inflammatory, anticoagulant, antioxidant and immunostimulant activities [4]. Camel milk is different from other ruminant milk, having low cholesterol, low sugar, high minerals (Na, K Fe, Cu, Zn, and Mg), high vitamin C, low protein and large concentration of insulin [5, 6]. Camel milk is used as a remedy for some diseases like tuberculosis, juvenile diabetes [7], liver cirrhosis, rickets, constipation, asthma [8], Camel milk has also shown antiviral [9], therapeutic [10] and antimicrobial activity [11]. In India, camel milk is used as therapeutically against dropsy, jaundice, problem of spleen, asthma, anaemia, pile and diabetes role of camel milk chronic pulmonary tuberculosis [5, 12]. Keeping in mind the biological activity of camel milk and oligosaccharide present therein, it was collected in bulk and was processed by method of Kobata and Ginsburg [13] followed by different chromatographic techniques like gel filtration, TLC, CC, HPLC etc. which resulted into the isolation of new milk oligosaccharides namely Dariose (D). The structures of purified milk oligosaccharides were elucidated by the using of the data generated from spectroscopic techniques like NMR (^{1}H, ^{13}C, COSY, TOCSY and HSQC) mass spectrometry and chemical degradation, chemical transformation. The present paper deals with structure elucidates of novel oligosaccharide from camel milk namely Dariose (D).

The name of this oligosaccharide was derived from Biological name of camel i.e. *Camelus Dromedarius.*

2 Experimental:

2.1 General procedures

General procedures were same as described in our previous article [14].

2.2 Isolation of Camel milk oligosaccharides by Kobata and Ginsberg method

10 litres milk was collected from a camel and was stored at -20°C until use. The milk was processed by the method of Kobata and Ginsberg **[13].** It was centrifuged for 15 min. at 5000 rpm at -4°C. The solidified lipid layer was removed by filtration through glass wool column in cold atmospheric condition. Ethanol was added to the clear filtrate (supernatant) to a final Concentration of 68% for precipitating out the lactose and proteins and the resulting solution was left overnight at 0°C. The white precipitate of lactose and protein was formed and removed by centrifugation for 15 min. at 5000 rpm at -4°C and washed twice with 68% ethanol. Further for complete removal of remaining lactose the supernatant was passed through a microfilter (0.24 μm) and lyophilized to get the crude oligosaccharide mixture (205g). The lyophilized material responded positively to Morgan-Elson test **[15]** and thiobarbituric-acid assay suggesting the presence of N-acetyl sugars in oligosaccharide mixture. This lyophilized material (mixture of oligosaccharide) was further purified by fractionating it on Sephadex G-25 chromatography using glass triple distilled water as eluant at a flow rate of 3 mL /m. Each fraction was analyzed by phenol sulphuric acid reagent **[16]** for the presence of neutral sugar.

2.3. Acetylation of oligosaccharide mixture

11g of crude oligosaccharide mixture was acetylated with pyridine (11 ml) and acetic anhydride (11 ml) at 600C and solution was stirred overnight. Further the mixture was evaporated under reduced pressure and the viscous residue was taken in CHCl3 (250ml) and washed with ice cold water (25 ml). The organic layer was dried over anhydrous Na2SO4, filtered and evaporated to dryness yielding the acetylated mixture (13g). The acetylation converted the free sugars into their nonpolar acetyl derivatives which were resolved nicely on TLC, giving eight spots on TLC i.e. A, B, C, D, E F, G and H of which one compound was finally separated by column chromatography over silica gel (60-120 mesh) using hexane: CHCl3 and MeOH: CHCl3 as eluents.

2.4. Purification of acetylated milk oligosaccharide

Separation of the acetylated products (10 g) was purified by column chromatography. The silica was used in the ratio of 1:100 using various proportions of Hexane $CHCl_3$, $CHCl_3$ and $CHCl_3$: MeOH mixture which resolved into eight fractions namely I(1.45 gm), II(1.10 gm), III(2.15 gm), IV(1.20 gm), V(2.30gm), VI(2.10gm), VII(1.40gm) and VIII(800 mg) respectively. These fractions were containing a mixture of three to four compounds. Repeated column chromatography of fraction IV, led to the isolation of one chromatographically pure compound D (204 mg).

2.5. Deacetylation of compounds

Compound D (98 mg) was dissolved in acetone (6 mL) and NH_3 (7 mL) and left overnight, in hydrolysis flask and ammonia was removed under reduced pressure, washed with $CHCl_3$ and was finally freeze dried giving the deacetylated oligosaccharide D (80 mg).

2.6. Killiani Hydrolysis:

Dry compounds D was placed separately in hydrolysis flask with 1 mL of Kiliani mixture (AcOH-H_2O-HCl, 7:11:2) and heated on a boiling water bath for half an hour. After evaporation they were checked by paper chromatography with the authentic samples of sugars. It was observed that compound A on Killiani hydrolysis **[17]** gave Glc, Gal, GalNAc, and GlcNAc, and compound D gave Glc, Gal, and GlcNAc confirming the presence of these sugar units.

Description of compounds

COMPOUND D (DARIOSE):

The presence of sugar units in compound **D** have been confirmed by ^1H, ^{13}C NMR, and Mass spectrometry.

^{1}H NMR of Dariose:

(D_2O, 400MHz): δ 5.55 [d, 1H, J=4.0 Hz, α-Glc (S-1) H-1], 5.15 [d, 1H, J=8.0Hz, β-Glc (S-1) H-1], 4.58 [d, 1H, J=8.0Hz, β-Glc (S-5), 4.45 [d, 2H, J=8.0 Hz, β-Gal (S-2), β-Gal (S-4) H-1], 4.35 [d, 2H, J=8.0 Hz, β-GlcNAc (S-3), β-GalNAc (S-6) H-1], 3.20 [t,1H, J=8.0Hz, β-Glc (S-1) H-2],1.95 [s, 3H, β-GlcNAc (S-3), $NHCOCH_3$] and δ1.90 [s, 3H, β-GalNAc (S-6), $NHCOCH_3$].

^{1}H NMR of Dariose Acetate:

($CDCl_3$, 400MHz): δ 6.15[d,1H, J=4.0Hz, α-Glc(S-1) H-1], 5.35 [d, 2H, J=8.0Hz, β-Glc (S-1), β-GlcNAc (S-3), H-1], 4.75[d, 1H, J=8.0Hz, β-Glc, (S-5), H-1], 4.50 [d, 2H, J=8.0Hz, β-Gal (S-2), β-Gal (S-4) H-1], 4.45 [d, 1H, J=8.0Hz, β-GalNAc(S-6), H-1], 4.10 [m, 1H, β-Gal (S-2), H-3], 3.90[m, 1H, β-Gal (S-4), H-3], 3.80[m, 1H, β-Glc (S-1), H-4].

^{13}C NMR of Dariose Acetate:

($CDCl_3$, 400MHz) δ 89.00 [1C, α-Glc (S-1)

C-1], 90.05 [2C, -Glc (S-1), β-GlcNAc (S-3) C-1], 95.19 [1C β-Glc (S-5) C-1], 100.91 [2C, β-Gal (S-2), β-Gal (S-4),C-1], 101.01 [1C β-GalNAc (S-6) C-1]

ES Mass

m/z 1111 $[M+K]^+$, 1095 $[M+Na]^+$, 1072 $[M]^+$, 1025, 1012, 965, 929, 919, 869, 838, 820, 804, 780, 747, 707, 676, 659, 586, 565, 545, 529, 516, 499, 422, 342, 324, 295, 180.

3. Theoretical studies

The quantum chemical calculations have been analyzed on basis set of B3LYP functional and 6-311+G (d, p). Geometry of compound D has been first optimized and the presence of positive wave numbers values for all the optimized geometry indicates stability of the compounds. The isolated compound was described by computational data using the Gaussian 09 program package **[18]**.

4 Result and discussion

4.1. Structure elucidations of the isolated Camel milk oligosaccharide

4.1.1. NMR spectroscopy

The isolated compounds have been identified and their structures were elucidated with the help of ^1H, ^{13}C NMR and 2D NMR of acetylated and natural oligosaccharide, mass spectrometry and chemical degradation. In the present study, analogies between chemical shift of certain 'structural reporter group resonances' and chemical shift difference between natural and acetylated oligosaccharide were also used to make proton resonance assignments as well as structural assignments of the oligosaccharides.

Compound 'D'(Dariose), $C_{40}H_{68}O_{31}N_2$, $[\alpha]_D$ $+55^0$ gave positive Phenol-sulphuric acid test **[16]**, Feigl test**[19]** and Morgon-Elson test**[15]** showing the presence of normal and amino sugar(s) in the moiety. The ^1H NMR spectrum of Dariose in D_2O at 400 MHz showed seven anomeric proton signals as doublets in its respective region at δ 5.55(1H), δ 5.15 (1H), δ 4.58(1H), δ 4.45(2H), δ 4.35(2H). The HSQC spectrum of acetylated Dariose also showed the presence of seven cross peaks of anomeric protons and carbons in the respective region at δ5.35 x 90.05, δ 5.35 x 90.05, δ 4.75 x 95, δ 4.50 x 100.91, δ 4.50 x 100.91, δ 4.35 x101.01 suggested the presence of seven anomeric protons and carbons in it. The presence of seven anomeric protons were also confirmed by the presence of seven anomeric doublets for seven anomeric protons at δ 6.15(1H), δ 5.35 (2H), δ 4.75 (1H), δ 4.50(2H), δ 4.45(1H) in the ^1H NMRspectrum of acetylated Dariose at 400 MHz in $CDCl_3$. The presence of seven anomeric protons/carbons may be defined as presence of hexasaccharide in its reducing form. The hexasaccharide nature of Dariose was further supported by seven anomeric carbon signals at δ 89.00(1C), 90.05(2C), 95.19(1C), 100.91(2C), 101.01(1C) in the ^{13}C NMR spectrum of Dariose acetate at 400 MHz in $CDCl_3$. The seven anomeric proton signals in the ^1H NMR spectrum of Dariose containing the matchable chemical shifts for α and anomers of glucose could be interpreted for the presence of a hexasaccharide in its reducing form, giving signals for α and β-anomers of glucose at its reducing end. The reducing nature of compound Dariose was further confirmed by its methyl glycosidation by $MeOH/H^+$ followed by its acid hydrolysis which led to the isolation of α and β methyl glucosides confirming the presence of glucose at the reducing end in the compound Dariose The six monosaccharides present in Dariose have been designated as S1, S2, S3, S4, S5 and S6 for convenience starting from the reducing end. To confirm the monosaccharide constituents in Dariose, it was hydrolyzed under strong acidic conditions (Kiliani hydrolysis) **[17]** followed by paper chromatography and TLC. In Kiliani hydrolysis the reducing

hexasaccharide gave four spots on TLC and paper chromatography, which were later identified as Glc, Gal, GlcNAc and GalNAc by co-chromatography with the authentic samples (paper chromatography) suggesting that the reducing hexasaccharide was made up of these monosaccharide unitsThe chemical shifts of anomeric carbons observed in ^{13}C NMR spectrum and of anomeric protons observed in ^{1}H NMR spectrum of Dariose were also in agreement with the reported values of ^{1}H and ^{13}C anomeric chemical shifts of Glc, Gal, GlcNAc and GalNAc suggesting the presence of these monosaccharides in the compound Dariose. The presence of two anomeric proton signals at δ 5.55 (1H, J = 4.0 Hz) and δ 5.15 (1H, J = 8.0 Hz) suggested the presence of α and β anomer of glucose (S-1) and hence confirmed the presence of glucose (S-1) at the reducing end **[20, 21]** in the ^{1}H NMR spectrum of Dariose in D_2O at 400 MHz in the compound Dariose. Further the presence of another anomeric proton doublet which appeared at δ 4.45 was assigned of β-Gal (S2) residue as the next monosaccharide unit. Further the appearance of a triplet at 3.20 (SRG) **[22, 23]** in the ^{1}H NMR of Dariose at 400 MHz in D_2O suggested the 1□4 linkage between β-Gal(S-2) and β-Glc (S-1) confirming the presence of Lactose type linkage i.e. β-Gal(1□4) β-Glc in the compound Dariose and hence the presence of lactosyl moiety**[24]** in it at the reducing end. The 1□4 linkage between β-Gal(S-2) and β-Glc (S-1) was further supported by the presence of β-Glc (S-1) H-4 signal at δ 3.80 in the ^{1}H NMR spectrum of acetylated Dariose in $CDCl_3$ at 400 MHz. The 1□4 linkage between β-Gal(S-2) and β-Glc (S-1) was further confirmed by the COSY TOCSY and HSQC spectrum of acetylated Dariose in $CDCl_3$ at 400 MHz. The large coupling constant value of β-Gal(S-2) i.e., J=8.0 Hz confirmed the β-glycosidic linkage between β-Gal(S-2) and β-Glc (S-1). Further the anomeric proton signal of β-Gal (S-2) at δ 4.45 showed two consequent complementary signal in the linkage region at δ 3.65 and δ 4.10 in TOCSY spectrum of Dariose acetate at 400 MHz in $CDCl_3$. The chemical shift of these methine protons at δ 3.65 and δ 4.10 suggested the OH groups of these positions may be involved in glycosidic linkages by next monosaccharide. These signals were later identified as H-2 and H-3 of β-Gal (S2) by COSY spectrum of Dariose acetate suggested that H-2 and H-3 of β-Gal (S2) were available for glycosidic linkage by the next monosaccharide units. The next anomeric proton signal which appeared at δ 4.35 along with a singlet of amide methyl at δ 1.95, was due to the presence of β-GlcNAc (S-3) moiety**[25]** as the next monosaccharide unit in the compound Dariose in D_2O at 400 MHz. As already assigned by ^{1}H NMR and TOCSY spectrum of acetylated Dariose in $CDCl_3$ at 400 MHz, that positions 2 and 3 of -Gal (S2) were present in glycosidic linkage region i.e., at δ 3.65 and δ 4.10 respectively indicating that two OH groups of β-Gal (S2) were available for glycosidic linkages. The downfield shifted H-4 proton of β-Gal (S-2) at δ 4.25 suggested that β-Gal (S-2) was glycosidically linked at C-3 position by β-GlcNAc (S-3) moiety (SRG) **[26, 27]** and hence suggested the 1□3 linkage between β-GlcNAc (S-3) and β-Gal (S-2) The (1□3) linkage between β-GlcNAc (S-3) and β-Gal (S-2) was further supported by the ^{1}H NMR spectrum of acetylated Dariose in which the signal for H-3 signal of β-Gal (S-2) appeared at δ 4.10 suggesting the (1□3) linkage between β-GlcNAc (S-3) and β-Gal (S-2).The (1□3) linkage between β-GlcNAc (S-3) and β-Gal (S-2) was later confirmed by the COSY and TOCSY spectrum of acetylated Dariose in $CDCl_3$ at 400 MHz. The coupling constant of anomeric signal -GlcNAc (S-3) with J value 8.0 Hz confirmed the β-configuration of β-GlcNAc (S-3) moiety and hence the β-glycosidic linkage between β-GlcNAc (S-3) and β-Gal (S-2).Another anomeric proton signal which appeared as a doublet at δ 4.35 in the ^{1}H NMR spectrum of Dariose in D_2O along with a singlet of $-NHCOCH_3$ (Methyl amide) at δ 1.90 suggested the presence of β-GalNAc moiety (S6) in

Dariose. Since it was ascertained by the COSY and TOCSY spectrum of Dariose acetate that the positions 2 and 3 of β-Gal (S-2) were available for glycosidic linkages and position 3 of β-Gal (S-2) was already linked with β-GlcNAc(S3), the leftover H-2 position of β-Gal (S-2) must be linked by β-GalNAc (S6). The position of linkage between β-GalNAc (S-6) and β-Gal (S-2) was further confirmed by the appearance of H-2 signal of β-Gal (S-2) at δ 3.65 in the ^{1}H NMR spectrum of Dariose acetate suggested the 1□2 linkage between β-GalNAc (S-6) and β-Gal (S-2). The 12 linkage between β-GalNAc (S-6) and β-Gal (S-2) was confirmed by COSY and TOCSY spectrum of Dariose acetate at 400 MHz in $CDCl_3$. The coupling constant of anomeric signal (S-6) with J value 8.0 Hz confirmed the β-configuration of β-GalNAc (S-6) moiety and hence the β-glycosidic linkage between β-GalNAc (S-6) and β-Gal (S-2). Further the presence of another anomeric signal which appeared as doublet at δ 4.45 (J=8.0Hz) was due to the presence of β-Gal (S4) moiety. The presence of H-3 proton of β-GlcNAc(S3) at δ 4.10 in the ^{1}H NMR spectrum of Dariose acetate suggested the 1□3 linkage between β-Gal (S-4) and β-GlcNAc (S-3). The 1□3 linkage between β-Gal (S-4) and -GlcNAc (S-3) was further determined by anomeric proton chemical shift value of β-Gal (S4) moiety, chemical shift of which was merged with the anomeric proton chemical shift value of β-Gal (S-2) of lactosyl moiety, which is a SRG**[23]** for 1□ 3 linkage between β-Gal (S-4) and β-GlcNAc (S-3) and hence, confirming the LNT moiety i.e., Gal-GlcNAc-Gal-Glc in compound Dariose. The 1□3 linkage between -Gal (S-4) and β-GlcNAc (S-3) was further confirmed by the COSY and TOCSY spectrum of acetylated Dariose at 400 MHz in $CDCl_3$ in which the signal for H-3 of β-GlcNAc (S-3) appeared at δ 4.10 i.e., in the glycosidic linkage region confirming the 1□ 3 linkage between β-Gal (S-4) and β-GlcNAc (S-3). The coupling constant of anomeric signal of β-Gal (S-4) with J value 8.0 Hz was confirms the β-configuration of β-Gal(S-4) moiety. Further another anomeric proton signal which appeared at δ 4.58 as a doublet in the ^{1}H NMR spectrum of Dariose in D_2O at 400 MHz could be interpreted for the presence of β-Glc (S-5). Since the anomeric signal of β-Gal (S-4) showed a cross peak at δ 3.90 in the TOCSY spectrum of acetylated Dariose suggested that this position was available for glycosidic linkage by next monosaccharide present. Later this signal was identified for H-3 of β-Gal (S-4) by COSY spectrum of Dariose acetate hence confirming that the H-3 of β-Gal(S-4) was available for glycosidic linkage hence confirming the linkage between β-Glc (S-5) and β-Gal(S-4) as 1□3. The large coupling constant of β-Glc (S-5) of J=8.0 Hz showed the β-glycosidic linkage between β-Glc (S-5) and β-Gal(S-4). The anomeric proton signals of β-Glc(S-5) and β-GalNAc (S-6) at δ 4.58 and δ 4.35 respectively present in Dariose does not have any methine signals in glycosidic linkage region i.e., δ 3-4 ppm in the TOCSY spectrum of Dariose acetate confirmed that none of their -OH group were involved in glycosidic linkages and hence, confirmed that β-Glc (S5) and β-GalNAc (S-6) were present at non-reducing ends and none of their OH were available for glycosidic linkages. All the ^{1}H NMR assignments for ring protons of monosaccharide units of Dariose were confirmed by HOMOCOSY and TOCSY experiments. The positions of glycosidation in the Dariose were confirmed by position of anomeric signals, S.R.G and comparison of the signals in ^{1}H and ^{13}C NMR of acetylated and deacetylated Dariose. The glycosidic linkages in Dariose were assigned by the cross peaks for glycosidically linked carbons with their protons in the HSQC spectrum of acetylated Dariose. The heteronuclear single quantum carbon atoms involved in glycosidation were also present in HSQC spectrum at δ 3.80 x 76 [Glc (S1) C_4 x H_4 showing 1□4 linkage], δ 3.65 x 72 [β-Gal (S2) C_2 x H_2 showing 1□2 linkage], δ 4.10 x 78 [β-Gal (S2) C_3 x H_3 showing 1□ 3 linkage], δ 4.10 x 68 [β-GlcNAc (S3) C_3 x H_3 showing 1□

3 linkage] δ 3.90 x 70 [β-Gal (S4) C_3 x H_3 showing 1□3 linkage]. Based on the pattern of chemical shift of ^1H, ^{13}C, HOMOCOSY **[28, 29]**, TOCSY **[30]** and HSQC **[27, 31]** NMR experiments, it was interpreted that the compound Dariose was a hexasaccharide comprised of two Glc, one GlcNAc, one GalNAc and two Gal moieties having the structure.

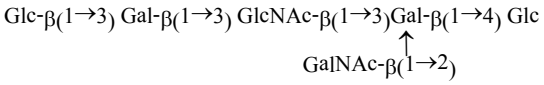


Table: 1- ^1H and ^{13}C NMR values of acetylated Dariose in $CDCl_3$-

Moieties	^1H NMR	^{13}C NMR	Coupling Const.(J)
α-Glc (S_1)	6.15	89	4.0
β-Glc (S'_1)	5.35	90.05	8.0
β-Gal (S_2)	4.50	100.91	8.0
β-GlcNAc (S_3)	5.35	90.05	8.0
β-Gal (S_4)	4.50	100.91	8.0
β-Glc (S_5)	4.75	95.19	8.0
β-GalNAc (S_6)	4.45	101.01	8.0

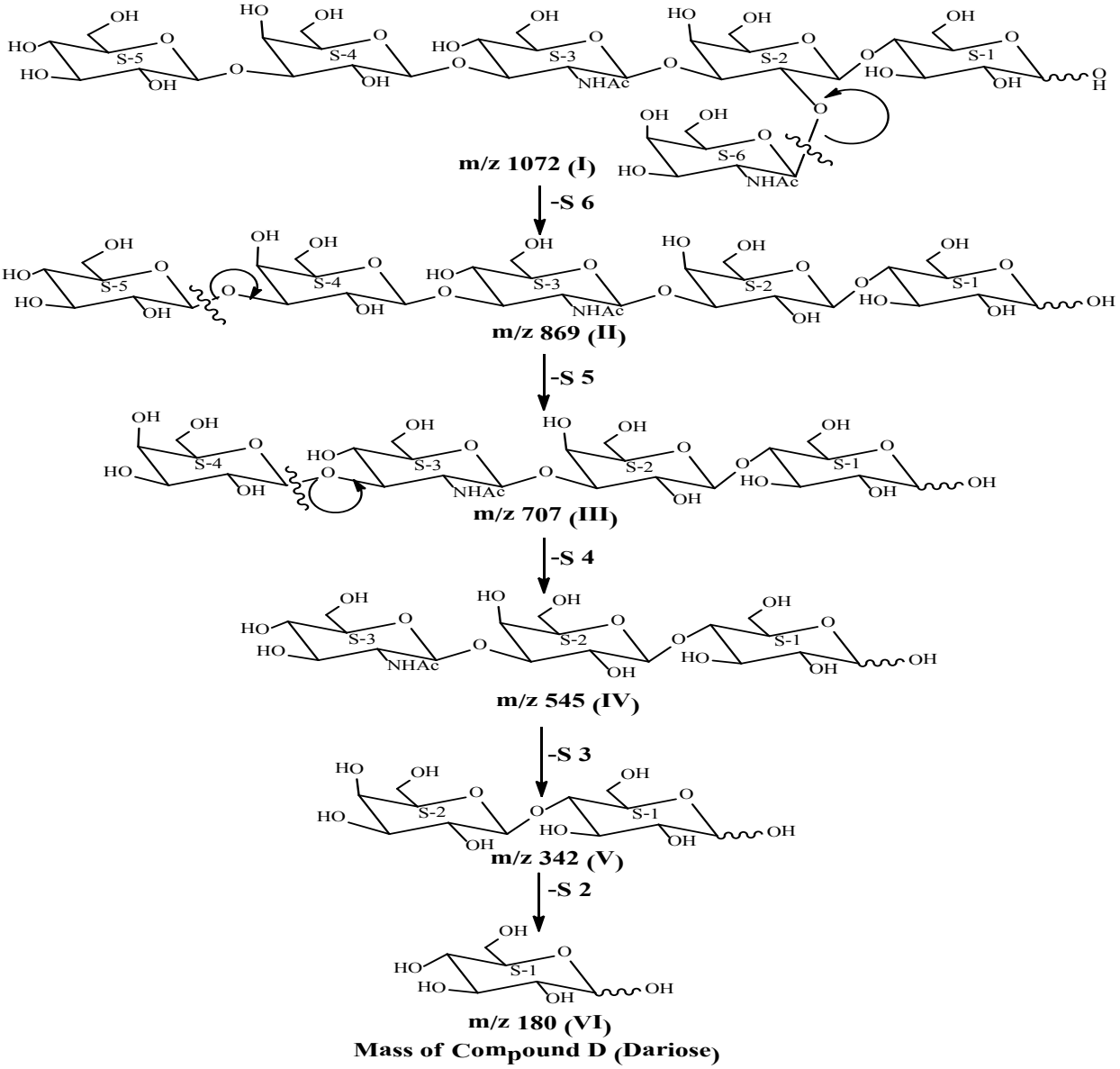
Table: 1- ^1H values of Dariose in D_2O-

Moieties	^1H NMR	Coupling Const.(J)
α-Glc (S_1)	5.55	4.0
β-Glc (S'_1)	5.15	8.0
β-Gal (S_2)	4.45	8.0
β-GlcNAc (S_3)	*4.35*	*8.0*
β-Gal (S_4)	*4.45*	*8.0*
β-Glc (S_5)	*4.58*	*8.0*
β-GalNAc (S_6)	*4.35*	*8.0*

4.1.3. Mass spectrometry of Compound D

The Electrospray Mass Spectrometry data of compound not only confirmed the derived structure but also supported the sequence of monosaccharide in Dariose. . In ESMS of compound D, (the highest mass ion peak were recorded at m/z 1111 and 1095 which was due to $[M+K]^+$ and $[M + Na]^+$ respectively, other mass ion peak recorded at m/z 1072 was due to $[M]^+$ confirming the molecular weight of Dariose as 1072 with molecular formula $C_{40}H_{68}O_{31}N_2$. Further, the mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less H_2O. The hexasaccharide at m/z 1072(I) fragmented to give ion peak at m/z 869 (II) [1072-S_6], which was the presence of pentasaccharide (II). It was further fragmented to give mass ion peak at m/z 707 (III) [869-S_5], confirmed tetrasaccharide (III). After fragmentation of compound (III) gave mass ion peak at m/z 545 (IV) [707-S_4], because of trisaccharide (IV), mass ion of compound (IV) 545 further fragmented to give ion peak at m/z 342 (V) [545-S_3] due to presence of disaccharide (V) moiety. This disaccharide further fragmented to give mass ion peak at m/z 180 (VI) [342-S_2]. These five mass ion peak II, III, IV, V and VI appeared due to the consequent loss of S_6, S_5, S_4, S_3 and S_2 from molecule. The mass spectrum also contain the mass ion peak at m/z 747, 586, 545, correspond to the mass ion fragments A, B and C which confirm the position of S_1 S_2, S_3, S_4, S_5 and S_6 . The other fragmentation pathway in ES Mass spectrum of compound Dariose at m/z 1072 shows the mass ion peak at 1025 [1072-H_2O, CHO], 1012 [1072-CH_2OHCHO], 965 [1012- H_2O, CHO], 919 [965- CH_2OH, OH], 820[838- H_2O], 582 [659-OH CH_2OHCHO], 565 [582- OH], 496 [527- CH_2OH], 295 [313-H_2O], 180 [342-S2]. Based on result obtained from chemical degradation/acid hydrolysis, chemical transformation, electrospray mass spectrometry and ^1H, ^{13}C NMR, HOMOCOSY, TOCSY and HSQC 2D NMR technique of Dariose and acetylated Dariose the structure and sequence of isolated Novel oligosaccharide molecule Dariose was confirmed.

Stability of Molecular geometries of the



isolated compound

The geometry optimization of Dariose has been done using B3LYP method at 6-311G basis set employing density functional theory (DFT). The theoretical calculations have been performed using Gaussian 09W package. The optimized geometry is visualized using Gauss View 5.0.9 utility software. All the monosaccharide rings are present in the most stable chair form. The Dariose molecule possesses C1 symmetry. The molecule is found to be highly polar in nature with the total dipole moment of 6.1844 Debye. The molecule has total energy of -4007.5215 a. u. The distribution of Mulliken charges shows that oxygen atom has maximum negative charge and atom has maximum positive charge. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of Dariose is shown in Fig-1 and Fig-2. The energy gap of the molecule is also shown. The molecular electrostatic potential map also shows the electron cloud distribution in the overall molecule. The red colored area shows the most electronegative region.

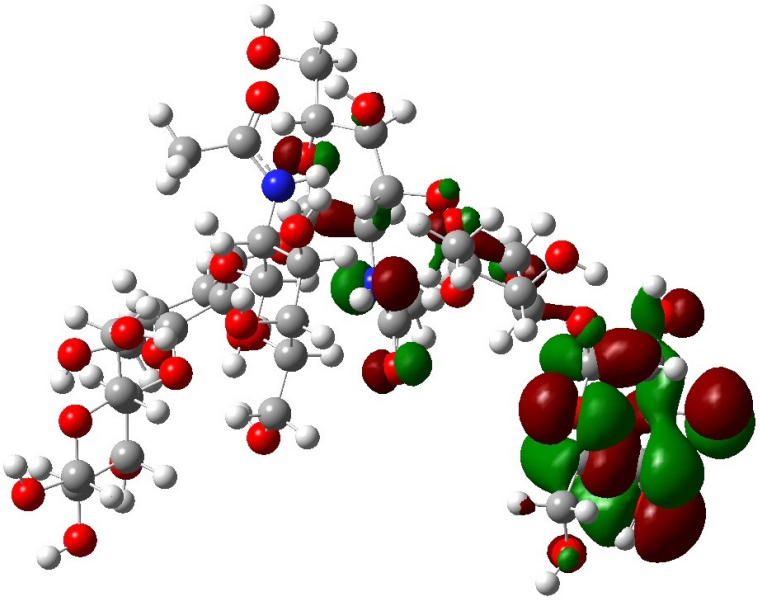


Fig-1. HOMO of Dariose

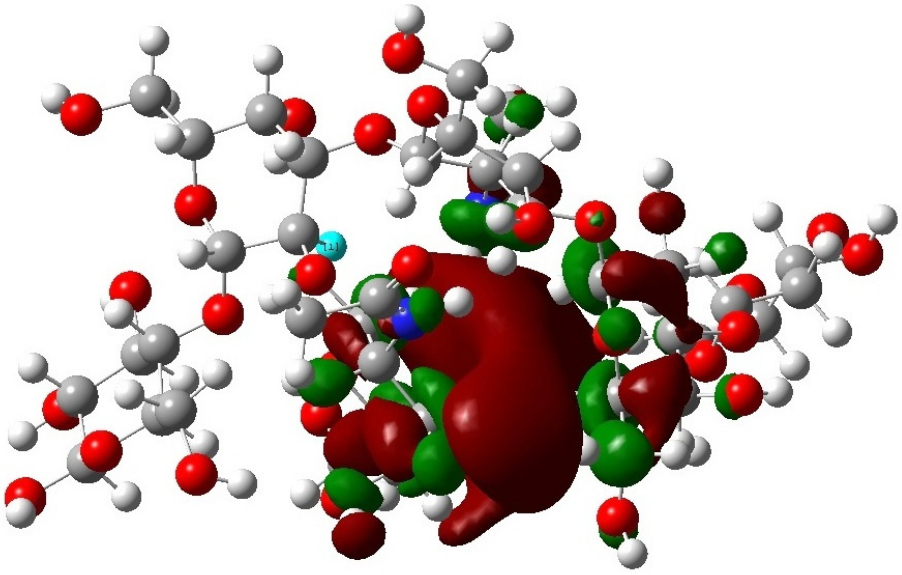


Fig-2. LUMO of Dariose

4. Conclusion

In summary, the two novel milk oligosaccharides namely as **D** (Dariose) have been isolated from camel milk and elucidated with the help of ^{1}H, ^{13}C, 2D NMR spectroscopy and mass spectrometry.

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