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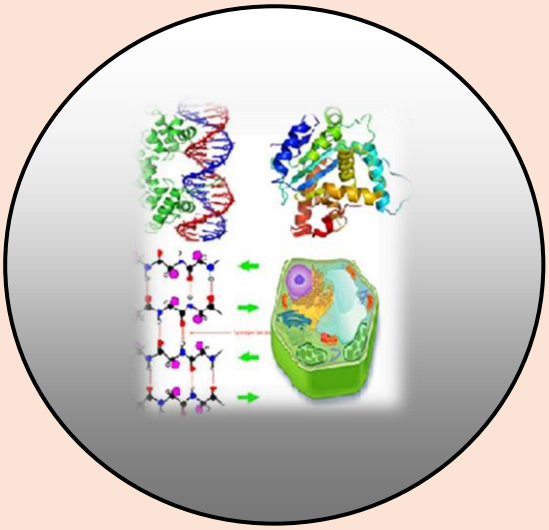

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REVIEW PAPER

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Isolation and Structure Elucidation of Ovis aries orientalis Milk Oligosaccharide 'Entaliose' and Its DFT Studies

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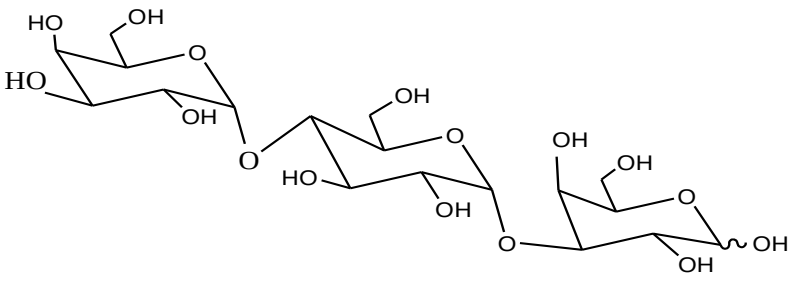
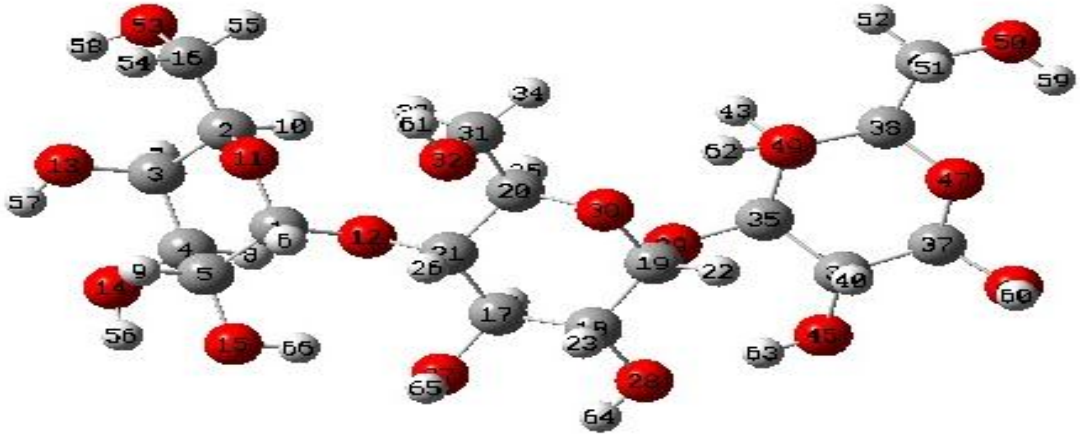
ABSTRACT

Oligosaccharide is an important natural component which can be obtained from milk, plants, micro-organisms, etc. Among all the resources milk is the source of prime importance which have rich quantity of oligosaccharides. Modified Method of Kobata and Ginsburg play a very crucial role for the isolation of milk oligosaccharides. This method includes - Collection, Preservation, Filtration, Evaporation and Lyophilisation followed by Acetylation and Separation by Column Chromatography. The structure of isolated milk oligosaccharide can be elucidate with the help of 1-D NMR (^{1}H and ^{13}C) and 2-D NMR spectroscopy. 2-D NMR spectroscopy includes – HSQC, COSY, TOCSY and HMBC techniques. After following the complete process a trisaccharide ENTALIOSE was isolated and its structure was elucidated. After structure elucidation of isolated trisaccharide its computational studies were also performed using Gaussian09W programme and B3LYP/6-31+G(d, p) basis set. The structure of isolated oligosaccharide was elucidated as under-



ENTALIOSE



OPTIMISED STRUCTURE OF ENTALIOSE

Keywords: Entaliose, Trisaccharide, Milk oligosaccharide, NMR and DFT Studies.

INTRODUCTION

Mammary glands of mammals produces a highly nutritious liquid i.e. Milk. A number of mammals like – human [Bode, 2015], sheep [Ranjan and Deepak 2015], goat [Kumar, 2016], mare [Maurya, 2017], cow [Gunjan Narain, 2016], buffalo [Gangwar, 2017], etc. produces milk with considerable concentration of oligosaccharides. These oligosaccharides are very good biological agents. They have bioactive properties. They work as cell marker for the antigens [Sinead, 2018] or foreign particles, they play an important role for blood group determination [Bruice, 2015] and show various medicinal properties, etc. To study the biological activities of oligosaccharides it is important to isolate them, then purification is required after isolation. The structure of isolated and purified oligosaccharides is elucidated with the help of 1-D i.e. ^{1}H, ^{13}C and 2-D i.e. HSQC (Heteronuclear spin quantum correlation) [Key et al., 1992], TOCSY (Total correlation spectroscopy) [Key et al., 1992], COSY [Dunkel, 1992] (Correlation spectroscopy), and HMBC (Heteronuclear multiple bond correlation) [Willker et al., 1993] NMR spectroscopic techniques.

In this study, the milk of *Ovis aries orientalis* (Gaddi sheep) was taken because the milk of gaddi sheep has many biological properties. Gaddi sheep's milk is sweet and hot in nature. It has the highest amount of calcium and phosphate. Sheep's milk has the natural ability to moisturize and nourish the skin and is safe on even most delicate skin. According to 'Ayurveda and Unani' system of medicine, the sheep's milk has various medicinal importance. Sheep's milk does not trigger lactose intolerance in humans. It decreases the fat. It is given in dengue fever to increase the blood platelets. It is used for wrinkle free skins and cosmetic products.

In this investigation, the milk of Gaddi Sheep was taken and processed by MMK and G [Kobata and Ginsburg, 1970]. After performing all the steps an oligosaccharide was isolated. Structure elucidation of this oligosaccharide shows that it is a *Trisaccharide*. The name '*Entaliose*' was given to the isolated oligosaccharide and it was originated from *orientalis* part of the zoological name of gaddi sheep.

When the structure elucidation was complete then the computational studies were performed by using Gauss 09 W programme and B3LYP basis set to obtained the optimised geometry of isolated trisaccharide Entaliose and also the Frontier Molecular Orbitals (FMO) [Amal et al., 2016] i.e. LUMO (Lowest unoccupied molecular orbital) and HOMO (Highest occupied molecular orbital) were studied.

MATERIAL AND METHODS

General Procedure

General procedure was same as described in our previous articles.

Isolation of Gaddi Sheep milk Oligosaccharide by Modified Method of Kobata and Ginsberg

Isolation of gaddi sheep milk oligosaccharide was done by modified method of Kobata and Ginsberg [Kumar et al., 2019] which was described in our previous communication [Khan et al., 2018]. The isolation was done from 10 litre of sheep milk which yielded 550 gm of oligosaccharide mixture.

Acetylation of Sheep milk Oligosaccharides [Singh et al., 2019]

12.8 gm of Oligosaccharides mixture was acetylated with pyridine (12.8 ml) and acetic anhydride (12.8 ml) at 60^{o}C and solution was stirred overnight. The mixture was evaporated under reduced pressure and viscous residue was taken in $CHCl_3$ (250 ml) and washed twice by ice cold water which was further evaporated to dryness yielding the acetylated mixture (13.0 gm). The acetylation converted the free sugars into their non-polar acetyl derivatives which were resolved nicely on TLC [Cai, 2014], giving 8 spots i.e. a, b, c, d, e, f, g, and h from which compound Entaliose acetate was finally separated by column chromatography over silica gel using varying proportions of hexane, chloroform and methanol as eluants.

Purification of Acetylated Oligosaccharide Mixture by Column Chromatography

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

Deacetylation of Compound Entaliose [Kumar et al., 2019]

Trisaccharide Entaliose acetate (32 mg) obtained from fractions 17 – 20 of column chromatography 2 of acetylated oligosaccharide mixture was dissolved in acetone (2ml) and 3 ml of NH_3 was added and left overnight in a stoppered hydrolysis flask. After 24 h ammonia was removed under reduced pressure and the compound was washed with (3 x 10ml) $CHCl_3$ and the water layer was finally freeze dried obtaining the deacetylated natural trisaccharide.

Methylglycosidation/ Acid Hydrolysis of Compound Entalios [Agnihotri et al., 2019]

Compound Entaliose (5mg) was refluxed with absolute MeOH (2 ml) at 70°C for 18h in the presence of cation exchange IR-120 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To a solution of methylglycoside of entaliose in 1,4-dioxane (1ml), 0.1 N H_2SO_4 (1 ml) was added and the solution was warmed for 30 minutes at 50^{0}C and solution was left over night. The hydrolysis was complete after 24h. The hydrolysate were neutralized with freshly prepared $BaCO_3$ filtered and concentrated under reduced pressure to afford α-and β-methylgalactosides along with the Glc, and Gal. Their identification was confirmed by comparison with authentic samples (TLC, PC).

Kiliani Hydrolysis of Compound Entaliose [Killiani, 1930]

Compound entaliose (4 mg) was dissolved in 2 ml Kiliani mixture (AcOH-H_2O-HCl, 7:11:2) and heated at 100^0C for 1 h followed by evaporation under reduced pressure. It was dissolved in 2 ml of H_2O and extracted twice with 3ml $CHCl_3$. The aqueous residual solution was made neutral by addition of 1-2 drops of 2N NaOH to it and was evaporated under reduced pressure to afford glucose and galactose on comparison with authentic samples of glucose and galactose.

DESCRIPTION OF COMPOUND ENTALIOSE

Compound Entaliose

On deacetylation of acetylated trisaccharide with NH_3/Acetone it afforded compound entaliose as a viscous mass. For experimental analysis, this compound was dried over P_2O_5 at 100^0 C and 0.1 mm pressure for 10 hr. It gave positive Phenol-sulphuric acid test and Morgon-Elson test.

$C_{18}H_{32}O_{16}$	%C	%H	%O
Calculated	42.85	6.34	50.79
Found	42.92	6.51	50.94

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

δ 5.69 [d, 1H, J=9.0 Hz β-Gal (S-1), H-1)], 4.50 [d, 1H, J=3.0 Hz, α-Glc (S-2) H-1], 5.11 [d, 1H, J=3.0 Hz, α-Gal (S-3), H-1], 3.80 [m, 1H, α-Glc (S-2), H-3), 3.82 [m, 1H, α-Gal (S-3), H-4].

^{13}C NMR of Entaliose acetate: δ in $CDCl_3$ at 300 MHz

δ 91.52 [1C, β-Gal (S-1), C-1], 101.15[1C, α-Glc (S-2), C-1], 100.89 [1C, α-Gal (S-3), C-1].

RESULT AND DISCUSSION

STRUCTURE ELUCIDATION OF ENTALIOSE BY SOLVING ITS SPECTRA

Compound Entaliose, $C_{18}H_{32}O_{16}$, isolated by repeated column chromatography of the oligosaccharide mixture, obtained from Gaddi sheep milk, gave positive Phenol-sulphuric acid test [Dubois et al., 1956] and Feigl test [Fiegl, 1975] showing the presence of normal sugars in the compound. The ^{1}H NMR spectrum of acetylated Entaliose in $CDCl_3$ at 300 MHz exhibited four doublets for three anomeric protons at δ 6.25 (1H), δ5.69 (1H), δ 4.50 (1H) and δ 5.11(1H) indicating that Entaliose may be a trisaccharide in its reducing form giving signals for α and β-anomers at the reducing end. The trisaccharide nature of Entaliose acetate was further confirmed by the presence of four signals for four anomeric carbons at δ88.94(1C), δ91.52(1C), δ101.15 (1C) and δ100.89(1C) in ^{13}C NMR of acetylated Entaliose at 300 MHz in $CDCl_3$.

Table for Anomeric Protons and Carbons of compound Entaliose

SUGAR	^{13}C	^{1}H
S_1	91.52	5.69
S_2	101.15	4.50
S_3	100.89	5.11

The reducing nature of compound Entaliose was again confirmed by its methylglycosydation followed by its acid hydrolysis ($MeOH/H^+$), which led to the isolation of α and β-methylgalactosides, along with Gal and Glc suggesting the presence of galactose at the reducing end and presence of Gal and Glc in the Entaliose. The HSQC spectrum of acetylated Entaliose at 300 MHz in $CDCl_3$ showed the presence of four cross peaks of anomeric protons and carbons in their respective region at δ 6.25 x 88.94, δ 5.69 x 91.52, δ 4.50 x 101.15 and δ 5.11 x 100.89 suggesting that compound Entaliose must be a trisaccharide in its reducing form. Thus ^1H and ^{13}C NMR spectra of acetylated Entaliose justify the four anomeric signals for trisaccharide. For convenience, starting from reducing end, the monosaccharides present in compound Entaliose have been designated as S-1, S-2 and S-3. To confirm the monosaccharide constituents in compound Entaliose, it was hydrolyzed under strong acidic conditions of Kiliani hydrolysis which gave two monosaccharides i.e. Gal and Glc which were found identical with the authentic samples of Gal and Glc by co-chromatography (TLC and PC), confirming that the trisaccharide compound Entaliose was consist of two types of monosaccharide units i.e. Gal and Glc. The molecular formula $C_{18}H_{32}O_{16}$ was in agreement with mass ion peak obtained from ES-MS spectrum of Compound Entaliose which showed the highest mass ion peak at m/z 504 $[M]^+$ for a trisaccharide.

The ^1H NMR spectrum of Entaliose acetate in $CDCl_3$ at 300 MHz contain two anomeric proton doublets at δ 6.25 and δ 5.69 for α and β-anomers of reducing monosaccharides (S-1) i.e. Gal. The anomeric protons signal present at δ5.69 in TOCSY Spectrum of Entaliose acetate assigned to β-Gal (S-1) gave three cross peaks at δ5.69 x 3.8, δ5.69 x δ 5.05 and δ5.69 x δ 5.28. These cross peaks i.e. δ5.69 x 5.05, δ5.69 x 3.8 and δ5.69 x 5.28 were later identified as H-2, H-3 and H-4 of reducing Gal respectively by COSY spectrum of acetylated Entaliose at 300 MHz in $CDCl_3$. The chemical shift of H-3 of S-1 at δ3.8 suggested that H-3 of S-1 was available for glycosidic linkage by next monosaccharide unit. Further the ^1H signal present at δ3.8 assigned to H-3 of reducing Gal (S-1) gave a cross peak at δ 3.8 x 101.15 in HMBC spectrum of Entaliose acetate which was between H-3 of reducing Gal and C-1 of S-2, confirmed the (1→3) linkage between Gal (S-1) and S-2. The anomeric carbon of S-2 at δ101.15 gave its complimentary anomeric proton signal at δ 4.50 (3.0 Hz) in the HSQC spectrum of Entaliose acetate. Further the coupling constant of anomeric signal (S-2) at δ 4.50 with J value of 3.0 Hz confirmed the α-configuration of the glycosidic linkage between (S2→S1) in Entaliose acetate. Further, the anomeric proton signal at δ 4.50 assigned to S-2 (α-Glc) showed three cross peaks at δ 4.50 x δ3.82, δ 4.50 x δ5.00 and δ 4.50 x δ 5.35 in the TOCSY spectrum of Entaliose acetate at 300 MHz. These cross peaks i.e. δ 4.50 x 5.35, δ 4.50 x 5.00 and δ 4.50 x 3.82 were later identified as H-2, H-3 and H-4 of α-Glc (S-2) respectively by COSY spectrum of acetylated Entaliose in $CDCl_3$ at 300 MHz. The chemical shift of H-4 of S-2 at δ3.82, suggested that H-4 was available for glycosidic linkage by next monosaccharide unit i.e S-3. Further the ^1H NMR signal present at δ 3.82 assigned to H-4 of α-Glc (S-2) gave a cross peak at δ 3.82 x 100.89 in HMBC spectra of Entaliose acetate which was between H-4 of α-Glc (S-2) and C-1 of S-3 confirmed a 1→4 linkage between S-2 and S-3. The anomeric carbon of S-3 at δ100.89 gave its complimentary anomeric proton signal at δ 5.11 in the HSQC spectrum of Entaliose acetate. The chemical shift values of anomeric carbon at δ100.89 and anomeric proton at δ5.11 were having resemblance with literature value of anomeric chemical shift value of Gal, confirming that S-3 was Gal [Rathore et al., 2022].

Further the coupling constant of anomeric signal of S-3 at δ 5.11 with J value of 3.0 Hz confirmed the α-configuration of the glycosidic linkage between (S3→S2) in Entaliose acetate. Further the anomeric proton signal at δ 5.11 (3.0 Hz) assigned for α-Gal (S-3) showed three cross peaks at δ 5.11 x δ 4.5, δ 5.11 x δ 4.9 and δ 5.11 x δ 5.35 in the TOCSY spectrum of acetylated Entaliose. These cross peaks i.e. δ 5.11 x 4.5, δ 5.11 x 5.35 and δ 5.11 x 4.9 were later identified as H-2, H-3 and H-4 of α-Gal (S-3) respectively by COSY spectrum of acetylated Entaliose in $CDCl_3$ at 300 MHz. Since The chemical shift values of ring protons of S-3 at δ 4.5, δ 5.35 and δ 4.9 does not reside in the linkage region and hence they did not show any cross peak in the linkage region i.e. δ 3.5-4.2 ppm confirming that α-Gal (S-3) was present at non-reducing end and none of its -OH group were engaged in glycosidic linkage, which was confirmed by the TOCSY and COSY spectra of acetylated Entaliose in $CDCl_3$ at 300 MHz. All the ^{1}H NMR assignments for ring protons of monosaccharide units of Entaliose were confirmed by COSY and TOCSY experiments. The positions of glycosidation in the oligosaccharide were confirmed by position of anomeric signals, Structure reporter groups (SRG)[26] and comparing the signals in ^{1}H and ^{13}C NMR of acetylated and deacetylated oligosaccharide. The glycosidic linkages in Entaliose were assigned by the cross peaks for glycosidically linked carbons with their protons in the HSQC and HMBC spectra of Entaliose acetate. All signals obtained in ^{1}H and ^{13}C NMR of compound Entaliose were in conformity with the assigned structure and their positions were confirmed by 2-D NMR viz. COSY, TOCSY, HSQC and HMBC experiments of Entaliose acetate.

Table for TOCSY spectra.

ORDER OF S_1	ORDER OF S_2	ORDER OF S_3
5.69	4.50	5.11
5.28	5.35	5.35
5.05	5.00	4.9
3.8	3.82	4.5

Correlation explained by COSY spectra

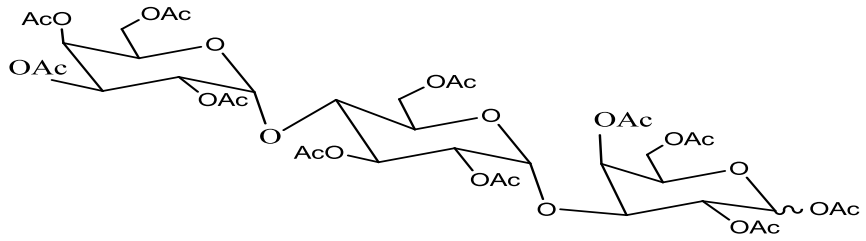
NO. OF PROTON	ORDER OF S_1	ORDER OF S_2	ORDER OF S_3
H_1	5.69	4.50	5.11
H_2	5.05	5.35	4.5
H_3	3.8	5.00	5.35
H_4	5.28	3.82	4.9

The heteronuclear single quantum coherence (HSQC) spectrum of acetylated compound Entaliose confirmed linkages in ^{1}H and ^{13}C NMR spectra by showing cross peaks of β Gal (S_1) H-3 and C-3 at (δ3.80 x 75.75) showed (1→3) linkage of S_2 and S_1 i.e. its 3-position of Gal (S_1) were involved in linkage, α-Glc (S_2) H-4 and C-4 (δ 3.82 x 75.62) showed (1→4) linkage of $S_3 \rightarrow S_2$. It was further confirmed by the presence of same peaks in COSY and TOCSY spectrum.

TABLE FOR LINKAGES

SUGAR	POSITION	1H VALUE	J-VALUE	LINKAGE TYPE
S_1	H_3	3.80	3.0	α
S_2	H_4	3.82	3.0	α

Thus based on the pattern of chemical shifts of ^{1}H, ^{13}C, HMBC, HOMOCOSY, TOCSY, HSQC and HMBC NMR experiments, it was interpreted that the compound Entaliose was a trisaccharide consist of one Glc, and two Gal moieties in it and having galactose at the reducing end. It was the first structure in which after complete study, galactose is reported at the reducing end. The deduced structure of entaliose is as following:



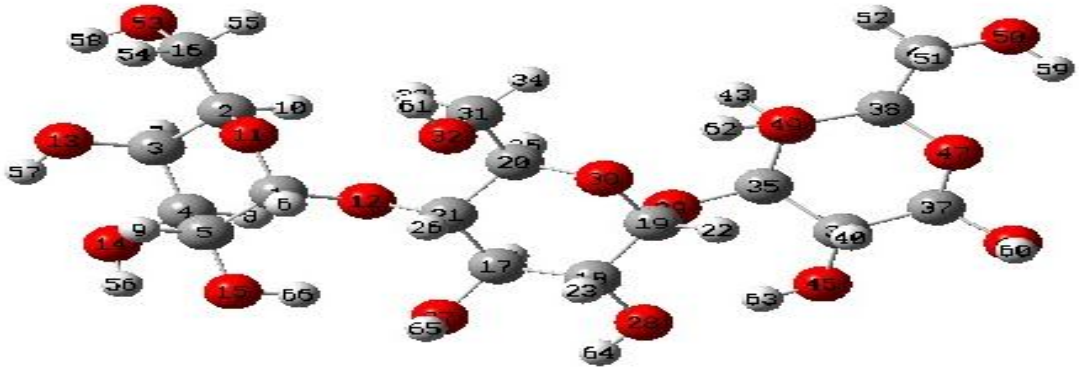
ENTALIOSE

COMPUTAIONAL ANALYSIS [Nikita et al., 2021]

The computational studies were performed on Entaliose using Density Functional Theory (DFT) of Gaussian09 W programme [Pence and Williams, 2010]. For this, structure of Entaliose was drawn on Gauss View 5.0 and was further optimized. The optimized structure was utilized for the calculations of molecular orbitals, i.e., HOMO and LUMO. Other evaluations like bond lengths, bond angles, molecular electrostatic potential and mulliken charges were also performed from the optimized geometry.

Optimised Structure of Entaliose

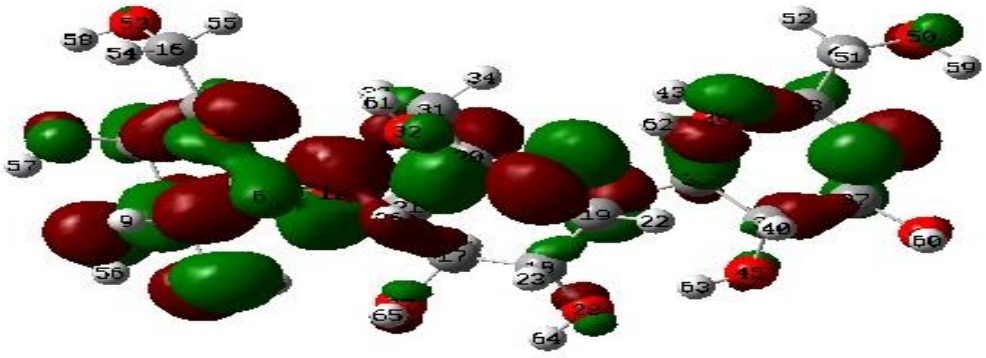
The structural geometry was optimized by minimizing its energies compared to all geometrical variables without forcing any molecular symmetry restrictions. The molecular structures of the optimized compounds were drawn by Gauss View 5.0.



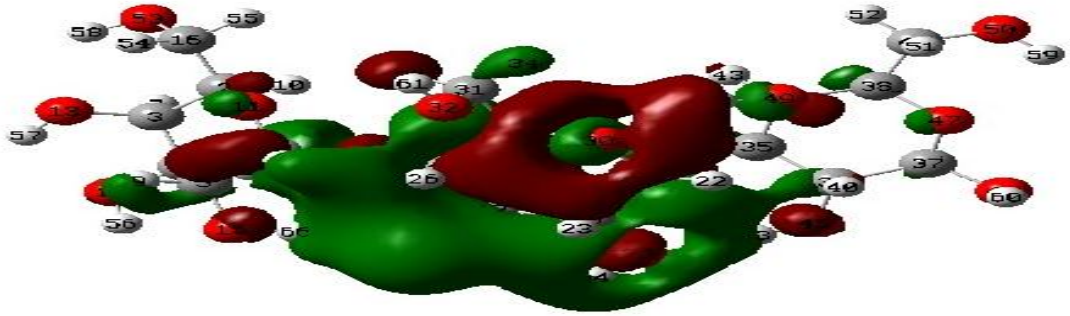
Frontier Molecular Orbitals

Frontier molecular orbitals (FMOs) are the highest occupied molecular orbital (HOMO) with electrons, so it is an electron donor and the lowest unoccupied molecular orbital (LUMO) that has a space to accept electrons, so it is an electron acceptor. These orbitals control the mode of the interaction of the compounds with the receptors [29]. Moreover, HOMO and LUMO are very important quantum chemical parameters to determine the reactivity of the molecules and are used to calculate many important parameters such as the chemical reactivity descriptors.

Structure of HOMO of Entaliose

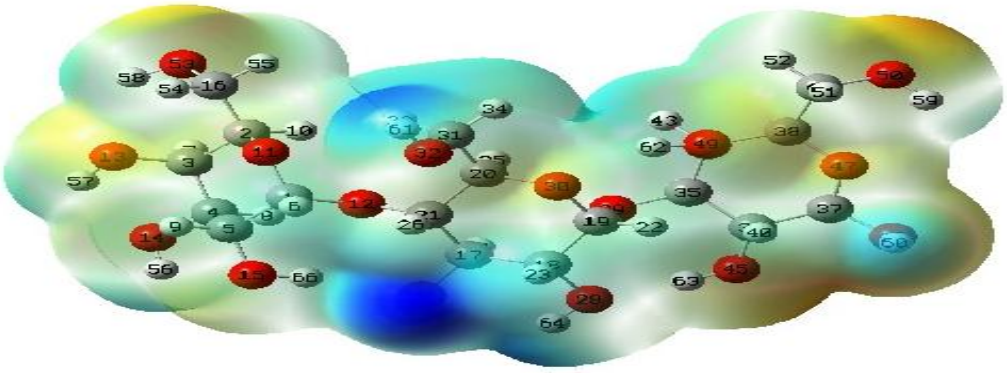


Structure of LUMO of Entaliose



MOLECULAR ELECTROSTATIC POTENTIAL

The MEP [Nikita et al., 2022] gives an indication about the molecular size and shape of the positive, negative as well as the neutral electrostatic potential. In the MEP, the maximum negative region is the preferred sites for electrophilic attack, indicated as red color. So, an attacking electrophile will be attracted by the negatively charged sites, and the opposite situation for the blue regions. It is obvious that the molecular size and the shape as well as the orientation of the negative, positive, and the neutral electrostatic potential varied according to the compound because of the type of the atoms and its electronic nature. The structure shows the total electron density is as under:

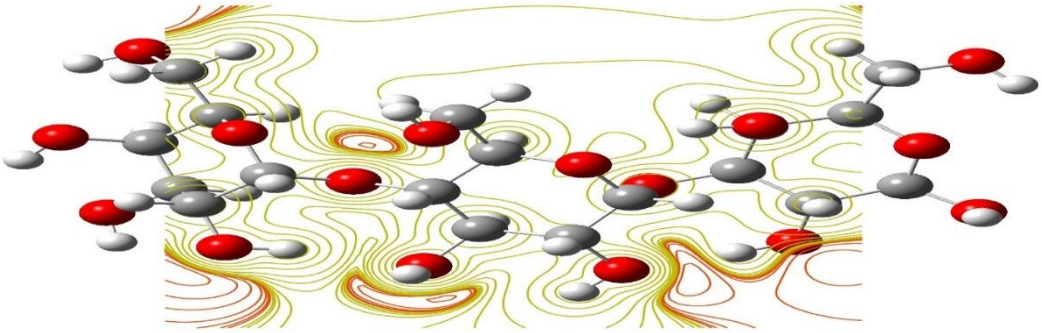


GLOBAL REACTIVITY DESCRIPTORS [Nikita and Anil, 2021]

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

Sugar	HOMO	LUMO	E	χ	η	S	ω	μ
Entaliose	-9.04	-1.749	7.29	5.39	3.64	0.09	1.480	5.35

Electrostatic Potential Contour Surfaces



MULLIKEN CHARGES

The Mulliken atomic charges of the estimated compounds were calculated by the DFT using B3LYP basis set, the data for compound is arranged in the following Table. It showed that the C5, C36 and C38 have negative charges but rest of the carbons have positive charges. All oxygens have negative charges only in Compound entaliose. The positively charged centres are the most susceptible sites for nucleophilic attacks. However, the most negatively charged centres are the most susceptible sites for electrophilic one. Sum of mulliken charges is -0.0000.

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

S. NO.	ATOM NO.	ATOM	CHARGES	S. NO.	ATOM NO.	ATOM	CHARGES
1	1	C	0.383433	18	28	O	-0.126299
2	2	C	0.291892	19	29	O	-0.323676
3	3	C	0.075587	20	30	O	-0.261978
4	4	C	0.292456	21	31	C	0.117202
5	5	C	-0.139755	22	32	O	-0.182311
6	11	O	-0.260796	23	35	C	0.332929

7	12	O	-0.297330	24	36	C	-0.165694
8	13	O	-0.206426	25	37	C	0.658068
9	14	O	-0.187063	26	38	C	-0.189421
10	15	O	-0.105725	27	39	C	0.408272
11	16	C	0.282185	28	45	O	-0.087536
12	17	C	0.368119	29	46	O	-0.100685
13	18	C	0.009476	30	47	O	-0.417140
14	19	C	0.274357	31	48	C	0.165199
15	20	C	0.004777	32	49	O	-0.141562
16	21	C	0.006397	33	50	O	-0.138737
17	27	O	-0.208106	34	53	O	-0.130108

BOND LENGTH

The theoretical calculation done by DFT using Gauss09W programme, the C-C, C-H and C-O bond lengths were obtained and arranged in the following table. The data shows that the bond length for C-C single bond is approx. 1.5299 A^{O} while bond length between C-H is approx. 1.1021.The bond length for O-H is 0.9688 while C-O bond length is 1.427.

PARAMETER	BOND LENGTH	PARAMETER	BOND LENGTH	PARAMETER	BOND LENGTH
R(1,5	1.5352	R(16,55	1.0935	R(32,61	0.965
R(1,6	1.0943	R(17,18	1.5268	R(35,36	1.5393
R(1,11	1.4038	R(17,21	1.5317	R(35,39	1.5391
R(1,12	1.4281	R(17,24	1.0935	R(35,41	1.0962
R(2,3	1.533	R(17,27	1.4363	R(36,37	1.5412
R(2,10	1.0982	R(18,19	1.5329	R(36,40	1.1017
R(2,11	1.4441	R(18,23	1.102	R(36,45	1.4122
R(2,16	1.5374	R(18,28	1.4219	R(37,44	1.0995
R(3,4	1.5313	R(19,22	1.0916	R(37,46	1.3871
R(3,7	1.0994	R(19,29	1.4036	R(37,47	1.4275
R(3,13	1.4273	R(19,30	1.4235	R(38,39	1.5359
R(4,5	1.5291	R(20,21	1.5451	R(38,42	1.1031
R(4,8	1.0996	R(20,25	1.0977	R(38,47	1.432
R(4,14	1.4268	R(20,30	1.4442	R(38,48	1.5254
R(5,9	1.094	R(20,31	1.5206	R(39,43	1.1014
R(5,15	1.4222	R(21,26	1.0981	R(39,49	1.4231
R(11,31	3.4068	R(27,65	0.97	R(45,63	0.9731
R(12,21	1.4265	R(28,64	0.9687	R(46,60	0.9684
R(13,57	0.9701	R(29,35	1.4425	R(48,50	1.4216
R(14,56	0.9692	R(30,49	2.8933	R(48,51	1.096
R(15,66	0.9754	R(31,32	1.427	R(48,52	1.0942
R(16,53	1.4203	R(31,33	1.0962	R(49,62	0.9717
R(16,54	1.0954	R(31,34	1.0986	R(50,59	0.9681

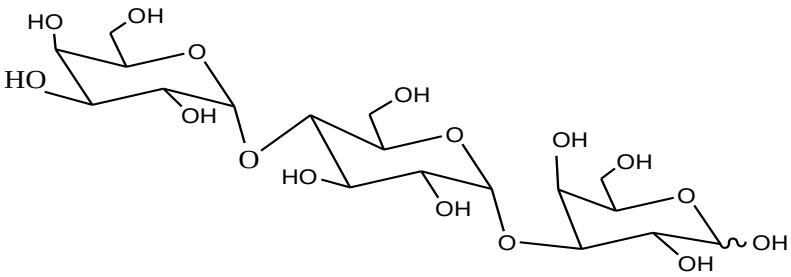
DIHEDRAL ANGLES [Josef, 2003]

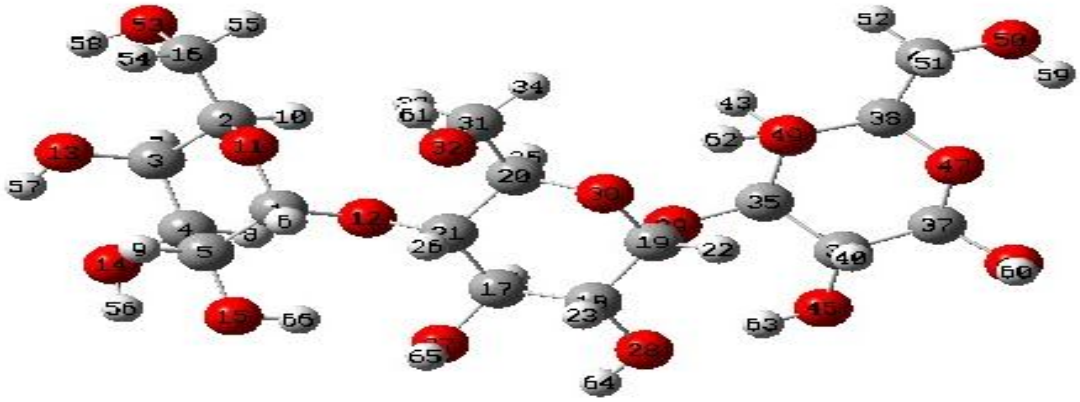
A dihedral angle is defined as the angle between two planes both of which pass through the same bond.There is much weaker preference for particular values of the dihedral angle around single bonds. Usually, the value of 0^o (eclipsed) is avoided, and values of around 60^o(staggered) to 90^o are somewhat preferred, depending on the number of lone pairs on the termini.The values of different dihedral angles in the isolated trisaccharide Entaliose are arranged in the following table:

Parameter	D. Angle	Parameter	D. Angle	Parameter	D. Angle
D(6,1,5,4)	172.6258	D(27,17,21,26	55.6468	D(42,38,39,43	-50.8484
D(6,1,5,9	53.9159	D(18,17,27,65	63.6749	D(42,38,39,49	-170.5922
D(6,1,5,15	-62.6719	D(21,17,27,65	-60.5264	D(47,38,39,35	-53.5666
D(11,1,5,4	55.5337	D(24,17,27,65	-178.8344	D(47,38,39,43	-170.326
D(11,1,5,9	-63.1762	D(17,18,19,22	172.7745	D(47,38,39,49	69.9302
D(11,1,5,15	-179.764	D(17,18,19,29	-64.1342	D(48,38,39,35	-173.5814
D(12,1,5,4	-67.3744	D(17,18,19,30	57.9924	D(48,38,39,43	69.6592
D(12,1,5,9	173.9157	D(23,18,19,22	54.2071	D(48,38,39,49	-50.0846
D(12,1,5,15	57.3279	D(23,18,19,29	177.2984	D(39,38,47,37	62.0724
D(5,1,11,2	-54.5929	D(23,18,19,30	-60.575	D(42,38,47,37	-56.9067
D(5,1,11,31	-169.4038	D(28,18,19,22	-63.922	D(48,38,47,37	-173.2644
D(6,1,11,2	-174.8135	D(28,18,19,29	59.1693	D(39,38,48,50	-174.3492
D(6,1,11,31	70.3756	D(28,18,19,30	-178.7041	D(39,38,48,51	62.0696
D(12,1,11,2	65.8642	D(17,18,28,64	-40.0376	D(39,38,48,52	-56.7211
D(12,1,11,31	-48.9467	D(19,18,28,64	-161.9404	D(42,38,48,50	-54.0385
D(5,1,12,21	-127.3078	D(23,18,28,64	81.6892	D(42,38,48,51	-177.6197
D(6,1,12,21	-6.5805	D(18,19,29,35	-140.3261	D(42,38,48,52	63.5897
D(11,1,12,21	110.3397	D(22,19,29,35	-18.3411	D(47,38,48,50	62.6468
D(10,2,3,4	72.6504	D(30,19,29,35	99.0827	D(47,38,48,51	-60.9344
D(10,2,3,7	-45.7086	D(18,19,30,20	-61.0493	D(47,38,48,52	-179.7251
D(10,2,3,13	-164.5567	D(18,19,30,49	171.707	D(35,39,49,30	-50.5191
D(11,2,3,4	-47.7215	D(22,19,30,20	-179.307	D(35,39,49,62	-70.1279
D(11,2,3,7	-166.0805	D(22,19,30,49	53.4493	D(38,39,49,30	-170.4786

CONCLUSION

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





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