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Stability of Conformationally Locked Free Fructose: Theoretical and Computational Insights

Milind M. Deshmukh,^{a*} Shridhar R. Gadre^b and Emilio J. Cocinero^{c*}

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Despite of many experimental and theoretical investigations, a quantitative explanation of the factors governing the stability of sugars is rather scarcely attempted in the literature. Quantitative understanding of such factors is important for correlating the stability of these molecules with their function. Recent experimental-theoretical studies report the global minimum structure of fructose and provide qualitative information about the predominant factors that determine the overall stability. In the present, we quantitatively show that the relative stability of different conformers of fructose in gas phase, albeit somewhat approximately, can be obtained in terms of collective effect of (i) the sum of the energies of all the hydrogen bonds in a given conformer, (ii) the strain energy of bare fructose ring, and (iii) the sum of anomeric stabilization (endo + exo). The combined effect of these three factors is indeed useful for explaining the conformational landscape of fructose. The calculated relative stability of fructose is in good agreement with the one obtained from the relative energies of these sugar molecules. The large energetic gap between pyranose and furanose conformers is also nicely explained. It is concluded that the small ring strain, the sufficiently large sum of the energies of the intramolecular hydrogen bonds and the higher stabilization due to anomeric interactions in **β -Fructo-Pyranose** makes it conformationally locked predominant structure in gas phase.

1. Introduction:

Carbohydrates are molecules of great importance due to their roles in many biological processes such as molecular recognition,¹ cell signaling in protein stabilization,² and cryoprotection.³ These recognition processes are mainly dependent on the nature, architecture, stereochemistry and conformational structure of these molecules. Thus, understanding the topology or stereochemistry of these molecules is of utmost importance to get insights into their biochemical functions. However, due to the large conformational flexibility, tautomerism, possibility of anomeric effects and non-covalent interactions such as hydrogen bonding (H-bonding) as well as hydrophobic effects,⁴⁻⁶ it is indeed a challenging task to understand and correlate the structures and functions of these molecules.

Many experimental⁷⁻²⁹ as well as theoretical³⁰⁻⁵⁷ attempts have been made for understanding the structure-stability relationship of

these molecules. The general consensus is that the various molecular forces contribute to the stability of the native structures of these sugars. The experimental methods mostly rely on X-ray^{7,8} or neutron diffraction⁹ and NMR¹⁰⁻¹⁵ studies. However, the structural information obtained using these experimental techniques is in the solid state, heavily perturbed by crystal packing forces and intermolecular interactions and usually does not match with the corresponding free molecule. Apart from these, the spectroscopic studies, for example, the NMR¹⁰⁻¹⁵ provide averaged structures in physiological environment (but affected by solvent). Some experimental studies in gas phase allow one to get insights into the intrinsic properties of carbohydrates. For instance, laser-vibrational¹⁶⁻²⁰ as well as rotational²¹⁻²⁹ spectroscopic techniques are useful for getting further insights into the bonding patterns among the different hydroxy groups of sugar molecules. The arrangement of hydroxy groups in these sugar molecules leads to a network or a chain of hydrogen bonds (H-bonds) resulting into a phenomenon called "cooperativity".^{30,31} This interconnected cooperative network of H-bonds is seen to affect the overall stability of a particular conformation as well as influence their ability to interact with other molecules such as pyridine,³² water,³³ and importantly proteins.³⁴ Understanding the cooperativity effects is hence immensely useful for probing the structure and stability of these biomolecules. The above mentioned experimental techniques are very useful for knowing the structure and stability of different conformers. However, they do not furnish information about the strength of each of individual H-bonds between the different hydroxy groups and the effect of cooperative networking. A detailed theoretical analysis of each of these factors is thus warranted. Apart from the above experimental techniques, the

^a Department of Chemistry, Dr. Harisingh Gour Central University, Sagar, 470003, India. Email: milind.deshmukh@gmail.com

^b Department of Chemistry, Indian Institute of Technology Kanpur, Kanpur 208 016, India.

^c Departamento de Química Física, Facultad de Ciencia y Tecnología, Universidad del País Vasco (UPV-EHU), Apartado 644, 48080 Bilbao, Spain. Email: emiliojose.cocinero@ehu.es

Supporting Information: Procedure for obtaining bare fructose rings (Figure S1). Hyperconjugation energy by the deletion and perturbation methods (Table S1). The relative stability of fructose conformers with group equivalence and deletion methods (Table S2). The relative stability of fructose conformers with conventional homodesmic and perturbation methods (Table S3). The relative stability of fructose conformers with conventional homodesmic and deletion methods (Table S4). See DOI: 10.1039/x0xx00000x

isothermal titration calorimetric (ITC) method³⁵⁻³⁷ has been routinely used for understating interaction between two different biomolecules such as protein and/or other small molecules.³⁸ Although, ITC can in principle provide quantitative information about the strength of interaction in terms of heat of formation or breaking of H-bond, it is beyond its applicability to quantify cooperative networking effects even for monosaccharides.

To the contrary, the theoretical modelling of these sugars has not only been applied to the structural analysis,³²⁻⁵² but also found to be successful in the recent past to provide the quantitative information about the nature of interactions involved in them.⁵³ Most of the theoretical studies related to the structural analysis of sugars provide useful information such as the orientation of hydroxy groups and the OH...O bond distances. Based on this structural information, relative strengths of hydrogen bonds were discussed. For instance, a molecular mechanics and ¹H-NMR study by López de la Paz *et al.*³² and the molecular dynamic (MD) simulation by Dashnau and coworkers,⁵² suggested that the axial-axial H-bonds in glucopyranose are stronger than their axial-equatorial counterparts. The equatorial-equatorial H-bonds are the weakest ones. Though such qualitative information about structure and strength of interactions is useful, the energetics of each of these H-bonds is essential to get further insights into the factors that govern the overall stability of these molecules.

The structural information of carbohydrates obtained from the above experimental techniques can be effectively coupled with those by theoretical modelling of these molecules. Recent combined experimental-theoretical studies by Cocinero and coworkers²⁶⁻²⁸ are indeed directed toward this goal. The experimental rotational parameters such as rotational constants and the relative intensity of rotational transitions in fructose and ribose were effectively used by these authors to discern the global minimum structure of these molecules.²⁶⁻²⁷ In addition, the experimental atomic coordinates of framework of fructose were obtained thanks to enriched samples and were further used for validating the theoretical methods.²⁷ Several conclusions emerged from their rotational works : (i) The free molecules of fructose and ribose preferentially adopt the β -pyranose structure in the gas phase. (ii) The enhanced stability of this conformer over the furanose was preliminarily attributed to various factors such as a presence of a six-membered pyranose ring, the extended interconnected network of H-bonds (cooperativity), and the occurrence of two anomeric effects. (iii) From the viewpoint of theoretical methods, the use of B3LYP density functional theory (DFT) was not recommended since method systematically favoured the open chains, and failed to detect the global minimum. Although these combined experimental-theoretical studies²⁶⁻²⁹ are indeed very interesting and useful for charactering the conformational landscape with the most predominant structures of these monosaccharides, there are certain questions which still remain unanswered. For instance; (i) How does the H-bond strength and the anomeric interactions vary among the few low-lying fructose conformers? (ii) How much is the contribution of cooperative networking toward the stability of a given conformer? (iii) Are there

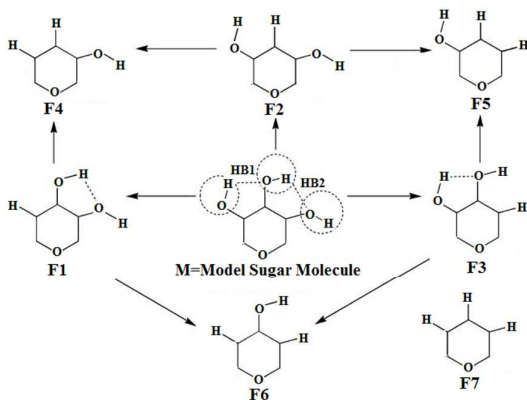
any other factors which also contribute significantly to the overall stability? and (iv) How does the collective effect of these factors determines the stability of the conformers?

To answer some of these questions, we conduct a detailed theoretical study of some of the low-lying conformers of the fructose. The purpose of the present theoretical study is to identify and quantify the most important factors that concerns for stability of carbohydrate and understand how these factors collectively govern the stability of these molecules. For this purpose, we evaluate the energy of individual H-bonds as well as their contributions toward cooperative networking using a method^{53,58} proposed by us, in the recent past, based on molecular tailoring approach (MTA).⁵⁹⁻⁶¹ This MTA-based method has been tested and validated for a variety of molecules ranging from simple diols^{54,55} to large macrocycles such as cyclodextrin⁵⁷ and calixarenes.⁵⁸ Apart from our MTA-based method, there are many other methodologies that have emerged in recent past⁶²⁻⁷⁰ for calculating the intramolecular H-bond energy. Also, there are some indirect measures of the H-bond strengths,⁷¹⁻⁸² such as the electron density value at bond critical point, vibrational O-H stretching frequency, ¹H-NMR chemical shift, etc. The detailed discussion and assessment of these methods/measures is beyond the scope of the present work and hence not discussed here. Apart from the H-bond energies, we also evaluate the contribution of anomeric effect in these fructose conformers and the strain energy of the various bare-fructose rings. In the present work, we have quantitatively explained how the combined effect of the three predominant factors enables us to understand the relative stability of the various fructose conformers.

2. Computational Details:

The geometries of three energetically lowest conformers each of α - and β -pyranose and α - and β -furanose forms of fructose (total 12 conformers) were obtained from reference 27. These geometries were already optimized at the MP2(full)/6-311++G(d,p) level and hence no further optimization was carried out. These structures were also verified²⁷ to be a local minimum on the potential energy surface (PES) by performing frequency calculations. Thus, in the present work, single point calculations were carried out at the MP2(full)/6-311++G(d,p) level using Gaussian G09 program package.⁸³ The OH...O intramolecular H-bond energy in these fructose conformers was calculated using MTA-based methodology.⁵³⁻⁵⁸ The general fragmentation procedure is illustrated here for a test sugar molecule having two H-bonds, HB1 and HB2, as shown in Scheme 1 (only two H-bonds are shown for illustrative purposes). The original sugar molecule (denoted as **M** in Scheme 1) is "cut" into three primary fragments F1, F2, and F3, which are obtained by replacing an -OH group with a hydrogen atom. The added H-atom is placed at the standard C-H distance (1.0 Å) along the direction of the C-O bond in the parent molecule. The scissored -OH regions are shown by dotted circles on the molecule **M** in Scheme 1. Fragments F4, F5, and F6 were obtained by taking

the intersection (a common structural backbone apart from added dummy H-atoms) of these primary fragments, i.e., (F1∩F2), (F2∩F3), and (F1∩F3), respectively. The fragment F7 is the intersection of three primary fragments F1, F2, and F3, i.e., (F1∩F2∩F3). A single point energies of all these fragments were evaluated at MP2(full)/6-311++G(d,p) level where 6d instead of 5d functions were used for better representation of diffuse nature of electron density on C and O atoms.



Scheme 1: A fragmentation scheme for a prototype sugar molecule (shown as M) for the estimation of intramolecular hydrogen bond energy, E_{HB1} , E_{HB2} . See text for details.

The fragments were not optimized to avoid the conformational changes in them (for a discussion on why fragments are not optimized, see reference 55). The total energy of the test sugar molecule is estimated as $E_{MTA} = E_{F1} + E_{F2} + E_{F3} - E_{F4} - E_{F5} - E_{F6} + E_{F7}$. The H-bond energies, E_{HB1} and E_{HB2} , are calculated as $E_{HB1} = (E_{F1} + E_{F2} - E_{F4}) - E_{MTA}$ and $E_{HB2} = (E_{F2} + E_{F3} - E_{F5}) - E_{MTA}$, respectively. The error in the total molecular energy may be estimated as $\Delta E_{error} = E_M - E_{MTA}$ where, E_M is the actual energy of test sugar **M**. Table 1 presents the E_M , E_{MTA} , and ΔE_{error} values for all the 12 fructose conformers. As seen from Table 1, the error in the estimated molecular energy is very small and we expect that the estimated H-bond energy values are also quite accurate.⁸⁴

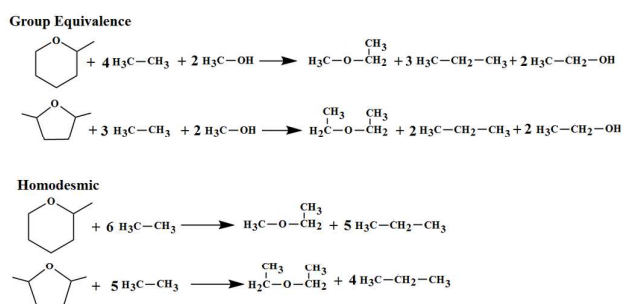
Table 1: The molecular energies were calculated at the MP2 level employing 6-311++G(d,p) basis set.

Molecule	Actual Energy (E_M)	Relative Stability	Energy by MTA (E_{MTA})	ΔE_{error} [§]
α -Fructo-Pyranose-1	-685.925461	2.92	-685.9253297	0.083
α -Fructo-Pyranose-2	-685.925329	3.00	-685.9253342	0.003
α -Fructo-Pyranose-3	-685.924588	3.46	-685.9245839	-0.003
β-Fructo-Pyranose-1	-685.930108	0.00	-685.9301102	0.001
β -Fructo-Pyranose-2	-685.925226	3.06	-685.9252284	0.001
β -Fructo-Pyranose-3	-685.927479	1.65	-685.9274832	-0.002
α -Fructo-Furanose-1	-685.922858	4.55	-685.9228556	-0.002
α -Fructo-Furanose-2	-685.922768	4.61	-685.9227698	0.001
α -Fructo-Furanose-3	-685.921326	5.51	-685.921327	0.001
β -Fructo-Furanose-1	-685.921489	5.41	-685.921494	0.003
β -Fructo-Furanose-2	-685.919365	6.74	-685.919361	-0.003
β -Fructo-Furanose-3	-685.920272	6.17	-685.920273	0.001

[§] Error = $\Delta E_{error} = E_M - E_{MTA}$ in kcal mol⁻¹. E_M and E_{MTA} values are in a.u. Relative Stability is in kcal mol⁻¹

For the calculation of ring strain energy in these conformers, we replace all the hydroxy groups on the fructose ring with the

hydrogen atoms keeping the ring backbone frozen as it is in the parent fructose; (see supporting information Figure S1). The hydrogen atoms were placed at an appropriate distance of 1.0 Å. The backbone rings of these fructose conformers thus obtained were employed for calculation of ring strain energy using two popular methods viz. the group equivalence method^{85,86} and the conventional homodesmotic reaction approach.^{87,88} The homodesmotic reaction maintains the same number of each type of bonds and also the same number of C, N, O, etc., atoms with zero, one, two or three attached hydrogens on either side of the reaction. The group equivalent approach is the modified homodesmotic reaction that identifies and conserves functional groups to be equivalents on either side of reaction. Both of these methods are well-summarized for ring system containing oxygen atom in reference 89. The appropriately constructed group equivalence homodesmotic and conventional homodesmotic reactions for a model pyranose and furanose rings are shown in Scheme 2.



Scheme 2: The representative group equivalence and the conventional homodesmotic reactions used for obtaining ring strain energy.

In the molecular orbital (MO) theory, the hyperconjugation interaction is commonly described as the interaction between the pairs of orbitals.⁹⁰⁻⁹⁵ In order to understand the anomeric effects in these fructose conformers, we have chosen $n(O^6) \rightarrow \sigma^*(C^2-O^2)$ and $n(O^2) \rightarrow \sigma^*(C^2-O^6)$ interactions at anomeric carbon (C^2), where O^6 is a ring oxygen, O^2 is hydroxy group at C^2 (anomeric) carbon which is either axial or equatorial, n is lone pair orbital on oxygen, and σ^* is the anti-bonding orbital of the C-O bond. The interactions between filled bonding and the empty antibonding orbitals represent the deviation of the molecule from the Lewis structure and generally used as a measure of delocalization. In the natural bond orbital (NBO)^{90,91} basis, there are generally two ways of obtaining these delocalization energy⁹² viz. the perturbation method and the deletion method. Since, the NBO analysis provides highly localized hybrid orbitals, the delocalizing interactions were obtained by a standard second order perturbation approach as shown in Eq. (1), where F_{ij} is the Fock matrix element between the NBOs i and j , ϵ_σ and ϵ_{σ^*} are the energies of the σ and σ^* orbitals, and n_σ is the population of the donor σ orbital.

$$E(2) = -n_\sigma \frac{\langle \sigma | F | \sigma^* \rangle^2}{\epsilon_{\sigma^*} - \epsilon_\sigma} = -n_\sigma \frac{\langle F_{ij} \rangle^2}{\epsilon_j - \epsilon_i} \quad (1)$$

This method is well explained in literature⁹² and the $E(2)$ values are generally obtained from standard NBO analysis output. In the deletion method, the off-diagonal elements of the Fock matrix

corresponding to the $n \rightarrow \sigma^*$ interactions, in the NBO basis are deleted and the energy is recalculated. The difference in the SCF energy with and without deletion of this interaction is referred as $E(\text{del.})$. In the present work, deletion energies were obtained by deleting the corresponding n and σ^* orbitals separately for each exo and endo interactions. Also the $E(2)$ and $E(\text{del.})$ energies were used for understanding the stabilization due to hyperconjugation interaction as described above.

3. Results and discussion:

3A. Geometries and the Relative Stability of Fructose: Although the geometries of various fructose conformers were taken from reference 27, it is necessary to discuss the H-bonding patterns and the relative stability of these conformers. Figure 1 displays the geometries of best three lowest lying energy conformers of each α - and β -Pyranose, with the corresponding energies presented in Table 1.

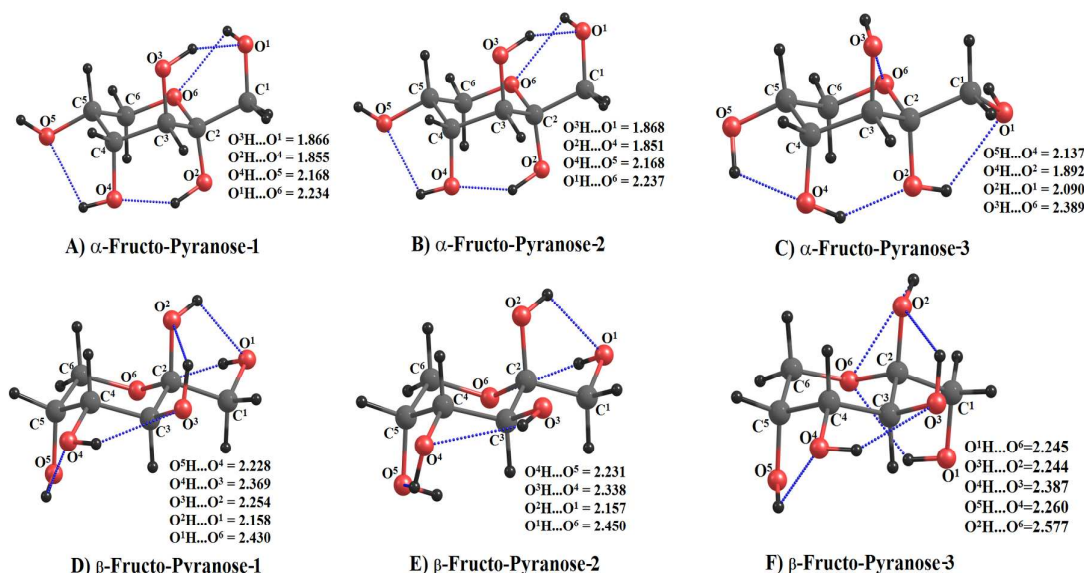


Figure 1: The MP2(full)/6-311++G(d,p) optimized geometries of various α - and β -anomers of Fructo-Pyranose.^a Bond lengths are in Angstrom unit.

As seen in Table 1, β -Fructo-Pyranose-1 is the most stable among the six pyranose conformers. Energetically second best conformer is the β -Fructo-Pyranose-3. The energy difference between the energetically best β -Fructo-Pyranose-1 and the second best β -Fructo-Pyranose-3 is about 1.65 kcal mol⁻¹. The furanose conformers are less stable than the pyranose ones (cf. Table 1). Figure 1 reveals that different orientations of hydroxy (-OH) groups generate different patterns of H-bonds. Among three α -Fructo-Pyranose conformers, the interconnected networks of H-bonds are seen to involve $-\text{O}^2\text{H}$, $-\text{O}^4\text{H}$ and $-\text{O}^5\text{H}$ hydroxy groups, whereas, $-\text{O}^1\text{H}$ group is also involved in the α -Fructo-Pyranose-3. This interconnected network of H-bonds is further extended in the β -Fructo-Pyranose conformers and is possible mostly because of the equatorial position of $-\text{O}^3\text{H}$ group. In the α -Fructo-pyranose, $-\text{O}^3\text{H}$

group is at axial position making it difficult to form an H-bond with O^2 -atom. Although such interconnected cooperative H-bond network is more feasible in the β -conformers, the $\text{OH}\dots\text{O}$ H-bond distances are in general longer in the β -conformers than those in the α -conformers. This suggests that the H-bonds in α -conformers are stronger than those in the β -ones, in spite of higher stability of later ones than the former ones. This leads to a tentative conjecture that it is not the individual hydrogen bond strengths but the extended networking of H-bonds in the β -Fructo-pyranose-1 makes it most stable than other conformers. It may be noted that there is one extra H-bond in the β -Fructo-Pyranose-1 than other conformers. Thus, it would be really interesting to quantify these H-bond strengths as well as the contribution due to cooperativity and see how the net effect of these correlates to the overall stability of these conformers.

In contrast to pyranose conformers, among six furanose conformers, the α -Fructo-Furanose-1 is the most stable one in spite of better networking of H-bonds in β -Furanose than in α -Furanose.

In general, the H-bond distances are longer in the β -conformers than in the α -ones except for the β -Fructo-Furanose-3 wherein, the H-bond distances are shortest among all twelve fructose conformers. Regardless of shorter H-bond distances and substantial cooperative networking of H-bonds in the β -Fructo-Furanose-3, this conformer is energetically not the most stable one. The reason for the reduced stability of the β -Fructo-Furanose-3 is discussed in subsequent Section. The long $\text{OH}\dots\text{O}$ distances in the β -Fructo-Furanose may be attributed to the H-bonds between the vicinal $-\text{OH}$ groups which results in formation of five-membered ring. The H-bonds formed between the axial-axial hydroxy groups are in general shorter in length (stronger) than those formed between equatorial-equatorial and axial-equatorial $-\text{OH}$ groups,^{32,52,53} due to formation of a six-membered ring. These shorter axial-axial H-bond

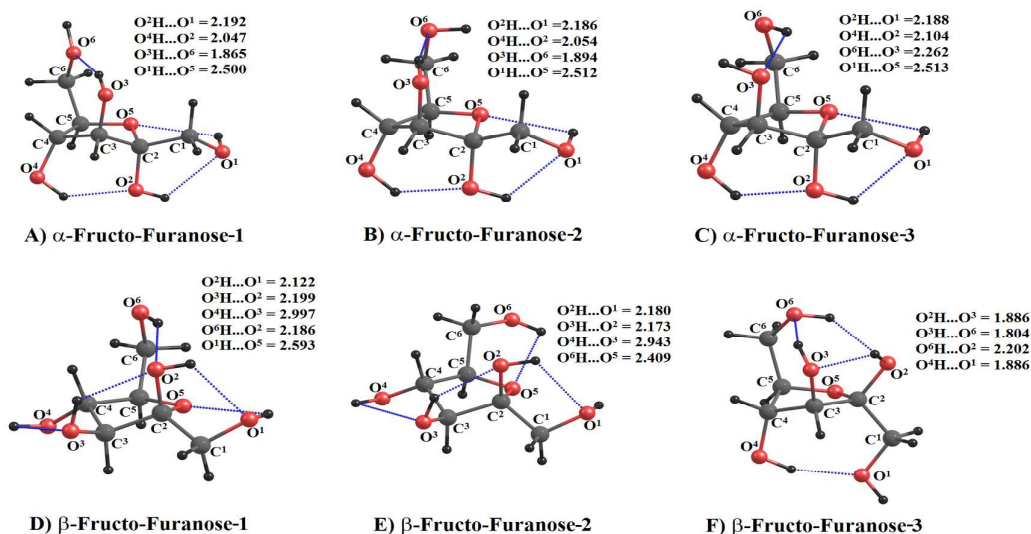


Figure 2: The MP2(full)/6-311++G(d,p) optimized geometries of various α - and β -anomers of Fructo-Furanose.^a Bond distances are in Angstrom units.

distances in the α -furanose could possibly dominate over the networking effects due to the vicinal equatorial-equatorial and/or axial-equatorial H-bonds in the β -furanose. This could be a plausible reason for α -Fructo-Furanose to be more stable than its β -counterpart. Hence, there is a need to quantify these H-bond strengths as well as the cooperativity contribution and see how the net effect of these correlates to the stability of these conformers.

3B. Intramolecular OH...O Hydrogen Bond Energy and Cooperativity in Fructose:

Tables 2 and 3 present the OH...O intramolecular H-bond energy values in the pyranose and the furanose conformers of fructose, respectively along with the corresponding OH...O distances. Note that the negative value of H-bond energy represents the stabilization. The contribution of intramolecular H-bond cooperativity towards the strength of the individual H-bond is assessed by estimating the corresponding OH...O interaction energy in the absence of an interconnected H-bond network.⁵³ For this purpose, a systematic replacement of one or more -OH groups with the hydrogen atom(s) is carried out in such a way that the OH...O bond whose energy is to be estimated is no longer a part of H-bond network. For instance, in the α -Fructo-Pyranose-3 (cf. Figure 1), on replacing -O¹H and -O⁵H groups with the H-atoms, the O⁴H...O² H-bond becomes isolated and is now free from the cooperative networking effect. The H-bond energy of all such isolated H-bonds is recalculated with MTA and is presented as the H-bond energy without network in the Tables 2 and 3. The difference in the H-bond energy with network and without network gives a quantitative estimate of contribution of cooperative networking towards each of these H-bonds. As seen in Tables 2 and 3, the estimated hydrogen H-bond energy values are in qualitative agreement with those expected from the respective H-bond distances. As discussed in previous section, among the six pyranose conformers, the H-bond distances in general are longer in the β -conformers than those in the α -conformers. The estimated H-bond energy values in these pyranose conformers are indeed in conformity with this general trend in H-bond distances; i.e. H-bond energy values are large (H bonds are stronger) in the α -conformers than those in the β -conformers.

Table 2: Intramolecular OH...O hydrogen bond energy and cooperative contribution (in kcal mol⁻¹) in Fructo-pyranose

Molecule	H-bond Distance	H-bond Energy with Network	H-bond Energy without Network	Cooperativity
α -Fructo-Pyranose-1	O ⁴ H...O ⁵ = 2.168	-3.19	-2.89	-0.30
	O ² H...O ⁴ = 1.855	-4.34	-4.04	-0.30
	O ³ H...O ¹ = 1.866	-3.17	-2.70	-0.46
	O ¹ H...O ⁶ = 2.234	-2.28	-1.17	-1.12
	Sum	-12.98	-10.80	-2.18
α -Fructo-Pyranose-2	O ⁴ H...O ⁵ = 2.168	-2.58	-2.23	-0.35
	O ² H...O ⁴ = 1.851	-4.43	-4.08	-0.35
	O ³ H...O ¹ = 1.868	-3.12	-2.66	-0.46
	O ¹ H...O ⁶ = 2.237	-2.68	-2.22	-0.46
	Sum	-12.80	-11.19	-1.61
α -Fructo-Pyranose-3	O ⁵ H...O ⁴ = 2.137	-3.38	-2.92	-0.46
	O ⁴ H...O ² = 1.892	-3.22	-2.76	-0.46
	O ² H...O ¹ = 2.090	-3.64	-2.94	-0.70
	O ³ H...O ⁶ = 2.389	-2.30	-1.77	-0.53
	Sum	-12.55	-10.40	-2.15
β -Fructo-Pyranose-1	O ⁵ H...O ⁴ = 2.228	-2.63	-2.43	-0.19
	O ⁴ H...O ³ = 2.369	-2.25	-1.93	-0.32
	O ³ H...O ² = 2.254	-2.91	-2.54	-0.38
	O ² H...O ¹ = 2.158	-3.03	-2.80	-0.24
	O ¹ H...O ⁶ = 2.430	-2.10	-1.82	-0.28
Sum	-12.92	-11.52	-1.41	
β -Fructo-Pyranose-2	O ⁴ H...O ⁵ = 2.231	-2.60	-2.44	-0.16
	O ³ H...O ⁴ = 2.338	-2.47	-2.36	-0.11
	O ² H...O ¹ = 2.157	-3.09	-3.08	-0.01
	O ¹ H...O ⁶ = 2.450	-2.11	-1.79	-0.33
	Sum	-10.27	-9.66	-0.61
β -Fructo-Pyranose-3	O ¹ H...O ⁶ = 2.245	-3.03	-2.15	-0.88
	O ³ H...O ² = 2.244	-2.25	-1.74	-0.51
	O ⁴ H...O ³ = 2.387	-2.25	-1.93	-0.32
	O ⁵ H...O ⁴ = 2.260	-2.66	-2.48	-0.18
	O ² H...O ⁶ = 2.577	-2.16	-2.00	-0.16
Sum	-12.35	-10.31	-2.04	

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Table 3: Intramolecular OH...O hydrogen bond energies and the cooperative contribution (in kcal mol⁻¹) in the various Fructo-furanose

Molecule	H-bond Distance	H-bond Energy with Network	H-bond Energy without Network	Cooperativity
α-Fructo-Furanose-1	O ³ H...O ⁶ = 1.865	-4.91	-4.80	-0.11
	O ⁴ H...O ² = 2.047	-3.01	-2.79	-0.22
	O ² H...O ¹ = 2.192	-2.79	-2.44	-0.36
	O ¹ H...O ⁵ = 2.500	-1.53	-1.48	-0.04
	Sum	-12.24	-11.51	-0.73
α-Fructo-Furanose-2	O ⁴ H...O ² = 2.054	-2.98	-2.76	-0.22
	O ² H...O ¹ = 2.186	-2.82	-2.60	-0.22
	O ³ H...O ⁵ = 1.894	-4.13	-3.89	-0.24
	O ¹ H...O ⁵ = 2.512	-1.59	-1.26	-0.34
	Sum	-11.52	-10.50	-1.02
α-Fructo-Furanose-3	O ⁶ H...O ³ = 2.262	-2.64	-2.82	0.18
	O ⁴ H...O ² = 2.104	-2.98	-2.77	-0.21
	O ² H...O ¹ = 2.188	-2.79	-2.32	-0.47
	O ¹ H...O ⁵ = 2.513	-1.00	-1.06	0.06
	Sum	-9.41	-8.97	-0.44
β-Fructo-Furanose-1	O ⁴ H...O ³ = 2.997	-1.16	-1.12	-0.04
	O ³ H...O ² = 2.199	-3.29	-3.11	-0.18
	O ² H...O ¹ = 2.122	-3.15	-2.60	-0.54
	O ⁶ H...O ² = 2.186	-2.26	-2.30	0.04
	O ¹ H...O ⁵ = 2.593	-1.44	-1.31	-0.13
Sum	-11.30	-10.45	-0.85	
β-Fructo-Furanose-2	O ⁴ H...O ² = 2.943	-1.34	-1.29	-0.05
	O ³ H...O ² = 2.173	-3.37	-3.03	-0.33
	O ² H...O ¹ = 2.180	-2.88	-2.50	-0.38
	O ⁶ H...O ⁵ = 2.409	-1.35	-2.00	0.65
	Sum	-8.93	-8.83	-0.11
β-Fructo-Furanose-3	O ² H...O ³ = 1.886	-4.64	-4.62	-0.02
	O ³ H...O ⁶ = 1.804	-5.63	-3.62	-2.01
	O ⁴ H...O ¹ = 1.886	-5.27	-3.44	-1.83
	O ⁶ H...O ² = 2.202	-4.72	-2.89	-1.83
	Sum	-20.26	-14.57	-5.69

Among the six furanose conformers, the OH...O distances are found to be the smallest in β-Fructo-Furanose-3 conformer (Figure 2). The corresponding estimated H-bond energy values are indeed the largest in this conformer (*cf.* Table 3). Also, the longer H-bond distances correspond to smaller H-bond energies (*cf.* Tables 2 and 3). For instance, the H-bond distance involving the ring oxygen is in general longer than the H-bonds involving other hydroxy groups. The corresponding estimated H-bond energy values are indeed small for all such OH...O H-bonds in these fructose conformers (*cf.* Tables 2 and 3). Although there is a qualitative correlation between the OH...O H-bond distances and H-bond energy values, the one-to-one quantitative correlation is difficult since H-bond energy value is not only dependent on H-bond distance but also on the O-H...O bond angle (i.e. directionality of the H-bond). Nevertheless, the quantitative correlation plots between the H-bond energy values estimated with MTA and the other indirect measure of H-bond strengths such as proton chemical shift, O-H stretching frequency, electron density value at the bond critical point (BCP), etc. were well-established in the recent past⁵³⁻⁵⁸ and hence the comparisons with other measures are not carried out in the present work.

The estimated values of the cooperativity contributions are also listed in Tables 2 and 3. Among the six pyranose conformers, the sum of the cooperativity value is the largest in the α-Fructo-Pyranose-1 despite of extended networking in the β-Fructo-pyranose-1 (five interconnected H-bonds). Though, the present

result appears against the naive expectation that large extended networking would lead to large cooperativity contribution, these cooperativity values are in agreement with our previous observation that the cooperativity contribution is large when there are strong H-bonds involved in the network.⁵³ In the β-Fructo-Pyranose-1, although there are five interconnected H-bonds involved in the network, the energy of each of these individual H-bonds is smaller than those in the α-Fructo-Pyranose-1. Thus, not only the number of H-bonds in the network, but also their strength is important for determining the cooperativity contribution. This general result also holds true in furanose conformers. For instance, the strongest H-bonds are found in the β-Fructo-Furanose-3, with the total cooperative contribution being large (~5.7 kcal mol⁻¹) and is the highest among all the twelve conformers of Fructose.

In spite of the strong H-bonds and the highest cooperativity contributions in the β-Fructo-Furanose-3, it is energetically less stable than other furanose conformers; A question arises, why? Also the β-Fructo-Pyranose-1 is more stable than the α-Fructo-Furanose-3 although the latter one has more strong H-bonds and the higher cooperative contribution. What are the other factors that contribute for governing the overall stability?

3C. The Strain Energy of the Bare-fructose Rings: Apart from the intramolecular H-bond energy and cooperativity, we also consider the effect of the cooperative networking of H-bonds on the pyranose and furanose rings. We believe that the strong cooperative networking that enhances the strength of individual H-bonds in the network; it also increases the rigidity of the structure i.e. the fructose ring is more distorted due to the formation of H-bonds, which in turn increases the strain of the backbone ring. In order to understand how relative stability varies among various conformers of fructose ring, we calculated the ring strain in these molecules.

Table 4: The strain energy (in kcal mol⁻¹) of the bare-fructose ring calculated at the MP2/6-311+G(d,p) level employing the conventional homodesmotic (CHD) and the group equivalence (GE) methods.

Fructose ring	Ring Strain Energy		Relative stability of fructose ring	
	CHD	GE	CHD	GE
α-Fructo-Pyranose-1	21.17	27.02	1.30	1.31
α-Fructo-Pyranose-2	21.40	27.25	1.53	1.54
α-Fructo-Pyranose-3	19.36	25.21	-0.51	-0.50
β-Fructo-Pyranose-1	19.87	25.71	0.00	0.00
β-Fructo-Pyranose-2	19.18	25.03	-0.69	-0.68
β-Fructo-Pyranose-3	19.58	25.43	-0.29	-0.28
α-Fructo-Furanose-1	21.85	27.70	1.98	1.99
α-Fructo-Furanose-2	22.01	27.86	2.14	2.15
α-Fructo-Furanose-3	22.08	27.93	2.21	2.22
β-Fructo-Furanose-1	22.05	27.90	2.18	2.19
β-Fructo-Furanose-2	21.89	27.74	2.02	2.03
β-Fructo-Furanose-3	23.45	29.30	3.58	3.59

In Table 4, the ring strain energies calculated by conventional homodesmotic (CHD) and group equivalence (GE) methods for fructose conformers are presented along with the relative stability of bare fructose ring with respect to that of the most stable β-Fructo-Pyranose-1 one. As seen from Table 4, the estimated values of ring strain energies are slightly higher (more positive) when

group equivalence method was employed than those calculated with conventional homodesmotic reaction approach. Nevertheless, the trends in the relative stability of fructose ring calculated by both of these methods is almost same. Further, these values show a good one-to-one correlation ($R^2=0.9999$ and slope=1.0006) between them as displayed in Figure 3. The values in Table 4 and Figure 3 indicate that both of these methods are useful for obtaining the ring strain energy.

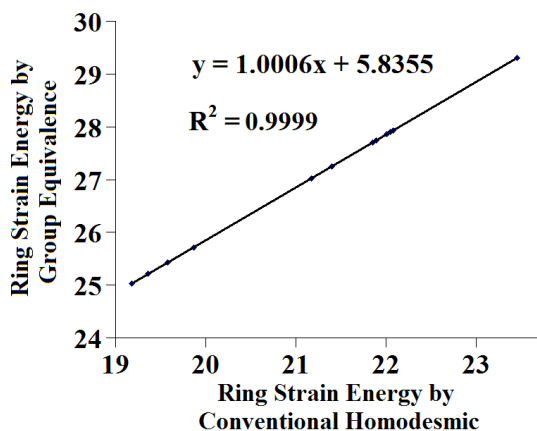


Figure 3: Correlation plot between the ring strain energies obtained by conventional homodesmotic and group equivalence homodesmotic reaction approach.

Among the twelve fructose conformers, the ring strain energies are higher in all the six furanose conformers than their pyranose counterparts. This is consistent with the general understanding that the six member pyranose ring is more stable than the five member furanose ring. Among the six pyranose conformers, although β -Fructo-Pyranose-1 is the most stable (see Table 1), the ring strain in this conformer is slightly higher than that in β -Fructo-Pyranose-2, the β -Fructo-Pyranose-3, and the α -Fructo-Pyranose-3 conformers by 0.7, 0.3, and 0.5 kcal mol⁻¹, respectively. The reason for slightly higher ring stability in these conformers than in the β -Fructo-Pyranose-1 is not clear at this moment. Nevertheless, the ring strain in the β -Fructo-Pyranose-1 is not very large compared to these conformers. The ring strain is highest in the α -Fructo-Pyranose-2 and in the α -Fructo-Pyranose-1. This is consistent with the smaller stability of these conformers as compared to the most stable β -Fructo-Pyranose-1.

Among the six furanose conformers, the α -Fructo-Furanose-1 is the most stable one (see Table 1) and the ring strain is also lowest in this conformer as compared to other five furanose conformers. The ring strain is the highest in the β -Fructo-Furanose-3 among all twelve fructose conformers and this may be attributed to short OH...O contacts which increase the distortion of furanose ring significantly (*cf.* Figure 2). The effect is more pronounced when more axial groups are involved in the formation of cooperative network of H-bonds. Here the O-H...O cooperative H-bond interactions are enhanced at the expense of ring strain. This could be one of the plausible reasons for the fact that despite strongest H-bonds and largest cooperativity contribution in the β -Fructo-Furanose-3, it is less stable than some of the other fructose conformers. We believe that the ring strain energy here, along with other factors significantly contributes to the overall stability of fructose conformers and as will be discussed in the subsequent Section.

3D. The Hyperconjugation / Steric Effect as a Measure of the Anomeric Stabilization in the Fructose Conformers:

The unusual preference of the axial form over the equatorial orientation in carbohydrates is known as anomeric effect and its physical origin still generates a considerable controversy nowadays.⁹²⁻⁹⁵ A way to tabulate this effect is via the hyperconjugation energies corresponding to the two anomeric $n(O)\rightarrow\sigma^*(C-O)$ interactions at the anomeric carbon (C²). The calculated values of hyperconjugation energies corresponding to these two anomeric interactions, by perturbation, E(2) and deletion, E(del.) methods are given in Table 5. In Table 5, the charge transfer (CT) from the lone pair of ring oxygen i.e. $n(O^6)$ to the anti-bonding orbital of C²-O² bond i.e. $\sigma^*(C^2-O^2)$ is denoted as "Endo" interaction and the CT from the lone pair orbital of the O² to the antibonding orbital of C²-O⁶ i.e. $n(O^2)\rightarrow\sigma^*(C^2-O^6)$ is represented as "Exo" interaction. Usually, there is a nice linear correlation between the E(del.) and E(2) energies reported in the literature.⁹² In the present work, we also obtained a very good correlation ($R^2=0.993$) between these two energies (See Figure 4) suggesting that either of the values can be used for the discussion of anomeric interactions.

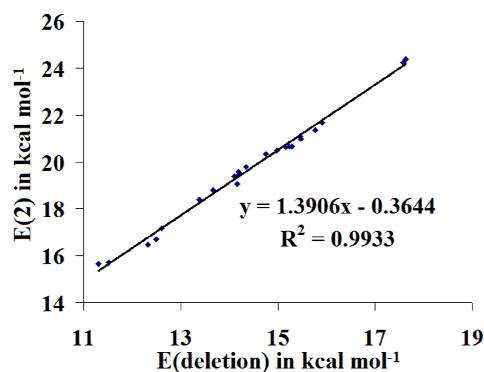


Figure 4: Correlation plot between the hyperconjugation energies obtained by deletion, E(deletion) and perturbation (E2) methods.

The corresponding E(deletion) and E(2) energy values are also reported in the supporting information Table S1. In Table S1, there are reported two entries for some of the $n\rightarrow\sigma^*$ interactions and these are denoted as LP(1) and LP(2) for the same antibonding (BD*) acceptor orbital. The sum of these two values is reported in Table 5 along with the average population of these two lone pair orbitals. This sum is taken as total interaction energy of the $n\rightarrow\sigma^*$ (Endo or Exo) interaction, hereafter.

As seen in Table 5, in general, the anomeric stabilization is larger for Exo anomeric interactions than for their Endo counterparts; β -Pry-3 and β -Fur-1 are exceptions. This is consistent with the previous report for sugar⁵⁰ and a model polypeptide.^{26-29, 93-95} Among the six pyranose conformers, the Endo anomeric interactions are slightly stronger in the β -pyranose than in the α -pyranose conformers than the β -pyranose ones. The sum of the Endo and Exo effect in pyranose conformers suggests that the anomeric stabilization is slightly higher in the α -pyranose conformers than that in the β -pyranose ones. This trend is reversed in furanose conformers. Although the Endo anomeric interactions in furanose conformers are similar to those pyranose conformers, the Exomeric interactions are similar between the α -furanose and in the β -furanose. Because of this, the

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Table 5: The hyperconjugation energy (kcal mol⁻¹) obtained by deletion method, E(del.) and perturbation E(2) method at the HF/6-311++G(d,p) level. See text for details.

Conformer	$n^{O(6)} \rightarrow \sigma^*_{C(2)-O(2)}$ (Endo)					$n^{O(2)} \rightarrow \sigma^*_{C(2)-O(6)}$ (Exo)					Endo + Exo	
	E(del.)	E(2)	Ej-Ei	Fij	n_{σ}^b	E(del.)	E(2)	Ej-Ei	Fij	n_{σ}^b	E(del.)	E(2)
α -Pry-1	12.498	16.71	2.45	0.154	1.95119	17.572	24.23	1.03	0.141	1.95895	30.070	40.94
α -Pry-2	13.040	16.47	2.46	0.154	1.95878	17.635	24.36	1.03	0.142	1.95149	30.675	40.83
α -Pry-3	12.611	17.16	2.39	0.158	1.95137	15.910	21.66	2.46	0.159	1.96114	28.521	38.82
β -Pry-1	13.673	18.80	2.37	0.164	1.94872	15.477	20.96	1.07	0.134	1.96503	29.150	39.76
β -Pry-2	14.208	19.55	2.35	0.167	1.95439	15.292	20.65	1.08	0.133	1.96065	29.500	40.20
β -Pry-3	14.194	19.57	2.35	0.160	1.94812	14.160	19.06	2.50	0.154	1.96700	28.354	38.63
	$n^{O(5)} \rightarrow \sigma^*_{C(2)-O(2)}$ (Endo)					$n^{O(2)} \rightarrow \sigma^*_{C(2)-O(5)}$ (Exo)					Endo + Exo	
	E(del.)	E(2)	Ej-Ei	Fij	n_{σ}^b	E(del.)	E(2)	Ej-Ei	Fij	n_{σ}^b	E(del.)	E(2)
α -Fur-1	14.099	19.37	2.39	0.150	1.95095	15.168	20.62	2.48	0.158	1.96476	29.267	39.99
α -Fur-2	13.387	18.39	1.05	0.124	1.95246	15.462	21.10	2.46	0.158	1.96413	28.849	39.49
α -Fur-3	14.188	19.43	2.4	0.154	1.95072	15.230	20.66	2.48	0.157	1.96486	29.418	40.09
β -Fur-1	14.973	20.47	1.03	0.130	1.95056	14.756	20.33	1.07	0.132	1.96418	29.729	40.80
β -Fur-2	14.343	19.78	1.04	0.128	1.95181	15.764	21.36	1.07	0.135	1.96424	30.107	41.14
β -Fur-3	11.521	15.71	1.05	0.116	1.94898	11.314	15.65	1.06	0.115	1.96347	22.835	31.36

^a There are two $n(O) \rightarrow \sigma^*(C-O)$ interactions in some of the endo and also exo interactions; **Sum** of these two is presented here.

^b The average population of two oxygen lone pair orbital is presented.

For details see, Supporting Information Table S1.

sum of endo and exo anomeric stabilization is higher in the β -furanose than the α -furanose conformers. However, it should be noted that the β -Fructo-furanose-3 is exceptional; in this conformer, both endo and exo stabilization is the smallest among the all twelve fructose conformers. This may be attributed to strong cooperative H-bonding in this conformer. We expect that due to this strong H-bonding interaction involving anomeric hydroxy group (O^2H), the lone pair on O^2 is less available and hence the anomeric stabilization is suppressed. In other words, the CT interaction between the electron donor (lone pair) oxygen and the electron acceptor (antibonding orbital) C^2-O^5 bond is small. This is consistent with the small Fock matrix element for both endo and exo anomeric interactions in this conformer (*cf.* Table 5). Thus, the strong cooperative intramolecular H-bonding, not only increases the ring strain, but also suppresses the anomeric stabilization in the β -Fructo-furanose 3.

In the next section, we shall discuss how the combined effect of the intramolecular H-bonding, the destabilization due to ring strain, and the anomeric stabilization, help us to understand the relative stability of the pyranose and furanose conformers.

3E. Plausible Reasons for the Highest Stability of the β -Fructo-Pyranose-1 and General Order of the Relative Stability of Fructose Conformers

In the rotational experimental studies,²⁶⁻²⁹ the populations in a jet expansion were modulated by collision kinetics, and conformational relaxation has been observed for affordable conversion barriers. It was thus admissible that the population of the β -epimer could be enhanced with respect to the equilibrium number densities. In addition, mutarotation needs breaking the C-O bond and is known to be slow without a catalyst. In consequence,

these experimental results might reflect to some degree the tautomers composition in its original form prior to entering the gas phase. Nevertheless, in both experimental studies²⁶⁻²⁹ (ribose and fructose) observed populations agree well with those predicted theoretically. As a result many fructose conformers are closely placed on the energy landscape i.e energy differences in them is small. Also there is an energy gap between these energetically closely placed conformers and the most stable one. Indeed, the calculated *ab initio* energies in Table 1 reflect this.

The discussion in the previous Sections about the different contributing factors for the stability has indeed prompted us to see how the combined effect of these contributing factors, albeit somewhat approximately. This helps us to obtain the order of the relative stability among these conformers, quantitatively. For this purpose, three important contributing factors were considered: (i) the sum of the energy of all the H-bonds in a given conformer, (ii) the corresponding strain energy of fructose backbone ring, and (iii) the sum of stabilization energies due to anomeric interactions (exo+endo). We believe that these three predominant factors are the most crucial ones for the stability of sugars apart from the other minor factors such as other possible $n \rightarrow \sigma^*$ interaction and C-H...O interactions which are relative much weaker than the OH...O interactions in these sugar molecules. Another kind of stabilizations called as Hassel-Ottar^{96,97} and delta-two effects,^{96,97} has a secondary role in monosaccharides. In the present work, we only concentrated on the above discussed three most important contributing factors. In Table 6, we present the total energetic stabilization as sum of the above discussed three important factors.

In Table 6, the strain energy calculated by group equivalence homodesmic method and the hyperconjugation energies calculated by perturbation approach are reported.

Table 6: The total energetic stabilization and the relative stability (both in kcal/mol) in various fructose conformers.

Fructose Conformer	Sum of all the H-bond energies	Ring Strain ^{a,b}	Hyperconjugation ^{c,d}	Total Energetic Stabilization	Relative Energy
α -Pry-1	-12.98	27.02	-40.94	-26.90	0.068
α -Pry-2	-12.80	27.25	-40.83	-26.39	0.575
α -Pry-3	-12.55	25.21	-38.82	-26.16	0.803
β -Pry-1	-12.92	25.71	-39.76	-26.97	0.000
β -Pry-2	-10.27	25.03	-40.20	-25.44	1.525
β -Pry-3	-12.35	25.43	-38.63	-25.55	1.415
α -Fur-1	-12.24	27.70	-39.99	-24.53	2.435
α -Fur-2	-11.52	27.86	-39.49	-23.15	3.813
α -Fur-3	-9.41	27.93	-40.09	-21.57	5.392
β -Fur-1	-11.30	27.90	-40.80	-24.20	2.765
β -Fur-2	-8.93	27.74	-41.14	-22.33	4.636
β -Fur-3	-20.26	29.30	-31.36	-22.32	4.644

^a Positive value of strain energy represents destabilization

^b Group equivalence homodesmic method was employed for evaluation of ring strain energy.

^c Negative values of hyperconjugation represents stabilization and these negative values were used in valuation of total energetic stabilization.

^d Hyperconjugation energies were evaluated with perturbation technique i.e. E(2) values are presented

The total energetic stabilization calculated by considering the conventional homodesmic approach for ring strain energy and the deletion method for hyperconjugation energies, respectively are reported in supporting information Tables S2 to S4. The results presented in Tables S2 and S4 are similar to with those in Table 6 and hence these results are not discussed here.

As seen in Table 6, the total stabilization energy values are in the range of -22.3 to -27.0 kcal mol⁻¹. The variation in the values of the sum of all the H-bond energies among these conformers is about 3 to 4 kcal mol⁻¹, β -Fructo-Furanose-3 is exceptional case as discussed earlier. The destabilization due to ring strain energy is in general similar in all fructose conformers except that in some of the β -Fructo-Pyranose conformers, these values are smaller by 2 kcal mol⁻¹. Also α -Pry-3 is exception to this. The variation in hyperconjugation energies is also about 2 kcal mol⁻¹. Thus, the variation in the total stabilization energy values among these conformers is about 5.3 kcal mol⁻¹. It may be noted from Table 6 that the total energetic stabilization is higher in pyranose conformers than those in furanose ones. This is consistent with the higher stability of the pyranose conformers over the furanose (cf. Tables 1 and 6). Also, the total energetic stabilization due these three important factors is the largest for the β -Fructo-Pyranose-1 than for other all fructose conformers and is smallest in the β -Fructo-Furanose-2 and β -Fructo-Furanose-3 conformers. Importantly, large energetic gap between pyranose and furanose conformers is nicely reflected. Also difference between total stabilization energies of the most stable (β -Fructo-Pyranose-1) and the second most stable (β -Fructo-Pyranose-3) is (1.4 kcal/mol) quite similar to that obtained from the molecular energies of these molecules (1.6 kcal/mol). However, the calculated order of the stability and energy difference among the six pyranose and among the six furanose conformers is not exactly reproduced. The reasons for this discrepancy may be attributed to the various factors such as the approximate nature of the methodologies that are employed for calculation of different energy contributions, the exclusion of

other possible n \rightarrow σ^* interaction, omission of possible, although weaker C-H \cdots O interactions, the Hassel-Ottar^{96,97} and delta-two effects.^{96,98} Nevertheless, the aim of the present work is not to get into the evaluation of these secondary factors but to point out the most important predominant determining factors governing the stability. Indeed, the order of the relative stability of few low-lying conformers is well reproduced by the collective effect of above three important factors.

Importantly, the higher stability of pyranose conformers than those of furanose conformers is well distinguished with the present approach. The reasons for the lesser stability of furanose conformers than the pyranose ones can be discussed as follows. In furanose conformers, the stabilization due to hyperconjugation, in general is although marginally larger than those in pyranose conformers, the sum of H-bond energies are in general smaller (except that in the **β -Fructo-Furanose-3**) than those in pyranose conformers. Also the destabilization due to the ring strain is largest in furanose conformer than those in their pyranose counterparts (cf. Table 6). Because of this large destabilization due to the ring strain and the small sum of the H-bond energies, the overall energetic stabilization in furanose conformers is smaller than those in pyranose one. These are the reasons why furanose conformers are in general less stable than their pyranose counterparts in gas phase.

We now discuss the special case of the β -Fructo-Furanose-3. In this conformer, the sum of H-bond energies (-20.26 kcal mol⁻¹) is the largest among all the 12 fructose conformers. However, as discussed in previous Sections, the destabilization due to the ring strain caused by the short O-H \cdots O contacts is also the largest and the stabilization due to the hyperconjugation interaction is smallest as compared to all other fructose conformers. Because of this large destabilization due to ring strain and the much smaller hyperconjugation stabilization, the β -Fructo-Furanose-3 is not the most stable among the six furanose conformers.

Among the six pyranose conformers, the sum of the energies of the H-bonds is seen to be the largest in the α -Fructo-Pyranose-1 ($-12.98 \text{ kcal mol}^{-1}$) and also in the β -Fructo-Pyranose-1 ($-12.92 \text{ kcal mol}^{-1}$) conformers. Though, the sum of the energies of the H-bonds in the α -Fructo-Pyranose-1 is marginally larger (by $0.06 \text{ kcal mol}^{-1}$) than that in the β -Fructo-Pyranose-1, the α -Fructo-Pyranose-1 is less stable than the β -Fructo-Pyranose-1 (cf. Table 1). Looking at the destability due to the ring strain, the pyranose ring of the α -Fructo-Pyranose-1 is less stable by $1.31 \text{ kcal mol}^{-1}$ than that of the β -Fructo-Pyranose-1. To the contrary, the stabilization energy due to the hyperconjugation at the anomeric carbon is larger in the α -Fructo-Pyranose-1 (by $1.18 \text{ kcal mol}^{-1}$) than in β -Fructo-Pyranose-1. Thus total stabilization is higher in the β -Fructo-Pyranose-1 than in the α -Fructo-Pyranose-1. The second most stable conformer is the β -Fructo-Pyranose-3 (cf. Table 1); in this conformer, the contributions due to sum of the energies of the H-bonds is marginally smaller than that in the β -Fructo-Pyranose-1 and the ring strain is also smaller in the β -Fructo-Pyranose-3 than that in the β -Fructo-Pyranose-1. Importantly, the stabilization due to the hyperconjugation is substantially larger (by $1.13 \text{ kcal mol}^{-1}$) in the β -Fructo-Pyranose-1 than in the β -Fructo-Pyranose-3. Thus, due to the smaller ring strain, the strong H-bonding and substantially strong hyperconjugation interaction, the total energetic stabilization is highest in the β -Fructo-Pyranose-1 than all other fructose conformers. These are the reasons why the β -Fructo-Pyranose-1 is most stable among the various fructose conformers and is hence conformationally locked in gas phase. In the end, we wish to emphasize here that the present approach not only brings out the quantitative understanding of predominant factors governing the stability of different fructose conformers, but also successfully explains the highest stability of the β -Fructo-Pyranose-1 in gas phase. The large energetic difference between the pyranose and furanose conformers is well-reproduced.

4. Concluding Remarks

In the present work, we have quantitatively discussed the important determining factors that govern the relative stability of different fructose conformers. For this purpose, the geometries of three energetically best conformers of each of the α - and β -pyranose and each of the α - and β -furanose forms of the fructose were taken from the recent combined experimental-theoretical work.²⁷ Among the different conformers of fructose, the pyranose conformers are more stable than the furanose ones. The difference in the energy of the most stable pyranose and the most stable furanose conformer is about $\sim 4.55 \text{ kcal mol}^{-1}$. Among the six pyranose conformers, the β -Fructo-Pyranose-1 is the most stable. The α -Fructo-Furanose-1 is the most stable among the six furanose conformers. The intramolecular OH...O H-bond energies in these twelve conformers of fructose were estimated by molecular tailoring based approach. The estimated H-bond energy values follow the trends expected from the corresponding OH...O bond distances. For example, the shortest OH...O distances are found in the β -Fructo-Furanose-3 and the corresponding H-bond energy values are indeed the largest in this conformer. Also, the longer H-bond distances are associated with the smaller H-bond energies. In order to understand the relative stability of various fructose conformers in gas phase, the collective effect of three important

measures were considered: (i) the sum of the energy of all the H-bonds in a given conformer, (ii) the corresponding strain energy of the fructose ring, and (iii) the sum of stabilization energies due to anomeric hyperconjugation interactions. The total energetic stabilization as sum of these energetic contributions was then used to obtain the relative stability in these fructose conformers. Thus, calculated relative stability is indeed in a good agreement with the one obtained from the relative energies of these fructose conformers. Importantly, the large energetic difference between the pyranose and furanose conformers is well reproduced. It is concluded that the appropriate balance between the sum of H-bond strengths, the smaller ring strain and the substantial anomeric stabilization in the β -Fructo-Pyranose-1 makes it most stable among these fructose conformers and hence is conformationally locked in the gas.

In summary, present work provides a quantitative explanation about the relative stability of different conformers of fructose for the first time. We trust that the methodology to obtain relative stability, suggested in this work is universal, not limited to this study of sugar molecules but can also be applied for understanding the stability of carbohydrate molecules in general. For example, the present methodology can also be effectively used to explore the conformational landscape of biologically important molecule,⁹⁹ viz. Inositols, a sixfold alcohol of cyclohexane.¹⁰⁰ Inositol derivatives function as intracellular signal transduction molecules^{101–104} and thus play important role in various biological activities.^{105–108}

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Graphical abstract for the Table of Content Graphics:

Total stabilization is governed by three predominant factors viz. sum of energy of all H-bonds, ring strain and anomeric stabilization.

