Solvent Effect on the Isomeric Equilibrium of Carbohydrates: The Superior Ability of 2,2,2-Trifluoroethanol for Intramolecular Hydrogen Bond Stabilization†

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Abstract: The higher aptitude of 2,2,2-trifluoroethanol for intramolecular hydrogen bond stabilization in carbohydrates is suggested. This belief, arising from the analysis by ^{1}H NMR spectroscopy of the solvent effect of D_2O, DMSO-d_6, and 2,2,2-trifluoroethanol-d_3 on the isomeric equilibrium of caryophyllose, was also confirmed by shifting of the conformational equilibria of β-ribopyranose and of its methyl glycoside.

Introduction

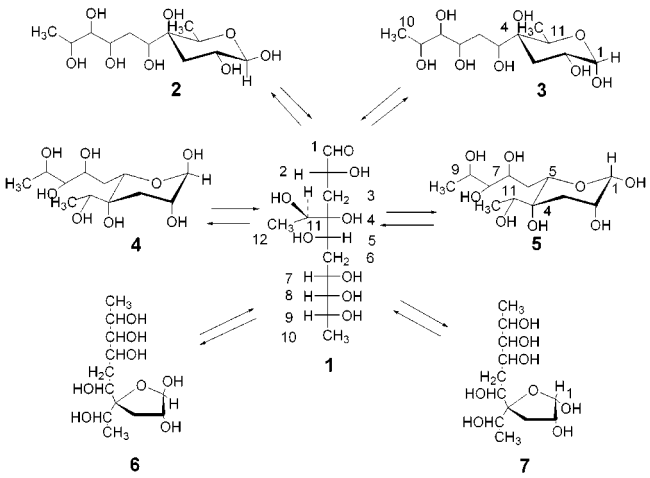
Intramolecular hydrogen bonding (IHB) plays a fundamental role in the determination of the active conformations of biological macromolecules, and thus, the finding of convenient structuring solvents that favor IHBs allows us to study more easily these active forms. In this regard, a lot of work has been done for peptides and proteins, while in the case of carbohydrates, IHBs have so far been investigated almost exclusively in DMSO or H_2O, obtaining apparently controversial results in some cases.[1,2] These conflicting data inter alia arise from the difficulty to evaluate the solvent effect on a complex system, such as that of carbohydrates, which is often constituted of both conformational and configurational forms. In the present study, we show that the isomeric equilibrium of caryophyllose (3,6,10-trideoxy-4-*C*-(D-*glycero*-1-hydroxyethyl)-D-*erythro*-D-*gulo*-decose) (**1**), a 12-carbon monosaccharide isolated from the LPS fraction of *Pseudomonas caryophylli*,[3] can be a convenient probe to analyze the influence of different solvents for IHB stabilization.

Its peculiar structure, which has been confirmed by synthesis,[4−6] allows it to give an isomeric equilibrium mixture consisting of two couples of anomeric pyranose forms **2**, **3** and **4**, **5** in addition to anomeric furanose **6** and **7** (see Scheme 1).

Molecular modeling for the pyranose forms, using the MM2 force field as implemented in the program Chem 3D,[7] gave the energy-minimized conformations depicted in Figure 1.

Those of **4** and **5** suggested the possibility of IHBs due to the syn-diaxial orientation of hydroxyls at C4 and C2[8,9] and to the hydrogen-bonding cooperative effect[10−12] of hydroxyls at C11, C7, and C9. This suggestion was in agreement with the electrostatic energy terms for **4** and **5**, which were calculated to be more negative than those for **3** and **2**, so that the stabilization *in a vacuum* of the former anomers with respect to the latter ones might be mainly due to the IHBs. Work is in progress on an accurate estimate of the steric energy of **2**−**5** tautomers.

Scheme 1. Equilibrium among Caryophyllose Isomers



The possibility offered by caryophyllose to have in equilibrium pyranose forms having different hydrogen bond requests, each in only one conformation, prompted us to investigate the effect of D_2O, DMSO-d_6, and 2,2,2-trifluoroethanol-d_3 (TFE-d_3) on the position of this equilibrium by ^{1}H NMR spectroscopy. The conclusions of this study were confirmed by analyzing the conformational equilibria of β ribopyranose and of its methyl glycoside.

Results and Discussion

Caryophyllose monosaccharide was studied in the following three solvents: D_2O, DMSO-d_6, and TFE-d_3 (or OH-d_2) and

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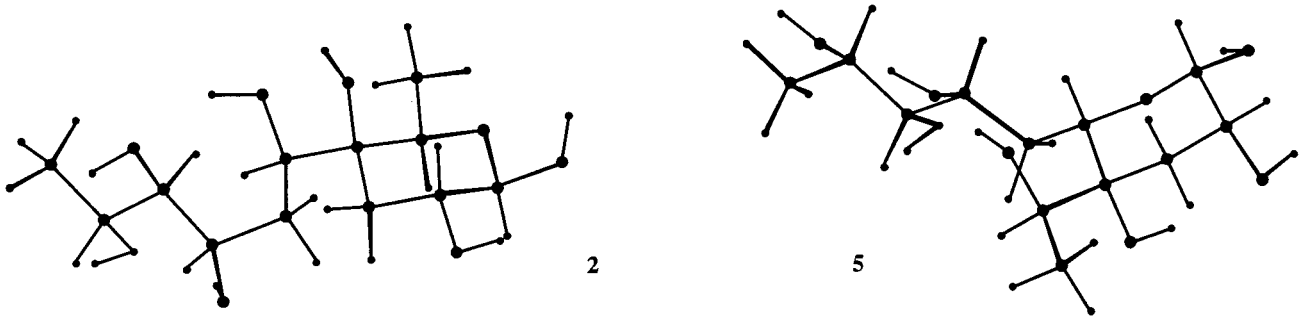

Figure 1. Minimized conformations for β anomers **5** and **2**. Those for α anomers **4** and **3** (not shown) are similar.

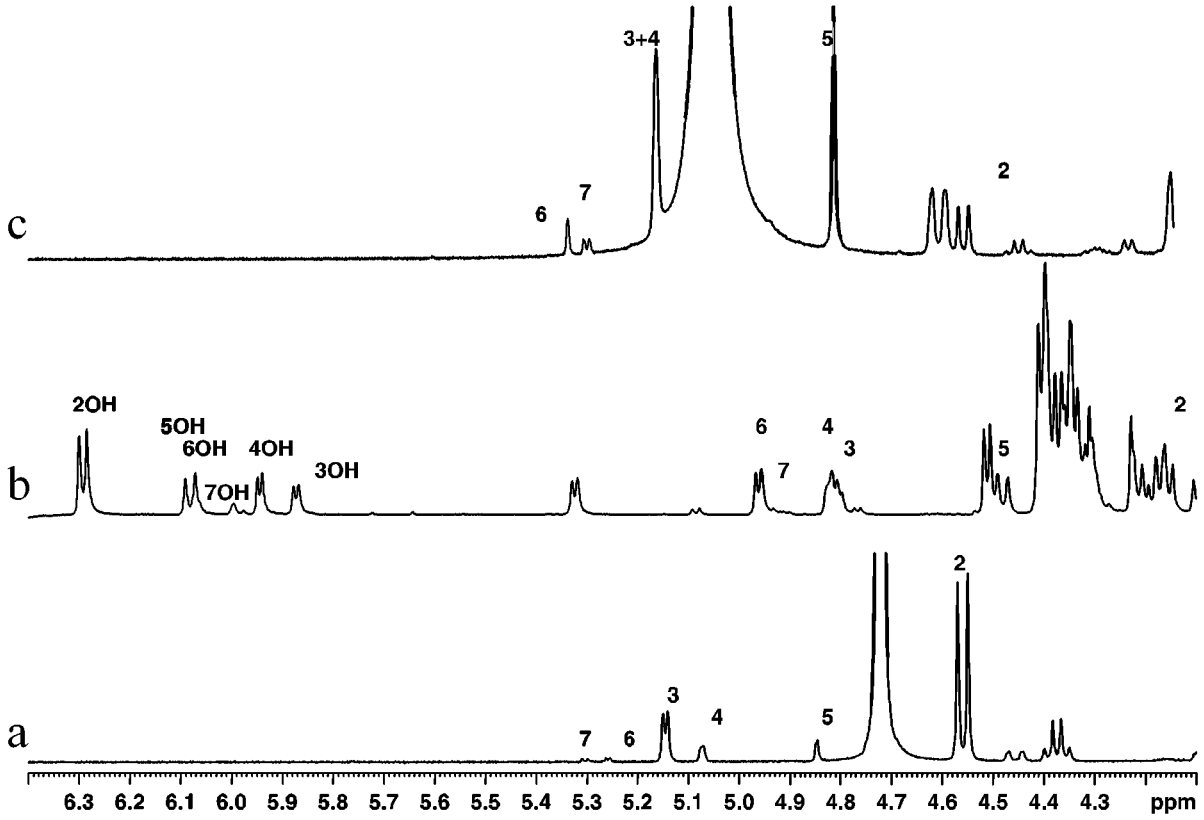

Figure 2. Anomeric regions of 400-MHz ^{1}H NMR spectra of caryophyllose isomeric mixture at 30 °C in D_2O (a), DMSO-d_6 (b), and TFE-d_3 (c) and anomeric hydroxyl region in DMSO-d_6.

all the isomers **2**−**7** were identified on the basis of NMR data arising from ^{2}D (COSY, HSQC, HMBC, TOCSY) and ^{1}D NMR NOE experiments. The relevant proton and carbon signals are shown in Table 1. Confirmatory evidence of the factual equilibrium among isomers **2**−**7** was inferred by the reversible alteration of their anomeric signal intensities induced by temperature variation.

As far as the anomeric region of the ^{1}H spectrum in D_2O (Figure 2a) is concerned, the most intense signals at δ 4.56 (d, 8.2 Hz) and 5.15 (d, 3.5 Hz), which were correlated to carbon signals at δ 98.5 ($^1J_{CH}$ = 162 Hz) and δ 91.6 ($^1J_{CH}$ = 174 Hz), had been previously assigned at **2** and **3**, respectively.[3] Of the other minor signals (less than 20% of the whole mixture), those occurring at δ 5.08 (d, 2.1 Hz) and 4.85 (d, 1.3 Hz), were assigned to pyranose forms **4** and **5**, respectively, on the basis of the chemical shifts[13,14] and $^1J_{CH}$ values[15,16] of their correlated carbon signals at δ 93.4 (170 Hz) and 95.0 ($^1J_{CH}$ 162 Hz). Finally, the very minor signals at δ 5.26 (d, 3.0 Hz) and 5.31 (d, 4.7 Hz), which were correlated to carbon signals at δ 103.6 and 96.7, were attributed to furanose forms **6** and **7**, respectively, on the basis of both proton[14] and carbon chemical shifts[13] and $^3J_{H1,H2}$ values.[14]

The isomeric distribution was measured by integration of anomeric proton signals (Table 2).

As for DMSO, a solvent where the presence of IHBs is usually measured for both oligo-[11,12,17] and monosaccharides,[18,19] the ^{1}H spectrum (Figure 2b) appeared very complex due to the additional presence of hydroxyl proton signals. The integration of the hydroxyl signals at lowest field prompted us to determine the isomeric distribution (Table 2).

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Table 1. Relevant NMR Data of **2**−**7** at 30 °C in DMSO-d_6, TFE-d_3, and D_2O[a]

	2			**3**			**4**			**5**			**6**			**7**		
HC	D_2O	DMSO	TFE	D_2O	DMSO	TFE	D_2O	DMSO	TFE	D_2O	DMSO	TFE	D_2O	DMSO	TFE	D_2O	DMSO	TFE
1	4.56 d (8.2) 98.5	4.19 d (7.7) 99.3 *6.33 d* (*5.9*)	4.53 d (7.8) 99.6	5.15 d (3.5) 91.6	4.84 d (3.3) 91.2 *5.91 d* (*4.0*)	5.12 d (3.5) 92.4	5.08 d (2.1) 93.4	4.86 d (1.1) 92.4 *5.98 d* (*4.0*)	5.12 bs 94.4	4.85 d (1.3) 95.0	4.51 d (1.0) 94.7 *6.12 d* (*6.2*)	4.77 d (1.3) 95.7	5.26 d (3.0) 103.6	4.99 d (1.8) 103.6 *6.11 d* (*5.7*)	5.29 bs 105.0	5.31 d (4.7) 96.7	4.95 d (4.3) 97.1 *6.03 d*	5.26 d (3.9) 97.5
2	3.66 68.1	3.44 67.4 *4.55 d* (*4.80*)	3.68 dd (16.0; 6.4)	4.02 65.2	3.72 64.6 *4.80*	4.00	3.90	3.63 67.6 *5.36 d* (*4.52*)	3.88	3.98	3.70 67.7 *4.86*	3.92 71.1	4.18	3.87 *5.12 d* (*6.2*)	4.19 dd (6.4; 1.4)	4.38	4.00 *4.56*	4.24 dt (6.9; 3.9)
3	1.74 2.04 dd (13.2; 5.2) 35.3	1.71 dd (5.4; 13.2) 1.54 dd (12.0; 13.2) 36.3	1.74 t (12.7) 2.07 dd (12.7; 5.4)	1.80 1.96 t (13.2) 30.3	1.82 t (12.4) 1.44 dd (5.3; 12.4) 31.4	1.84 1.93 t (12.7)	1.80 dd (14.7; 5.4) 2.13 dd (14.7; 3.4)	1.96 dd (3.1; 13.9) 28.0 1.46	1.80 dd (15.2; 1.5) 28.7 2.09 dd (15.2; 3.9)	1.95 2.05	1.72 1.52	1.89dd (15.6; 5.4) 2.06 dd (15.6; 5.4) 32.9	2.00 2.36 dd (14.5;6.6)	2.15 dd (7.2; 13.6) 1.72	1.94 d (13.2) 2.39 dd (15.1; 6.8)	1.95 2.30 dd (14.5; 8.5)		2.02 dd (13.6; 6.9) 2.39 dd (13.6; 8.3)
4	75.2	*4.09 s* 73.5		75.2	*4.02 s*			*4.35 s*	*4.79 s* 74.7			*4.62 s* 74.2						
5	3.79 70.2	3.53 70.0		3.79 69.9	70.0			4.33 dd (10.2; 2.0) 67.2	4.56 dd (10.8; 2.0) 68.6		3.90 dd (10.5; 2.1) 75.2	4.09 dd (9.9; 2.1) 76.7						
6	1.74 33.0	1.58 34.2		1.74 32.5	33.9			1.81; 1.52 30.7	2.02; 1.85 31.2		1.87; 1.50	2.02; 1.87 30.9						
7	3.85 68.5	3.60 67.9			67.9			3.65 67.9	3.96 71.1		67.9	4.00 71.1						
8	3.54 78.2	3.15 78.0		3.54 78.2	3.15 78.0			3.15 78.0	3.50 78.6		3.15 78.0	3.47 78.3						
9	3.96 68.0	3.72 69.3		3.96 68.0	69.3			69.3	3.98 70.7		69.3	3.94 70.7						
10	1.23 d (6.5) 17.4	1.09 18.6		1.28 d (6.5) 17.4					1.26 17.7			1.27 17.9						
11	4.06 q (6.5) 75.2	3.77 73.9		4.38 q (6.5) 67.0	4.21 66.2				3.79 70.4			3.75 70.4						
12	1.20 d (6.5) 13.0	1.06 13.7		1.15 d (6.5) 13.2	0.99 d (6.3) 13.6				1.24 16.0			1.24 15.7						

[a] Chemical shift in ppm. $^3J_{H,H}$.in parentheses (Hz). Hydroxyl protons (italic), hydroxyls' coupling constants (parentheses and italic).

Table 2. Percentage of Caryophyllose Equilibrium Isomers **2**−**7** at 30 °C in D_2O, DMSO-d_6, and TFE-d_3 and Their Ratios

solvent	**2**	**3**	**4**	**5**	**6**	**7**	**4+5/2+3**	**3/2**	**4/5**	**3+4/2+5**
D_2O	66.2	20.5	5.8	5.2	1.1	1.2	0.13	0.31	1.11	0.37
DMSO-d_6	49.7	19.0	20.0	6.2	3.4	1.7	0.38	0.38	3.22	0.70
TFE-d_3	14.2	5.4	31.8	40.4	4.7	3.5	3.68	0.38	0.78	0.68

These data were confirmed by the integration of anomeric proton signals of all isomers in a spectrum where the intensities of the hydroxyl signals were strongly reduced by exchange with D_2O.

Finally, we used 2,2,2-trifluoroethanol (TFE), a well-known structuring solvent for linear peptides owing to its adequacy in preserving inter alia intramolecular hydrogen bonds.[20,21] The ^{1}H spectrum of caryophyllose measured in TFE-d_3 showed a very different isomeric equilibrium position with respect to those found in D_2O and in DMSO-d_6 (Figure 2c). The integration of the anomeric proton signals gave the isomer distribution (Table 2) confirming the actual shift in TFE-d_3 of the caryophyllose equilibrium toward the isomers **4** and **5**. ^{1}H NMR spectra of caryophyllose in TFE-d_3 with increasing amounts of D_2O showed a change of the relative intensities of the anomeric signals of the isomeric mixture in accordance with the shift of equilibrium toward the aqueous situation. These experiments allowed us to correlate the anomeric signal assignment of each isomer in the two solvents as well.

To support the involvement of IHBs in the stabilization of **4** and **5**, we performed some ^{1}H NMR spectra in CF_3CD_2OH (TFE-OH-d_2) at temperatures lower than 30 °C in order to obtain hydroxyl signals as sharp patterns. In the best condition[22] at 15 °C, we obtained a proton spectrum where two sharp singlets occurred at δ 4.69 and 4.62 besides a very broad signal centered at δ 4.45. All of these signals were attributed to hydroxyl protons since they were missing in the proton spectrum measured in TFE-d_3 and they were the exchange-correlated signals occurring in a ^{1}D NOE spectrum obtained by selective excitation of the solvent OH signal. The singlets at δ 4.69 and 4.62 were assigned to the C4 hydroxyl protons of **4** and **5**, respectively, on the basis of their long-range correlation with the C4, C3, and C11 carbon signals for both the isomers (Figure 3). The appearance of these protons as sharp signals indicated a reduced exchange rate with solvent, suggesting their involvement in hydrogen bonds. Small temperature coefficients of hydroxyl proton signals are usually exploited to ascertain their involvement in hydrogen bonds.

For the singlets at δ 4.69 and 4.62, values of 11.4 and 10.6 ppb deg^{-1}, respectively, were measured. Since all of the other hydroxyl signals occurred in close proximity, we were unable to perform comparative measurements of temperature coefficients. However, even though they are higher than the value of ∼3 ppb deg^{-1}, which was reported in DMSO solution for hydroxyl protons involved in strong hydrogen bonds,[23] the above values are close to 9.1 ppb deg^{-1}, a value found for a hydrogen-bonding hydroxyl proton in H_2O/CD_3COCD_3 solution.[23] The above results suggested the involvement of C4 hydroxyls in hydrogen bonds very probably in an intramolecular way with the C2 hydroxyls as occurs for analogous molecules with syn-diaxial orientated hydroxyls in solvents such as DMSO.[9,19]

The data of Table 2 clearly show that the values of pyranose from ratio **(4 + 5)/(2 + 3)**, which are not significantly altered by neglecting the amounts of furanose forms, increase in the order H_2O < DMSO ≪ TFE, suggesting that TFE stabilizes the IHBs more than do DMSO and H_2O. The different aptitudes of these solvents for stabilizing of IHB formation can be explained by comparing their hydrogen-bonding acceptor and donor abilities. Taking into account the acidity and basicity features of these solvents, it can be easily established that their hydrogen-bonding acceptor character decreases progressively from DMSO to H_2O to TFE, whereas the hydrogen-bonding donor character increases.[21,24,25]

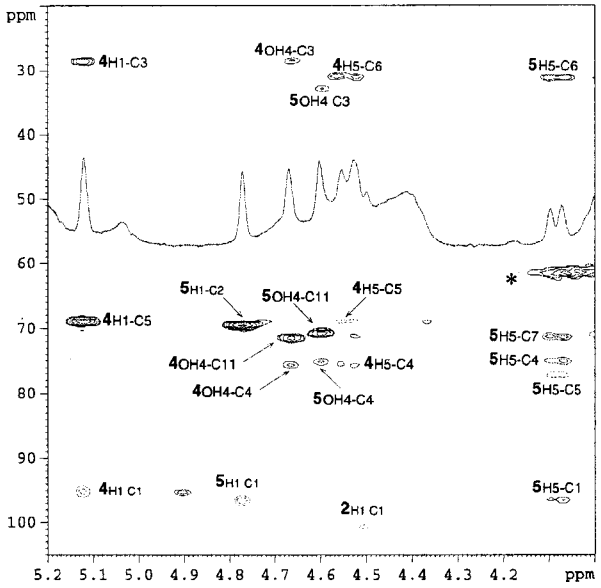

Figure 3. Low-field region of ^{1}H NMR, HMBC (boldface), and HSQC (dotted) spectra of caryophyllose isomeric mixture in TFE-OH-d_2 at 15 °C, measured with standard Bruker software. Asterisk indicates an artificial cross-peak. A delay of 60 ms was used for HMBC spectrum.

The different role played by the hydrogen acceptor and donor character of a solvent can be rationalized by considering that the oxygen has two hydrogen acceptor sites, and thus, it could accept a hydrogen bond without necessarily cleaving possible preexisting IHBs. As a consequence, the hydrogen acceptor character of a solvent is more significant for the cleavage of IHBs than its donor capacity. The above hypothesis was suggested to explain the structuring effect on peptides and proteins by TFE/H_2O mixtures. In this solvent, the amide carbonyl groups would form bifurcated hydrogen bonds preserving IHBs.[21,26] In this way, the opposite position of the isomeric caryophyllose equilibrium, which is shifted toward isomers **4 + 5** in TFE and isomers **2 + 3** in DMSO and H_2O, is in agreement with the higher hydrogen acceptor character of the latter solvents, which should prevent the formation of IHBs.[27] Less clear, at first glance, appear the factors responsible for the isomeric distribution of caryophyllose in DMSO and water. Actually, the **(4 + 5)/(2 + 3)** ratio is higher in DMSO than in

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water even though the former is more basic than the latter and therefore has a more marked hydrogen bond acceptor ability. This apparently conflicting result is supported by other data in the literature. Actually, strong IHBs between the syn-diaxial hydroxyls at C2 and C4 of talopyranoses in DMSO are reported.[19] In addition, there are many instances of carbohydrates that give IHBs in DMSO but not in water.[2,17,18] This would indicate a lesser ability of water than of DMSO to stabilize IHBs, likely due to the high polarity of water.[26,28] On the other hand, IHBs for oligosaccharides in water have been described as well.[1,23] This conflicting behavior indicates that other factors ought to be considered.

In this regard, it would be valuable to consider the data of Table 2 about the ratios of α/β anomers. As can be seen, **4/5** is ~3 times higher in DMSO than in D_2O, which is a trend similar to that occurring for the α-to-β ratio for D-talopyranose when the solvent changed from water to DMSO.[19,29] In those reports, the involvement of IHBs was ruled out as the cause of this trend, and although many factors were considered, no other explanation was given.

A possible explanation might stem from the different hydrogen bond requirements of equatorial and axial hydroxyls: the former can act as both hydrogen donor and acceptor, and the latter only as donors.[27] Therefore, these last are solvated only by hydrogen bond acceptor solvents and in this regard DMSO is better than H_2O, which as a hydrogen bond donor cannot solvate the axial hydroxyls but only the equatorial ones. This gives an increased solvation energy in DMSO to the isomer with the highest number of axial hydroxyls, as occurs for isomer **4**. In addition, the trans orientation of C1 and C2 hydroxyls makes them less sensitive to the large size of DMSO,[18] which instead reduces the solvation of the same hydroxyls in **5**, where they have a cis orientation. With respect to DMSO, water has a smaller molecular size, less hydrogen bond acceptor capacity, and a hydrogen bond donor character that enables it to solvate the equatorial anomeric hydroxyl of **5**. All these factors explain the lower value of the **4**/**5** ratio in water. Going to TFE, we found the lowest value of the ratio **4**/**5**, indicating an increased solvation energy for the equatorial anomer according to the higher hydrogen bond donor ability of this solvent. In this case, the steric hindrance of the TFE molecule, which is ~9 times the size of the water molecule,[21] seems to be less important than TFE's higher hydrogen bond donor ability than that of water.

As for the **3**/**2** ratio, the situation is completely different: in this case, its value is equal in both DMSO and TFE and in all cases the β-anomer predominates over the α anomer. The solvation energy in DMSO of the axial hydroxyls of **3** is reduced both for steric reasons (cis orientation of C1 and C2 hydroxyls) and for the weakness of the hydrogen bond formed by the anomeric hydroxyl group.[19] Going to the hydrogen bond donor solvents, water and TFE, the prevalence of **2** over **3** can be easily explained by the predominance of equatorial hydroxyls in the former isomer.

Solvent polarity is another factor that influences the α/β ratio. The equatorial orientation of anomeric hydroxyl is favored in solvents with a higher dielectric constant.[30] This is in agreement with the values of α/β ratios for (**3** + **4**)/(**2** + **5**) and of **3**/**2** (Table 3), which show higher values for the less polar solvents DMSO ($\epsilon = 46.4$) and TFE ($\epsilon = 26.67$) with respect to the more polar H_2O ($\epsilon = 78.54$).

The behavior of the caryophyllose isomeric equilibrium in TFE/water mixture was also investigated at 65 °C. The modification of caryophyllose isomeric distribution by increasing the water content up to 70% (v/v; Table 3) indicates that the shift of the equilibrium toward **2** and **3** does not occur in a regular way but varies widely up to ~30% the water content, so that further water additions, up to 70%, do not change the isomeric distribution.

Table 3. Caryophyllose Isomeric Distributions for Different Percentages (v/v) of TFE-d_3/D_2O, Measured by Anomeric Signal Integration of ^{1}H-NMR Spectra at 65 °C

TFE-d_3	**2**	**3**	**4**	**5**	**6**+**7**
100	15.3	5.2	26.4	38.4	14.7
85	25.3	8.0	25.0	30.8	10.9
80	26.6	8.7	25.3	28.9	10.5
75	31.9	11.2	20.8	25.1	11.0
65	37.8	13.4	17.9	20.5	10.4
60	38.5	13.5	18.0	20.0	10.0
50	39.5	13.5	17.9	19.2	9.9
40	39.6	13.3	18.2	18.9	10.0
30	39.6	13.3	17.8	19.0	10.3
0	60.6	17.4	7.5	9.1	5.4

The comparison of these isomeric distributions with that measured at the same temperature in pure D_2O suggests that low amounts, up to 30%, of TFE in D_2O determine a marked variation in the isomeric distribution as well. This behavior seems to be similar to that of peptides for which percentages higher than 30% (v/v) of TFE in water do not determine an increase in the structuring ability of the solvent mixture.[31] As matter of fact, additional factors have been taken into account to explain the structuring effect of the TFE/water mixture on peptides. They may also play a role in determining caryophyllose isomeric distribution. For example, the higher polarity of water should favor the cleavage of IHBs,[21,28] and the higher hydrophobicity of TFE would make it more difficult for the water molecules to approach TFE-solvated IHBs and to determine their severing. This effect could be enhanced by the steric hindrance of the TFE molecule.[21] In any case, when solvent mixtures are used, it is important to remember that preferential solvation can occur and that the properties of a solvent mixture can be very different from those of single components.[32]

Due to the low yields of furanose forms, any serious speculation about them would be hazardous. In any case, the increase of 1,2-*trans*-furanose isomer **6** on changing the solvent from water to DMSO to TFE may be due to the larger size of DMSO and TFE with respect to that of H_2O, so that the first solvents solvate the trans hydroxyl of **6** better than the crowded cis hydroxyl of **7**.[29]

To verify whether the results arising from caryophyllose are of general applicability, we investigated the isomeric equilibrium in TFE of ribose, a much more common sugar, which received attention in the past for its conformation properties in DMSO-d_6 and D_2O.[19,29,33−35]

In solution, this sugar gives a complex mixture in which both the conformations $^4C_1$ and $^1C_4$ chair of ribopyranose anomers **8** and **9** are present, in addition to the two furanose forms **10** and **11** (see Scheme 2).

On the basis of the $^3J_{H,H}$ coupling constant value of anomeric protons, it is possible to determine the amount of $^1C_4$ and $^4C_1$

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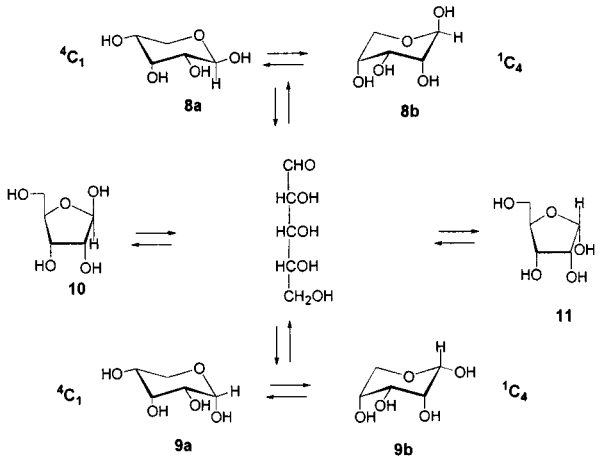
Table 4. ^{1}H NMR Chemical Shifts of Anomeric Protons (δ, Italic) and Percentage of Ribose Equilibrium Isomers **8**−**11** at 30 °C in D_2O, DMSO-d_6, and TFE-d_3 and $^3J_{vic}$ of Anomeric Protons (Hz, Parentheses)[a]

solvent	**8**	**9**	**10**	**11**	ref
D_2O	*4.90*, 58.7 (6.5) [77.0]	*4.84*, 25.0 (2.1)	*5.22*, 9.9	*5.35*, 6.4	34
	56 (6.4)[b]	20 (2.1)[b]	18[b]	6[b]	33
	55 [74.5]	23 [100 **9a**]	14	8	35
	54 (5.7)[c] (6.2)	18 (2.1)[c]	16 (1.0)[c] (1.4)	12[c]	35
DMSO	*4,71*, 62 (5.4)[51.6]	*4.64*, 27.5 (1.6)	*4.92*, 8.6 (2.3)	*5.06*, 1.9 (3.8)	
	54 [55.5]	16 [100 **9a**]	24	6	33
TFE	*5.95*, 61.0 (3.83)[36.0]	*5.60*, 34.5 (1.8)	*6.08*, 0.6 (3.6)	*6.17*, 3.7 (1.8)	

[a] In the bracket is indicated the percentage of $^4C_1$ conformation; literature data are reported for comparison. [b] At 35 °C. [c] At 70 °C.

Scheme 2. Equilibrium among Ribose Isomers



conformation of β-ribose **8**.[33] As a matter of fact, the interconversion of β-ribopyranose into the two possible conformers is fast with respect to the NMR time scale, leading to the observation of an averaged set of signals as well as of coupling constants; from this analysis, it was clear that β-ribopyranose anomer **8** exists in conformational equilibrium in both D_2O and DMSO-d_6.[33]

Unfortunately, the same approach cannot be applied to the α anomer of ribopyranose **9** because of the very low coupling constant values associated with α-ribopyranose in both conformations.

The data in Table 4 show that the ^3J values of **8** decrease going from D_2O to TFE-d_3, indicating the shift of the conformational equilibrium toward the $^1C_4$ conformation **8b**. This shift is in agreement with the surmised greater ability of TFE to favor the intramolecular hydrogen bonds between syn-axial hydroxyls 2 and 4. The same trend was found for the conformational methyl β-ribopyranoside equilibrium, which shifts toward the $^1C_4$ conformation going from D_2O (^1H δ 4.67) to TFE-d_3 (^1H δ 4.72) as supported by the decrease of the ^3J value from 5.3 to 2.3 Hz, respectively, and consequently the amounts of $^1C_4$ chair conformation were 42 and 88%, respectively.

Experimental Section

NMR Data Acquisition. All spectra were recorded on a Bruker DRX 400 Avance spectrometer, using a 5-mm multinuclear inverse Z-grad probe. One- and two-dimensional spectra (gradient-selected COSY, HSQC, and HMBC and phase-sensitive TOCSY) were performed using standard pulse sequences available in the Bruker Xwin-nmr 1.3 software. For ^{1}D-selective NOE, the HOD signal was presaturated in order to transfer the magnetization to the other hydroxyl protons, and a mixing time of 300 ms was used.

All deuterium-enriched solvents were purchased from Aldrich, except TFE-OH-d_2, which was available only from Cortec.

Chemical shifts in D_2O were expressed relative to the acetone signal (δ 2.22); in DMSO-d_6, the spectrum was calibrated on the methyl protons of the solvent (δ 2.49) as well as in TFE where the methylenic protons were set to δ 3.88.

Caryophyllose monosaccharide was isolated from the O-chain produced by the phytopathogenic bacterium *Burkholderia caryophylli*, as reported;[3] ribose was from a commercial source (Aldrich) whereas methyl D-ribopyranose was prepared by refluxing the corresponding monosaccharide with 1 M hydrochloridric methanol at 80 °C for 4 h and by successive purification from the other glycosides by silica gel chromatography with chloroform and increasing amounts of methanol.

Each monosaccharide (2−3 mg) was solved in the opportune deuterated solvent (500 μL) and analyzed as described in the text.

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