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Stereoelectronic Effects and H-Bonding in the Conformational Equilibrium of Serine and Threonine Methyl Esters

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Abstract: An extensive conformational search through the rotation of the dihedral angles χ_1 and χ_2 of serine (Ser-OMe) and threonine (Thr-OMe) methyl esters - two molecules of relative complexity that do not present a zwitterionic form in solution - was performed. The identified energy minima were subjected to Quantum Theory of Atoms in Molecules and Natural Bond Orbital analyses. In addition, the experimental $^3J_{HH}$ spin–spin coupling constants obtained in several solvents were compared with the calculated values for each conformer and provided insights into the conformational changes induced by each solvent. It was found that steric and hyperconjugative effects rule the conformational equilibrium of Ser-OMe and Thr-OMe, whereas the influence of H-bonding on both systems is a secondary effect.

Keywords: Serine and threonine methyl esters; Conformational analysis; Stereoelectronic effects, Spin-spin coupling constants

1. Introduction

The behaviour of proteins and peptides in biological systems is an important area to understand biological functions at the cellular and molecular levels, [1] as well as for the development of new drugs. However, macromolecules exhibit highly complex spectral properties [2] and require higher computational costs for theoretical calculations. Therefore, achieving a detailed conformational analysis of these compounds is notably difficult.

However, amino acids (AAs) are simple systems that represent the building blocks for proteins and peptides and thus can be used as models to study inter- and intramolecular interactions in biomolecules. Recently, the conformational equilibrium of some AAs in an isolated phase has been investigated. It was found that ubiquitous steric and hyperconjugative interactions, and not H-bonding, are responsible for the conformational preferences of glycine,[3] alanine,[4] sarcosine and *N*,*N*-dimethylglycine. [5]

However, AAs in the gaseous phase adopt the nonionic form that is significantly different from the *in-situ* conditions of the condensed medium in which biological reactions take place. [6] Moreover, these compounds exhibit thermal stability and low vapour pressure and decompose before melting. [7] In an aqueous solution and in crystals, AAs present

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a zwitterionic structure, *i.e.*, a bipolar form of the type (H_3N^+-CHRCOO$^-$), which does not occur in the polypeptide chain.[8] Thus, experimental studies in organic solvents and even in water are rather scarce due to either the zwitterionic form or the low solubility of the compounds.

Serine and threonine are AAs that have structurally similar side-chain polar (-OH) group, differing only by the substitution of one hydrogen atom from the side chain in serine by a methyl group in threonine (Figure 1). In both systems, the hydroxyl group can establish additional intramolecular hydrogen bonds as a proton donor to the amino group or to the carboxyl group or as a proton acceptor through the nonbonding electron pair at the group's oxygen atom. [9]

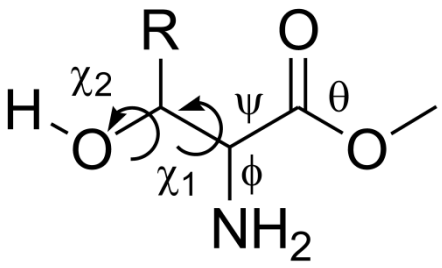

Fig. 1 Rotation of dihedral angles χ_1 and χ_2 provided the conformers of Ser-OMe (R= H) and Thr-OMe (R= CH_3).

In addition, the presence of the methyl group in threonine in comparison with serine can be used as a probe to evaluate the importance of steric effects on the conformational preferences on these systems. For this reason, the conformational analyses of these AAs and their derivatives have been extensively studied in the literature. [1,7,9-25] The most complete theoretical study for serine was performed by Gronert and O'Hair. [12] This study found 324 conformers (at the semiempirical AM1 level) by systematic rotations around the five rotations axes. The conformer geometries were then optimised at the 3-21G* and 6-31G* levels, resulting in 51 minima with relative energy differences that were less than 12 kcal mol^{-1}. Subsequently, Lambie *et al*. [7] evaluated the conformational equilibrium of serine through experimental matrix-isolation FT-IR spectroscopy and density functional theory (DFT) calculations and concluded that H-bonding drives the conformational equilibrium of this system. Accordingly, vibrational circular dichroism and absorption spectroscopy, together with DFT calculations at the B3LYP/6-311++G(d, p) level, were employed to demonstrate the importance of H-bonding on the conformational preferences of serine in an aqueous solution at several pHs.[24] Additionally, the conformational equilibrium of serine and threonine has been analysed in an aqueous solution by Hernández *et al.* [13] using Raman and FT-IR attenuated total reflectance spectra together with theoretical calculations (B3LYP/6-311++G level), and the same results were found. However, in all these cases, steric and hyperconjugation effects, which are important interactions present in biomolecules, have not been recognised. Recently, the conformational preferences of several amino esters [26-29], which are compounds that are soluble in organic solvents and do not show zwitterionic forms, have been investigated. The results indicated that stereoelectronic effects are important interactions present in these systems and should not be ignored. In this paper, the conformational analysis of serine (Ser-OMe) and threonine (Thr-OMe) methyl esters is investigated using ^{1}H NMR spin–spin coupling constants ($^3J_{HH}$) together with DFT theoretical calculations, the quantum theory of atoms in molecules (QTAIM) [30-34] and natural bond orbital (NBO) methods. [35]

2. Materials and Methods

2.1. Deprotonation

Ser-OMe and Thr-OMe were prepared by the deprotonation of serine methyl ester hydrochloride and of threonine methyl ester hydrochloride (from Sigma–Aldrich Company), respectively. Commercial zinc powder (100 mg) was added in one portion to a suspension of the Thr-OMe.HCl (1 mmol) in 10 mL of THF. The mixture was stirred for approximately 10 min. Subsequently, the mixture was filtered, and the solvent was evaporated. The Thr-OMe was obtained as a free ester crystalline solid. [36] Na_2CO_3 (0.5 mmol) was added in one portion to a suspension of Ser-OMe.HCl (1 mmol) in methanol (10 mL). The mixture was stirred for 2 h at room temperature. After completion of the reaction, the mixture was filtered and evaporated *in vacuo*. The free ester was obtained after extraction using acetonitrile.

2.2. NMR Experiments

The solvents (acetonitrile-d_3, methanol-d_4, pyridine-d_5 and DMSO-d_6) were commercially available and used without further purification. ^{1}H NMR spectra were recorded on a Bruker Avance III spectrometer equipped with a 5-mm probe operating at 600 MHz for ^{1}H. The internal reference used was TMS. The typical conditions for the ^{1}H spectra were as follows: eight transients and a spectral width of 6394 Hz with 32k data points, resulting in an acquisition time of 2.56 s. The resolution of the spectra obtained by these parameters is 0.4 Hz.

2.3. Computational Details

To obtain the energy minima of Ser-OMe and Thr-OMe, the six more-stable geometries of alanine methyl ester [27] were used as starting points to build three-dimensional potential energy surfaces (at the B3LYP/cc-pVDZ theoretical level) through the simultaneous rotation of the χ_1 [H_2N-C_α-C_β-O(H)] and χ_2 [C_α-C_β-O-H] torsion angles, *e.g.*, scanning χ_1 from 0º to 360º in steps of 10º and then holding χ_2 fixed at 0º, 10º, *etc*. (Figure 1). Moreover, the dihedral angles ϕ [LP-N-C-C(O)] (LP represents the nonbonding electron pair) and ψ [N-C-C=O], which were previously optimised for alanine methyl ester, [27] were held constant. In addition, the dihedral angle θ [O=C-O-CH_3] was also keep fixed (*cis* form); this arrangement is the most stable θ dihedral angle geometry, as demonstrated for several methyl esters. [37] Thus, a total of 6×37×37= 8214 conformers were initially considered for each system.

The local minima obtained were optimised at the B3LYP/aug-cc-pVDZ theoretical level with unconstrained dihedral angles. This level of theory has been tested satisfactorily in other amino esters [26, 27] and has already shown good agreement with CCSD/CBS and experimental data for glycine and alanine amino acids. [38, 39] The zero point energy (ZPE) correction was added to these calculated energies. Frequency calculations were performed to ensure that no imaginary frequencies were present. Then, each Ser-OMe and Thr-OMe energy minimum was reoptimised by considering solvent effects through the conductor-like polarisable continuum model (CPCM) [40,41] in the methanol, acetonitrile, pyridine and DMSO as solvents. The NBO analysis was performed over the wave functions obtained from the B3LYP/aug-cc-pVDZ optimisations in the same level of theory. The spin–spin coupling constant calculations were performed at the B3LYP functional and with the EPR-III (for C and H) and the aug-cc-pVDZ (for O and N) basis sets. All calculations were performed using the Gaussian 09 program. [42] In addition to NBO analysis, quantum theory of atoms in molecules (QTAIM) calculations was applied to search for hydrogen bonds and the stabilities of these bonds using the AIMAll program. [43]

3. Results and Discussion

From 6 three-dimensional potential energy surfaces built for each system, 27 and 41 local minima were obtained for Ser-OMe (Figure S1 and S2) and Thr-OMe (Figure S3 and S4), respectively. However, after optimisation in the isolated phase and taking into account the solvent effect, it was found that the conformational equilibrium of these systems is governed by not more than four conformers. The remaining conformers either migrated to one of the existing structures or showed relative energies above 2 kcal mol^{-1}, *i.e.*, are not expected to exist appreciably. In fact, the experimental identification of conformers with a larger relative energies is very difficult or even impossible. [25] However, a fifth conformer, which is relatively unstable and does not exhibit a suitable geometry for H-bonding formation, was used as a reference. Thus, 5 conformers (named by the increasing order of energy) were considered in this work (Figure 2).

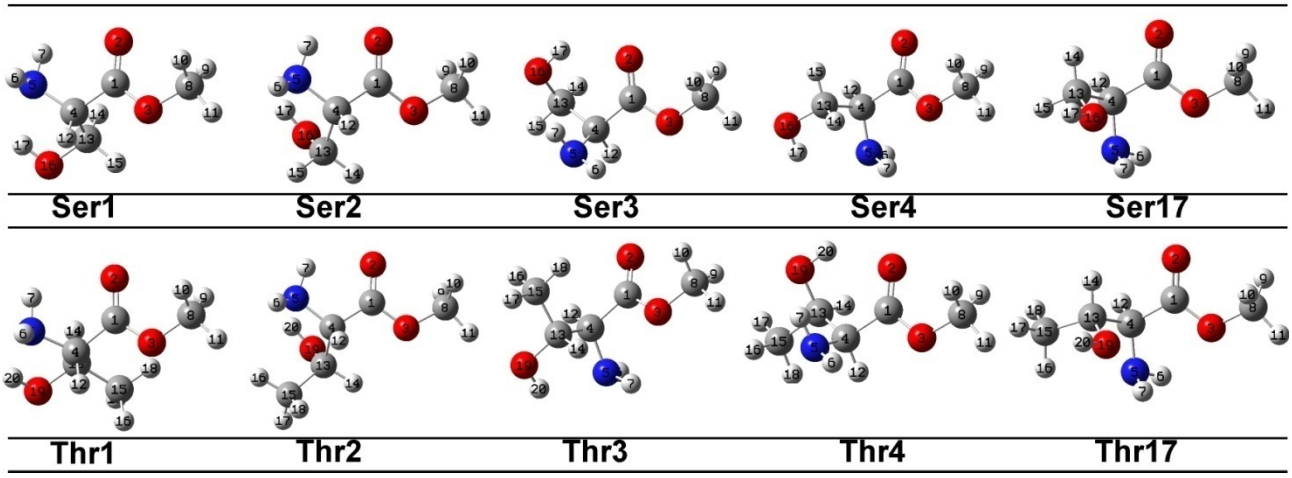

Fig. 2 Five conformers of the Ser-OMe and Thr-OMe presently studied.

The introduction of a methyl group in serine to produce threonine would enhance the hindrance effect that gives rise to the Lewis energy as well as a hyperconjugation increment that would change the conformational equilibrium of this system. In this sense, the dihedral angles represent important structural parameters to characterise and compare different conformers. Table 1 lists the main dihedral angles of the five energy minima obtained for each system. A comparative analysis of the various conformations of both systems reveals very small deviations for the dihedrals angles χ_1, χ_2, ψ and ϕ. In addition, the relative energies for **Ser1** and **Ser2** are, respectively, identical to the conformers **Thr1** and **Thr2**. However, **Thr4** and **Thr3**, corresponding to the conformers **Ser3** and **Ser4**, respectively, exhibit differences in the stability order. Even so, the energy difference (isolated phase, Table 1) between **Thr3** and **Thr4** conformers is very small (0.10 kcal mol^{-1}). These results suggest that a single methyl group would not seem sufficiently large to cause a sufficient disturbance in Thr-OMe and thus does not show a marked importance for the conformational preferences of this system.

Table 1 Dihedral angles (º) and relative energies (kcal mol^{-1}) of five conformers of Ser-OMe and Thr-OMe obtained at B3LYP/aug-cc-pVDZ level.

	Conformer									
	Ser1	**Ser2**	**Ser3**	**Ser4**	**Ser17**	**Thr1**	**Thr2**	**Thr3**	**Thr4**	**Thr17**
χ_1	54.8	305.3	300.1	55.6	297.5	53.8	306.2	54.4	301.9	298.5
χ_2	317.0	45.6	297.0	316.8	180.7	318.2	46.4	318.7	299.6	179.5
ψ	17.9	351.3	100.6	196.3	1.0	38.0	354.5	231.0	94.4	2.5
ϕ	203.3	240.7	170.7	196.3	171.5	199.5	255.7	193.2	172.3	174.5
E_{rel}	0.00	0.86	1.31	1.69	4.11	0.00	0.86	1.22	1.32	3.39

H-bonding formation is the interaction commonly used to explain the relative energy values of AAs and their derivatives. Thus, the Popelier criteria,[34] which are based in QTAIM, were employed. According to Popelier, when two atoms are linked through an H-bond, the electronic density ρ and its Laplacian ($\nabla^2\rho$) in the bond critical point (BCP) should fall between 0.002 and 0.034 a.u and between 0.024 and 0.139 a.u, respectively. In addition, a hydrogen atom involved in an H-bond should lose electronic charge q(H) while the atomic first dipole moment M_1(H) and atomic volume V(H) decrease and the atomic energy E(H) increases in magnitude when compared with an isolated H-bond – an uninvolved hydrogen atom. According to the molecular graphs (Figure 3) and the QTAIM parameters (listed in Tables S1 and S2), all the Popelier criteria are met by the main conformers of Ser-OMe and Thr-OMe (except **Ser17** and **Thr17,** which do not participate with any possible H-bonding and were used as references), and thus, these conformers have a consistent topology for the formation of H-bonds. However, as reported by Bader,[30] the proximity between an H-bonding BCP (green dots) and RCP (Ring Critical Points, red dots) suggests a catastrophe point, the point at which the BCP and RCP coalesce; these critical points then no longer exist as well as, obviously, the H-bond. Therefore, in the QTAIM viewpoint, only **Ser3** and the corresponding conformer **Thr4** (which are not the global minima) show stable H-bonding. In addition, only these conformers show H-bonds forming a stable six-membered ring, whereas the remaining conformers correspond to a strained five-membered ring.

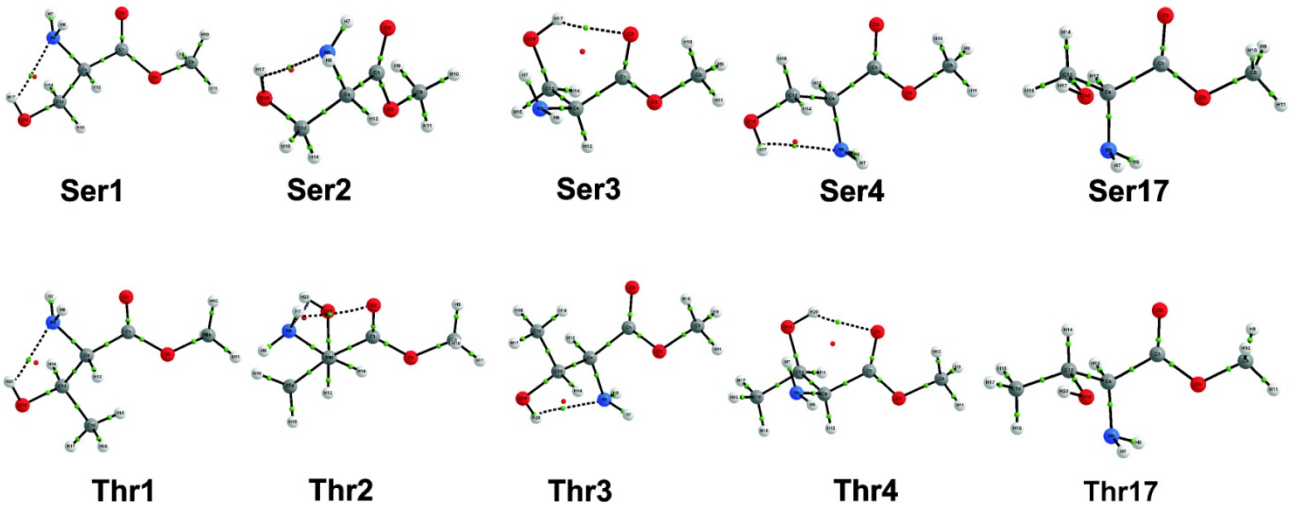

Fig. 3 Molecular graphs of Ser-OMe and Thr-OMe conformers. Green points represent the bond critical points (BCP) and red points the ring critical points (RCP).

To evaluate the contributions of steric effects and hyperconjugation, NBO analysis was employed. In this methodology, the full energy of a given system may be decomposed into a Lewis structure, represented by electrostatic and steric effects, and a non-Lewis structure, referred to hyperconjugation. The energy corresponding to each interaction can be obtained by deleting the interactions involving antibonding and Rydberg-type orbitals and by subsequently comparing the resulting energy, corresponding to an ideal Lewis structure, with the full energy of the system. Table 2 gives the relative contributions from the hyperconjugation and Lewis-type interactions, indicating that **Ser1** experiences lower steric effects than the remaining conformers. In this conformation, LP_{N5}, $LP_{O(H)}$, LP_{O2} and LP_{O3} remain far from each other, decreasing the steric effects among them. However, this figure changes when a methyl group is incorporated on the side chain, *i.e.*, the repulsive interaction between LP_{O3} and the $-CH_3$ group increases the ΔE_{Lewis} in **Thr1**. Nonetheless, this geometry remains the most stable among the energy minima of Thr-OMe due to the $\sigma_{C4\text{-}N5} \rightarrow \sigma^*_{C13\text{-}C15}$ (2.02 kcal mol^{-1}) hyperconjugative interaction that occurs in **Thr1** instead of the lower-intensity $\sigma_{C4\text{-}N5} \rightarrow \sigma^*_{C13\text{-}H5}$ (1.19 kcal mol^{-1}) present in **Ser1**. **Ser17** experiences the more significant contribution of $LP_{N5} \rightarrow \sigma^*_{C1\text{-}C4}$ (9.83 kcal mol^{-1}) but shows a higher Lewis energy due to the superposition of the electronic clouds of N5 and O(H) and thus represents a highly unstable geometry.

Table 2 Relative energy (ΔE_{rel}), Lewis energy (ΔE_{Lewis}), hyperconjugative energy (ΔE_{hyp}) and main hyperconjugative interactions (kcal mol^{-1}) for Ser-OMe and Thr-OMe (B3LYP/aug-cc-pVDZ theoretical level).

	conformer									
parameter	**Ser1**	**Ser2**	**Ser3**	**Ser4**	**Ser17**	**Thr1**	**Thr2**	**Thr3**	**Th4**	**Thr17**
ΔE_{rel}	0.00	0.86	1.31	1.69	4.11	0.00	0.86	1.22	1.32	3.39
ΔE_{Lewis}	0.00	1.65	2.89	0.81	3.55	1.26	0.63	0.00	1.88	1.88
ΔE_{Hyp}	0.87	1.67	2.46	0.00	0.31	2.75	0.76	0.04	1.68	0.00
$LP(1)_{O2} \rightarrow \sigma^*_{C1\text{-}C4}$	2.49	2.56	2.90	2.55	2.51	2.62	2.59	2.75	3.04	2.59
$LP(2)_{O2} \rightarrow \sigma^*_{C1\text{-}C4}$	18.10	18.44	16.87	17.81	18.89	17.55	18.23	17.71	16.56	18.94
$LP(1)_{O2} \rightarrow \sigma^*_{C1\text{-}O3}$	1.55	1.47	1.10	1.54	1.53	1.52	1.54	1.55	1.05	1.62
$LP(2)_{O2} \rightarrow \pi^*_{C1\text{-}O3}$	34.73	33.99	34.13	36.29	36.22	34.96	35.89	36.49	34.16	35.18
$LP(1)_{O3} \rightarrow \sigma^*_{C1\text{-}O2}$	9.43	9.41	9.37	8.78	8.69	9.52	8.76	8.88	9.38	9.05
$LP(1)_{O3} \rightarrow \sigma^*_{C1\text{-}C4}$	0.70	0.77	0.64	0.74	0.82	0.72	0.79	0.64	0.60	0.64
$LP(2)_{O3} \rightarrow \pi^*_{C1\text{-}O2}$	49.62	50.56	50.56	45.36	44.46	49.68	47.29	46.12	50.35	47.95
$LP(2)_{O2} \rightarrow \sigma^*_{N5\text{-}H7}$	0.71	1.81	-----	-----	-----	0.61	2.14	------	------	-----
$LP(2)_{O2} \rightarrow \sigma^*_{O\text{-}H}$	----	-----	3.53	-----	-----	-----	-----	------	4.27	-----
$LP(1)_{O(H)} \rightarrow \sigma^*_{C4\text{-}C13}$	1.58	1.82	1.07	1.58	1.25	1.53	2.35	1.58	1.17	1.16
$LP(2)_{O(H)} \rightarrow \sigma^*_{C4\text{-}C13}$	4.69	4.78	6.90	4.56	-----	4.75	3.87	4.54	6.81	-----
$LP_{N5} \rightarrow \sigma^*_{C4\text{-}C13}$	2.52	3.63	1.93	2.18	0.56	2.16	3.48	1.93	0.53	0.60
$LP_{N5} \rightarrow \sigma^*_{C1\text{-}C4}$	6.98	-----	8.13	8.54	9.83	6.78	-----	7.67	7.89	8.95
$LP_{N5} \rightarrow \sigma^*_{C4\text{-}H12}$	-----	5.68	1.79	-----	1.30	-----	5.88	1.38	1.56	1.41
$LP_{N5} \rightarrow \sigma^*_{O\text{-}H}$	3.41	3.14	-----	3.23	-----	4.40	3.79	4.00	-----	----
$\sigma_{C4\text{-}N5} \rightarrow \sigma^*_{C13\text{-}H15}$	1.19	-----	-----	1.19	-----	-----	-----	------	-----	----
$\sigma_{C4\text{-}N5} \rightarrow \sigma^*_{C13\text{-}C15}$	-----	-----	-----	-----	-----	2.02	-----	1.98	-----	----

In addition, the electronic delocalisation $LP_{N5}\rightarrow\sigma^*_{C1\text{-}C4}$ is also operative for **Ser1** (6.98 kcal mol^{-1}), **Ser3** (8.13 kcal mol^{-1}) and **Ser4** (8.54 kcal mol^{-1}), whereas **Ser2** is largely stabilised by the $LP_{N5}\rightarrow\sigma^*_{C4\text{-}H12}$ (5.68 kcal mol^{-1}). H-bonding may be attributed to hyperconjugative interactions, [44] as shown by the NBO calculations. In this sense, in agreement with the QTAIM calculations, the NBO analysis shows that the through-space electron delocalisation between the non-bonding electrons LP_{O2} and anti-bonding orbital (σ^*_{OH}) has a marked importance for the stabilisation of **Ser3** (3.53 kcal mol^{-1}) whereas for **Ser1**, **Ser2** and **Ser4**, where an H-bond $H_2N\ldots H\text{-}O$ does not occur, the values of the $LP_{N5}\rightarrow\sigma^*_{O\text{-}H}$ interactions are practically identical (3.41, 3.14 and 3.23 kcal mol^{-1} respectively). The removal of these interaction energies from the values of the relative energy (ΔE_{rel}), *i.e.*, the failure to account for $LP(2)_{O2}\rightarrow\sigma^*_{O\text{-}H}$ and $LP_{N5}\rightarrow\sigma^*_{O\text{-}H}$ for the respective conformers, does not alter the stability order among these values. Thus, from NBO results, H-bonding cannot be invoked to explain the stabilisation order of the energy minima. Indeed, the equilibrium between the stereoelectronic effects may be observed for all conformers of Ser-OMe and Thr-OMe. Therefore, these results suggest that an interplay between the hyperconjugation and steric effects governs the conformational equilibrium in both systems.

The spin-spin coupling constant $^3J_{H\alpha H\beta}$ between the α and β hydrogen atoms of Ser-OMe and Thr-OMe may give insight into the rotational isomerism in these systems according to the well-known Karplus relationship. [45] Coupling constant values between *anti* α and β hydrogens ($^3J_{anti}$) are expected to be large due to the antiperiplanar arrangement, whereas lower values of $^3J_{gauche}$ are expected from hydrogen atoms in a *gauche* arrangement. In this sense, **Ser1**, **Ser3** and **Ser4** should have large $^3J_{H\alpha H\beta1}$ and small $^3J_{H\alpha H\beta2}$ coupling constants. Otherwise, two small values should be observed for $^3J_{H\alpha H\beta1}$ and $^3J_{H\alpha H\beta2}$ in **Ser2** (see Figure 2). In fact, the observed coupling constant is an average of the intrinsic coupling constants $^3J_{anti}$ and $^3J_{gauche}$, and thus, intermediated values are experimentally obtained. This analogy may also be employed for Thr-OMe conformers; however, in this case, only $^3J_{H\alpha H\beta1}$ should be considered. Table 4 lists the coupling constants between α and β hydrogens for both systems in the solvents acetonitrile, pyridine, methanol and DMSO. Because the observed coupling constants did not vary uniformly when the solvents were changed, it appears that the conformer's population does not vary significantly. The NMR data also show that the introduction of a methyl group to replace one hydrogen on the side chain does not change the conformational equilibrium for Thr-OMe, once the values of $^3J_{H\alpha H\beta1}$ have the same magnitude as those observed for Ser-OMe.

Table 4 Coupling constants (J, in Hz) and Chemical shifts (δ, in ppm) to Ser-OMe and Thr-OMe rotamers in solvents of different dielectric constants (ε).

Solvent	ε	δ_α	$\delta_{\beta1}$	$\delta_{\beta2}$	$^3J_{H\alpha H\beta1}$	$^3J_{H\alpha H\beta2}$
			Ser-OMe			
Pyridine-d_5	12.3	3.85	4.15	3.20	4.44	4.74
MeOD	32.7	3.52	3.73	3.79	4.14	4.74
CD_3CN	37.5	3.45	3.61	3.63	4.38	5.04
DMSO-d_6	46.7	3.36	3.50	3.52	4.86	4.80
			Thr-OMe			
Pyridine-d_5	12.3	3.62	4.08	----	4.26	----
MeOD	32.7	3.73	4.45	----	3.84	----
CD_3CN	37.5	3.63	4.16	----	4.44	----
DMSO-d_6	46.7	3.20	3.90	----	4.40	----

4. Conclusion

The detailed conformational analysis of the dominant Ser-OMe and Thr-OMe conformers have been investigated using ^{1}H NMR spectroscopy together with QTAIM and NBO analysis. The results showed that the replacement of hydrogen (Ser-OMe) by a methyl group (Thr-OMe) increases the Lewis energy, due the increment of steric interactions between mean and side chains. However, this modification is counterbalanced by hyperconjugative effects, which decrease of the relative energy of ThrOMe conformers, when compared with energy minima of SerOMe. Therefore, the equilibrium between these stereoeletronic effects is responsible for conformational stability of both systems. In addition, the general consensus about the role H-bonding as interaction responsible for stability of these systems is equivocated, once that it appears only in one conformer relativity stable. Moreover, the remotion of this interaction does not change the stability order of the conformers and thus, H-bonding plays a secondary role in the conformational preferences of these systems.

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References

[1]. M. R. Watry, G L. Richmond, J. Phys. Chem. B 106 (2002) 12517-12523.

[2]. A. Barth, Biochim. Biophys. Acta 1767 (2007) 1073-1101.

[3]. R. A. Cormanich, L.C. Ducatti, R. Rittner, J. Chem. Phys. 387 (2011) 85–91.

[4]. R. A. Cormanich, L.C. Ducatti, R. Rittner, J. Mol. Struct. 1014 (2012) 12–16.

[5]. R. A. Cormanich, L.C. Ducati, C.F. Tormena, R. Rittner, Chem. Phys. 421 (2013) 32-38.

[6]. A. Lesarri, S. Mata, E. J. Cocinero, S. Blanco, J. C. López, J. L. Alonso, Angew. Chem. Int. Ed. 41 (2002) 4673-4676.

[7]. B. Lambie, R. Ramaekers, G. Maes, J. Phys. Chem. A 108 (2004) 10426-10433.

[8]. A. Lesarri, E. J. Cocinero, J. C. López, J. L. Alonso, Angew. Chem. Int. Ed. 43 (2004) 605-610.

[9]. S. Blanco, M. E. Sanz, J. C. Lopes, J. L. Alonso, Proc. Natl. Acad. Sci. USA 18 (2007) 20183-20188.

[10]. F. Corzana, J. H. Busto, G. Jiménez-Osés, M. G. Luis, J. L. Asensio, J. Jiménez-Barbero, J. M. Peregrina, A. Avenoza, J. Am. Chem. Soc. 129 (2007) 9458-9467.

[11]. A. Gil, S. Simon, L. Rodríguez-Santiago, J. Bertrán, M. Sodupe, J. Chem. Theory Comput. 3 (2007) 2210-2220.

[12]. S. Gronert, R. A. J. O'Hair, J. Am. Chem. Soc. 117 (1995) 2071-2081

[13]. B. Hernández, F. Pflüger, A. Adenier, M. Nsangou, S. G. Kruglik, M. Ghomi, J. Chem. Phys. 98 (2011) 1-7.

[14]. S. Jarmelo, I. Reva, M. Rozenberg, M. R. Silva, A. M. M. Beja, R. Fausto, J. Phys. Chem. B 112 (2008) 8032-8041.

[15]. D. Peters, J. Peters, J. Mol. Struct. THEOCHEM, 90 (1982) 305-320.

[16]. D. Peters, J. Peters, J. Mol. Struct. THEOCHEM, 90 (1982) 321-334.

[17]. M. A. Sahai, S. S. Motiwala, G. A. Chass, E. F. Pai, B. Penke, I. G. Csizmadia, Mol. Struct. THEOCHEM 251 (2003) 666-667.

[18]. A. Fernández-Tejada, F. Corzana, J. H. Busto, A. Avenoza, J. M. Peregrina, J. Org. Chem. 74 (2009) 9305-9313.

[19]. O. Kikuchi, T. Matsuoka, H. Sawahata, O. Takahashi, J. Mol. Struct. THEOCHEM 305 (1994) 79-87.

[20]. R. Miao, C. Jin, G. Yang, J. Hong, C. Zhao, L. Zhu, J. Phys. Chem. A 109 (2005) 2340-2349.

[21]. M. Noguera, L. Rodríguez-Santiago, M. Sodupe, J. Bertran, J. Mol. Struct. THEOCHEM 537 (2001) 307-308.

[22]. A. Pawlukojc´, J. Leciejewicz , J. Tomkinson, S.F. Parker, Spectrochim. Acta A. 58 (2002) 2897-2904.

[23]. A. Pawlukojc´, J. Leciejewicz, J. Tomkinson, S.F. Parker, Spectrochim. Acta A. 57 (2001) 2513-2523.

[24]. P. Zhu, G. Yang, M. R. Poopari, Z. Bie, Y. Xu, ChemPhysChem. 13 (2012) 1272-1281.

[25]. B. Lambie, R. Ramaekers, G. Maes, Spectrochim. Acta A 59 (2003) 1387-1393.

[26]. C. J. Duarte, R. A. Cormanich, L. C. Ducati, R Rittner, J. Mol. Struct. 1050 (2013) 174-179.

[27]. R. A. Cormanich, L. C. Ducati, C. F. Tormena, R. Rittner, J. Phys. Org. Chem. 26 (2013) 849-857.

[28]. R. A. Cormanich, L. C. Ducati, C. F. Tormena, R. Rittner, Spectrochim Acta A 123 (2014) 482.

[29]. C. B. Braga, L. C. Ducati, C. F. Tormena, R. Rittner, J. Phys. Chem. A 118 (2014) 1748.

[30]. R.F.W. Bader, Atoms in Molecules: A Quantum Theory, Oxford University Press, Oxford, 1990.

[31]. R. F. W. Bader, Chem. Rev. 91 (1991) 893-928.

[32]. R. F. W. Bader, J. Phys. Chem. A 102 (1998) 7314-7323.

[33]. S. J. Grabowski, Chem. Rev. 111 (2011) 2597-2625.

[34]. U. Koch, P. L. A. Popelier, J. Phys. Chem. 99 (1995) 9747-9754.

[35]. NBO 6.0, E.D. Glendening, J. K. Badenhoop, A. E. Reed, J. E. Carpenter, J. A. Bohmann, C. M. Morales, C. R. Landis, F. Weinhold, Theoretical Chemistry Institute, University of Wisconsin, Madison, 2013.

[36]. K. Ananda, V. V. S. Babu, J. Pept. Res. 57 (2001) 223-226.

[37]. C.F. Tormena, T.R.F. Yoshinaga, R. Rittner, Spectrochim. Acta A63 (2006) 511-517.

[38]. A. G. Csaszar, J. Phys. Chem. 100 (1996) 3541-3551.

[39]. R. M. Balabin, Theor. Chem. 965 (2011) 15-21.

[40]. V. Barone and M. Cossi, J. Phys. Chem. A, 102 (1998)1995-2001.

[41]. M. Cossi, N. Rega, G. Scalmani, V. Barone, J Comput Chem 24 (2003) 669-681.

[42]. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford CT, 2013.

[43]. T. A. Keith, AIMAll (Version 11.12.19), 2011.

[44]. C. J. Duarte, M. P. Freitas, J. Mol. Struct. 930 (2009) 135-139.

[45]. M. Karplus, Chem. Phys. 30 (1959) 11-15.