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How Many Conformers of the 1, 2, 3-Propanetriol Triacetate are Present in Gas Phase and in Aqueous Solution ?

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Abstract: We found 109 rotamers within 3 kcal from the global minimum of the triglyceride in vacuo and only 6 water-solvated rotamers within this range. Hydration restricts triacetin's conformational flexibility which is an unusual situation for small polar molecules. The lowest solvated conformation forms a system of three ring-like formations as the three carbonyl oxygens approach the hydrogen of the mid carbon of the glyceride skeleton. The structures of the global minima were subjected to geometry optimization with ab initio methods in order to validate the results.

INTRODUCTION

The importance of triglycerides in the biological cycle can hardly be overestimated. Between other functions they serve as structural components of the cell membranes where they are in close interaction with water. The mode of organization of lipids in biomembranes is crucially dependent on the conformation of the glycerol moiety. A recent study[1] of a fluid-phase dipalmitoyl phosphatidylcholine lipid bilayer in water and of neat hexadecane concluded that "interactions involving the glycerol headgroup and surface water, principally modulate translational diffusion, hence analytic theories should explicitly take into account the dynamic and structural features of the lipid-water interface". Time-averaged structures have been validated with the wealth of data on isolated organic molecules in the gas phase and in crystals, but lack of good structural data in less homogeneous media continues to limit the understanding of conformational preferences in condensed phase environments e.g. in solution or at an interface. The differences between in vacuo and solvent calculations may be so notable that one could select totally different shapes to represent low-energy conformers under the two conditions.[2,3]

The triglyceride 1, 2, 3-propanetriol triacetate known as triacetin, is the simplest of this class of compounds. Triacetin is amphipathic in nature, due to the presence of the polar glyceride ester group and the non-polar methyl groups. In particular, triacetin is studied in vacuo and in the presence of explicit water molecules. The results of the present work show that although triacetin is an inherently flexible molecule, water solvation restricts considerably its conformational freedom. We found only 6 triacetin-water clusters within 3 kcal from the global minimum, whereas 109 conformers are present in gas phase. This is highly unusual, since polar molecules tend to increase their conformational flexibility upon solvation.[3,4,5] The most stable triacetin-water cluster forms three intramolecular ring-like "hydrogen bonded" arrangements around the propane-frame, two of them being six-membered rings and one five-membered ring.

COMPUTATIONAL METHODS

One of the most difficult problems in molecular modeling is that the conformers of physical interest correspond to the few local energy minima above the best conformer or "global minimum". The total number of minima apparently increases exponentially with the size of the molecule.[2]

We used a global minimization strategy, that mainly follows the stochastic procedure described by Saunders.[6] Allinger's MM2 (87)[7,8] force field has been employed to calculate the conformational energy. For the local minimizations the Merlin package[9,10] was used, while the global strategy was coded in MCL.[11] Merlin is a portable optimization system that implements six different minimization algorithms. Its actions can be controlled by the user either interactively, or through MCL, a high level programming language designed to automate minimization sessions and aid the implementation of complex minimization strategies.

The method generates a starting geometry by applying a random displacement of the coordinates, or "kick", to every atom of a known conformation. Even with small kick sizes the steric energy raises by hundreds of kilocalories. We found that a maximum kick size of 6Å covers satisfactorily the conformational space of these particular molecular systems. The MM2 energy function is then minimized by three successive steps:

a) At the beginning only the stretch, bend, stretch-bend and torsion parts of the MM2 potential are minimized. The distortions in bond lengths and angles responsible for most of the steric energy are thus rapidly removed, while the computational cost of a call is significantly reduced compared to full potential evaluation. We have found that for such short minimization sessions, the conjugate gradient method of Polak and Ribiere[12] performs better than the other methods implemented in Merlin.

b) The MM2 potential that mimics the Van der Waals interactions is added and minimization proceeds by the Broyden-Fletcher-Goldfarb-Shanno (BFGS)[12,13] method.

c) Finally electrostatic interactions are added and the complete energy functional is further minimized by the BFGS method. MM2(87) supplies a block diagonal second derivative matrix of the potential which is used as an initial estimate for the BFGS routine of Merlin.

At the end of each minimization step the energy is examined and the conformer is rejected if it exceeds a given threshold. The newly found geometry is compared against all existing conformations and discarded if it matches any one of them. The procedure is repeated by selecting an existing conformer as a starting geometry for the kick. Termination occurs when a given number of kicks is reached or when the estimated probability[14] of finding every conformer within an energy range (about 3 kcal from the global minimum) reaches a preset value (typically 0.999).

The above algorithm is implemented using the Merlin/MCL[15] system and is quite general. It has been applied to the cycloheptadecane $(CH_2)_{17}$ as a test case. This 17-membered ring has been proposed[16] as a benchmark for the effectiveness of new methods for searching conformational space as they are developed. The computer program reproduced the low lying conformers found in ref. 16, requiring one third of the cpu time, taking into account the relative performance of the machines involved. Methods in which deformations of the coordinates are followed by local minimizations, are known[4,16] to have performances (in terms of number of conformers and cpu time) better than that of molecular dynamics or distance geometry searches.

The same code has been applied to triacetin in vacuo and to triacetin along with 15 water molecules. In the case of the triacetin-water cluster, spatial constraints must be imposed in order to keep the water molecules near the triglyceride after the kick. Merlin is an unconstrained optimization system, it can however handle simple bounds on the minimization parameters. After the initial kick, the atoms are confined in a box around C_2 (see Fig. 1). As the minimization proceeds, the atomic coordinates change and their bounds are adjusted in order to keep C_2 in the center of the box.

An important characteristic of the method is that a number of kicks can be simultaneously processed in different machines, maximizing the use of a local computer network and reducing the overall real time. The conformer search within the supermolecule approach[2] was considered prohibitive due to the large numbers of atoms involved. Exhaustive search is currently impossible even for systems of one hundred atoms[17,18]; this method renders the task of handling such a molecular system, possible. It is worth noting that within the MM2(87) framework lone electron pairs are treated as independent entities, hence the conformer search of the solute-solvent cluster was set to 110 atoms. The importance of the distributed nature of this procedure is that molecular systems with a large number of atoms can be treated by simply spreading the computational load to a larger number of machines which is possible since the communication overheads are negligible.

Ten thousand random kicks in the Cartesian space were executed for each molecular system, and a further run of another 10000 kicks did not succeed in finding a new low-energy triacetin-water cluster. Overall performance is thought to be satisfactory since the average cpu time required for one kick of the triacetin molecule on a 25 MHz MIPS R3000 workstation is 20 secs, while 100 cpu secs are required for the hydrated triacetin. All calculations were carried out on a network of 12 workstations.

The HONDO-7[19] suite of programs was used to perform the ab initio quantum mechanical calculations at the Hartee-Fock (HF) level on a multiprocessor MIPS computer after being modified for Unix environment. Geometry optimizations were performed at the MM2 best triacetin conformer, in C1 symmetry, with the split valence basis set 4-31G plus polarization functions. This basis set is known to give satisfactory results for hydrogen bond geometries[20] with small basis set superposition error.[20,21] The MM2 global minimum conformation of triacetin was subsequently optimized with total relaxation of all the geometrical parameters. An ab initio 4-31G geometry optimization was performed on a reduced three water-triacetin complex, derived from the MM2 lowest energy triacetin-water cluster, after the removal of the water molecules except the three most tightly bound to the triacetin molecule.

Table 1. MM2 energies (kcal/mol), dihedral angles and Boltzmann distributions for the ten lowest in energy conformers of the in vacuo triacetin. For the purpose of the analysis, the dihedral angles are divided in three groups: gauche plus (g+), 0^o-120^o, gauche minus (g-), -1^o-(-119^o) and trans (t), 121^o-$(-120)^o$. For the global minimum the dihedral angles are: $\theta_1 = 177.7$, $\theta_3 = -176.0$, $\theta_2 = -63.2$, $\theta_4 = 64.0$, $\alpha_1 = -78.0$, $\beta_1 = -142.9$, $\gamma_1 = -179.0$, $\alpha_2 = 176.6$, $\beta_2 = 179.7$, $\gamma_2 = -180.0$

	Energy	θ_1	θ_3	θ_2	θ_4	α_1	β_1	γ_1	α_2	β_2	γ_2	Boltz. %
1	15.87	t	t	g-	g+	g-	t	t	t	t	t	6.15
2	16.05	t	t	g-	g+	g-	t	g-	t	t	t	4.51
3	16.13	t	t	g-	g+	t	t	t	t	t	t	3.96
4	16.18	t	g+	g-	g-	g-	t	t	t	t	t	3.63
5	16.21	t	t	g-	g+	g+	t	t	t	t	t	3.43
6	16.23	g-	t	g+	g+	g-	t	t	t	t	t	3.31
7	16.33	t	g+	g-	g-	t	t	t	t	t	t	2.81
8	16.35	t	g+	g-	g-	g+	t	t	t	t	t	2.71
9	16.36	t	t	g-	g+	g+	g-	t	t	t	t	2.69
10	16.39	g-	t	g+	g+	t	t	t	t	t	t	2.54

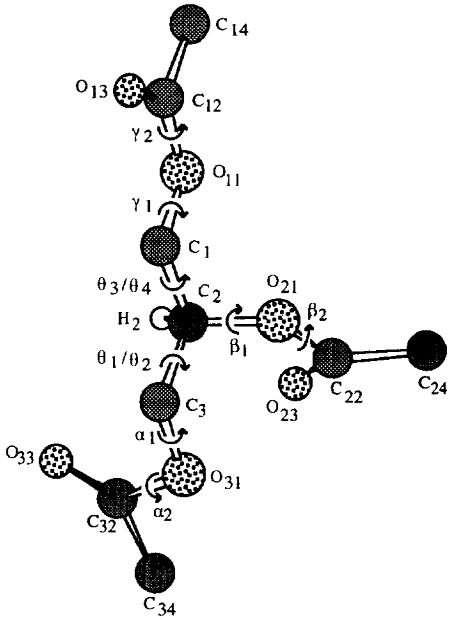

Fig. 1. The lowest triacetin conformer. Except for the mid-carbon hydrogen, the others are omitted for the sake of clarity.

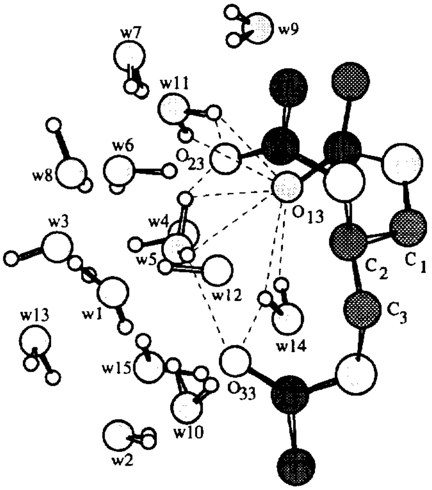

Fig. 2. The lowest triacetin-water cluster. Hydrogen bonds of the three most important waters are shown in dotted lines.

RESULTS AND DISCUSSION

The triglycerides in vacuo like other flexible structures, are molecules whose properties are not primarily dependent upon its global minimum energy conformer. Within 1 kcal there are 30 rotamers corresponding to a 67% Boltzmann distribution at room temperature. The probability of finding any of the triacetin conformers using the formula of Saunders[14] is found to be equal to 0.997. Fig. 1 depicts the lowest energy conformer using stereospecific numbering.[22] Table 1 describes the ten energetically lowest conformers with the usual trans-gauche notation for their angles, steric energies and Boltzmann distributions.

Among the low-lying conformers in Table 1, there is a significant number of close contacts between the carbonyl oxygens and the triglyceride backbone hydrogens. The global minimum forms two loose five-membered intramolecular O···H closures; O_{23} is within 2.40Å from H_2, and O_{33} is within 2.51 Å from a hydrogen of C_3. The ab initio 4-31G* geometry optimization study shortened these non bonded interactions to be 2.35 and 2.41 Å correspondingly. Within 3 kcal there are altogether 198 five-membered rings out of 109 conformers, hence on average 1.8 five-membered rings for every low-lying conformer. The six-membered ring-like configurations are much less frequent, 0.5 ring per conformer. The β branch shows a higher tendency to participate in such a ring-like close contact.

In a recent study[23] of eleven conformers of 1, 2, 3-propanetriol (glycerol) optimized at different parts of the conformational space using HF/4-21G, HF/6-311G** and second order Moller-Plesset MP2/6-311G**, all three calculations were able to predict differences between internal coordinates to within typical experimental inaccuracies. The ab initio optimized propane-skeletal dihedral angles $[\theta_1, \theta_3, \theta_2, \theta_4]$ of one of the conformers of glycerol produced the set of values [-171.0, 168.9, -52.8, 52.7] which is closely related to our 4-31G* optimized triacetin's set of values (Table 2). Hence it is safe to suggest that the substitution of the three alcoholic hydrogens with the $(CH_3)C{=}O$ group maintains the gross features of the parental three-dimensional structure. The previously mentioned glycerol conformers were input[23] to another molecular mechanics program CHARMm[24], re-optimized, and as a consequence the relative energies of the conformers changed. The lowest energy glycerol conformer as predicted by the CHARMm force field, has been identified to have the generic configuration of the best triacetin rotamer. The molecular mechanics force field tended to favor those glycerol conformers with trans O-C-C-C torsions ($\pm 170^{\circ}$) whereas the MP2 ab initio optimizations had favored gauche angles (±70). A comparison of these HF and MP2 calculations in glycerol shows that $C(sp^3)$-$C(sp^3)$ bonds match within 0.004Å, $C(sp^3)$-$O(sp^3)$ bonds are 0.016-0.020 Å longer at the MP2 level, and the angles of the glycerol headgroup (C_1-C_2-C_3, C_1-C_2-O_{21}, O_{11}-C_1-C_2, C_3-C_2-O_{21}) are smaller by $\approx 1^{\circ}$ at the HF level. These values provide estimates of the expected modifications (as compared to our HF results in table 2) in skeletal bond lengths and angles of triacetin at the MP2 level.

The reported experimental data[25] for the enthalpy of formation for 1, 2, 3-propanetriol triacetate is $\Delta_f H^{o}(g) = -298.46 \pm 1.02$ kcal/mol; the calculated value using the 109 lowest conformers that are present at $25^{o}C$ is -285.72 kcal/mol.

Table 3 describes the low energy conformers of the hydrated triacetin, along with the corresponding Boltzmann distributions. It can been seen that while the triacetin in vacuo has 10 conformers within 0.5 kcal with a total 35.7% Boltzmann distributions, a different picture is emerging for the hydrated triacetin. The global minimum (fig. 2) is dominant at room temperature with an 86% Boltzmann distribution, while the

Table 2. 4-31G* optimized geometry for the MM2 global minimum energy conformer of triacetin.

Bonds		Angles		Dihedral angles	
C_{14}- C_{12}	= 1.500	C_{14}-C_{12}-O_{11}	= 111.5	$\theta_1 = C_1$- C_2- C_3- O_{31}	= -179.7
C_{12}- O_{13}	= 1.185	C_{12}-O_{11}-C_1	= 116.8	$\theta_3 = C_3$- C_2- C_1- O_{11}	= -174.9
C_{12}- O_{11}	= 1.328	C_1- C_2- C_3	= 110.6	$\theta_2 = O_{21}$- C_2- C_3- O_{31}	= -62.0
O_{11}- C_1	= 1.413	C_2- C_3-O_{31}	= 111.3	$\theta_4 = O_{11}$- C_1- C_2- O_{21}	= 66.5
C_1- C_2	= 1.511	C_3 - O_{31}- C_{32}	= 117.5	$\alpha_1 = C_2$-C_3- O_{31}- C_{32}	= -80.4
C_2-C_3	= 1.519	O_{31}- C_{32}- C_{34}	= 111.3	$\beta_1 = C_1$- C_2- O_{21}- C_{22}	= -144.5
C_2-H_2	= 1.076	O_{31}- C_{32}- O_{33}	= 123.0	$\gamma_1 = C_2$-C_1-O_{11}- C_{12}	= 179.5
C_3- O_{31}	= 1.411	C_1- C_2- O_{21}	= 107.0	$\alpha_2 = C_3$-O_{31}-C_{32} - C_{34}	= 177.5
O_{31}- C_{32}	= 1.333	C_2- O_{21}- C_{22}	= 119.6	$\beta_2 = C_2$-O_{21}-C_{22}- C_{24}	= 179.4
C_{32}- C_{34}	= 1.519	O_{21}- C_{22}- C_{24}	= 110.8	$\gamma_2 = C_1$-O_{11}-C_{12}- C_{14}	= 179.6
C_{32}- O_{33}	= 1.185	O_{11} -C_1- C_2	= 107.7		
C_2- O_{21}	= 1.421	C_3 - C_2- O_{21}	= 109.0		
O_{21}- C_{22}	= 1.328				
C_{22}- C_{24}	= 1.502				
C_{22}- O_{23}	= 1.185				

Table 3. MM2 energies (kcal/mol), dihedral angles and Boltzmann distributions for the ten lowest in energy conformers of the triacetin-water cluster. For the global minimum the dihedral angles are: $\theta_1 = 68.0$, $\theta_3 = 176.2$, $\theta_2 = -170.3$, $\theta_4 = 55.0$, $\alpha_1 = 67.9$, $\beta_1 = -123.3$, $\gamma_1 = 57.0$, $\alpha_2 = 176.0$, $\beta_2 = -176.7$, $\gamma_2 = 172.2$. This is a ZZZ configuration since α_2, β_2 and γ_2 are ≈ 180°. The 5th and 8th conformers are both E at the γ branch ($\gamma_2 \approx 0°$).

	Energy	θ_1	θ_3	θ_2	θ_4	α_1	β_1	γ_1	α_2	β_2	γ_2	Boltz. %
1	-203.22	g+	t	t	g+	g+	t	g+	t	t	t	86.00
2	-201.64	g+	t	t	g+	g+	t	g+	t	t	t	5.98
3	-201.12	g+	t	t	g+	g+	t	g+	t	t	t	2.46
4	-201.09	g+	t	t	g+	g+	t	g+	t	t	t	2.34
5	-200.74	g+	g+	t	g-	g-	g+	t	t	t	g+	1.31
6	-200.39	g+	t	t	g+	g+	t	g+	t	t	t	0.72
7	-200.17	g+	t	t	g+	g+	t	g+	t	t	t	0.49
8	-199.78	g+	g+	t	g-	t	g+	t	t	t	g+	0.26
9	-199.64	g+	t	t	g+	g+	t	g+	t	t	t	0.20
10	-199.52	g+	t	t	g+	g+	g-	g+	t	t	t	0.16

global minimum triacetin in vacuo corresponds to only 6.15%. The energy range of the in vacuo conformers is densely populated while the hydrated clusters are more sparsely distributed.

The lowest solvated configuration of triacetin forms a system of three rings with the C_2-H_2 bond in common (fig. 3). One is a five-membered ring, whereas the other two are six-membered rings. The distances between H_2 and O_{13}, O_{23}, O_{33} are found to be 2.28, 2.32 and 2.37 Å correspondingly. Hence the hydrated global conformer has a shorter intramolecular hydrogen bonding than the global conformer in vacuo. The six-membered rings are of the half chair form due to the near planarity of the C-O-C=O part while the five-membered ring is nearly planar. It could be that the formation of these rings is a characteristic of the global energy minimum. Note that the system of the three rings is present for the2nd, 3rd and 4th lowest conformer.

Table 4 gives the energy decomposition of the ten lowest hydrated triglycerides. The lowest ten structures, after stripping the water molecules and minimizing the energy, end up to three different minima, as shown by E_{tm}. Averages of the number of water-hydrogens present at a distance less than 2.3 Å from the oxygens of the triglyceride were calculated taking into account all 390 lowest in energy clusters. Structures that end up to the 16.98 and 18.04 energy minima exhibit 6.4 and 6.7 water-hydrogens per carbonyl oxygen, and no appreciable number of water-hydrogens per carboxyl oxygen. However the structures that fall in the 23.51 energy minimum exhibit 7.2 water-hydrogens per carbonyl oxygen and 0.86 water-hydrogens per carboxyl oxygen, which can be interpreted that triacetin's E-configured ester oxygen is approachable from the water-solvent. A recent work[26] on the aqueous solvation of the triglyceride 1, 2, 3-propanetriol tridodecanoate (trilaurin) using molecular dynamics found five nearest neighbors water oxygens around the carbonyl oxygens O_{13} and O_{33}. Four nearest neighbors water oxygens were counted around O_{23} in the middle chain. The "tuning fork"-like solid state structure (two of the hydrophobic chains lying in parallel planes and the third in an antiparallel direction) was found to undergo a slow drift in solution toward a more compact geometry, but it was not possible to simulate the system long enough to reach an equilibrium geometry. The water solvation of the triacetin-carbonyl groups is comparable to trilaurin's solvation as it is deduced from the above cited pair distribution function values. The energetic order of the in vacuo rotamers is changed drastically by hydration. For example, the global hydrated conformer contains a triacetin molecule with a geometry corresponding to a gas phase rotamer 1.1 kcal above the global minimum.

It can be seen that the solvent-solute interaction (table 4) is energetically favoring E-configured ester structures. For instance the 5th and 8th conformers are both E for the γ branch, and they appear to have very efficient solvation (lowest E_{tw} value), which is balanced by the high steric energy of the solute. An inspection of the four lowest conformers shows that the water molecules are placed in such a way as to build a hydrogen bond network inside the triacetin.

Table 4. Energy components (in kcal/mol) of the triacetin-water cluster: $E = E_t + E_w + E_{tw}$. The MM2 steric energy for one water molecule is $E_{w1} = 0.2$ kcal/mol and hence the water-water interaction is calculated as: $E_{ww} = E_w - 15E_{wl}$

	cluster energy (E_0-E_i)	triacetin only (E_t)	triacetin minimized (E_{tm})	water only (E_w)	triacetin-water interaction (E_{tw})	water-water interaction (E_{ww})
1	0.0	22.26	16.98	-103.25	-122.24	-106.25
2	1.58	25.83	18.04	-106.89	-120.59	-109.90
3	2.10	24.56	18.04	-110.92	-114.76	-113.92
4	2.13	20.55	18.04	-116.97	-104.67	-119.97
5	2.48	32.11	23.51	-98.78	-134.07	-101.79
6	2.83	23.18	18.04	-107.86	-115.71	-110.87
7	3.06	24.72	18.04	-95.38	-129.51	-98.39
8	3.44	29.68	23.51	-98.19	-131.27	-101.20
9	3.58	23.05	18.04	-114.38	-108.31	-117.38
10	3.70	19.70	16.98	-119.96	-99.26	-122.96

Taking into account the conformation as depicted in fig. 2, we observe that the water molecules aggregate in the inner region around the carbonyl oxygens. A similar case[27] is observed for the $C_6H_6 \cdot 2\ H_2O$ cluster in which both molecules reside on the same side of the ring in a slightly modified water dimer geometry. In some sense, the cluster has taken the first step along the way towards immiscibility.

In the case of the global conformer, there is one water-hydrogen tightly bound (≈ 2.1 Å) to each carbonyl oxygen. Only three water molecules are not involved in direct hydrogen bonding to the triacetin. According to ref. 28, more than two water molecules actually solvate the formaldehyde molecule and the solvation above and below the molecular plane in the region of the carbonyl π-bond appears inevitable. An interpretation of the thermochemistry of dilute solutions of ethyl acetate in water is based on the complex formation of the type $C_4H_8O_2 \cdot 2\ H_2O$ and $C_4H_8O_2 \cdot 4\ H_2O$.[29] The later is in agreement with the four water molecules per carbonyl oxygen found in the global conformation of hydrated triacetin. From NMR experiments of glycerol trivalerate in $CDCl_3$ solution coupled with classical potential function[30] calculations, it is concluded[31] that two equally probable conformations exist (θ_1, θ_3) = (g+, g+) and (g-, t) while lysolecithin (which has only one hydrocarbon chain) also exhibits the same two conformations in aqueous dispersions. Kosugi et al.[32] from a combination of NMR studies on triacetin in $CDCl_3$ solution and MM2 in vacuo calculations reach the conclusion that in addition to (g+, g+) and (g-, t) another conformation is also of importance (θ_1, θ_3) = (t, t). Hence it appears that $CDCl_3$ acts as an intermediate between the gas phase and the water solvent, by picking triacetin conformations from both environments (table 1 and table 3). Low-lying conformers containing the (g+, g-) type of segment that is known[33] to deform, are present only in the triacetin-water study involving the dihedrals (θ_1, α_1) and (γ_1, θ_3). These sequences contain an sp^3 oxygen and the water environment compensates for the higher torsional strain of the triglyceride with more favorable electrostatic energy.

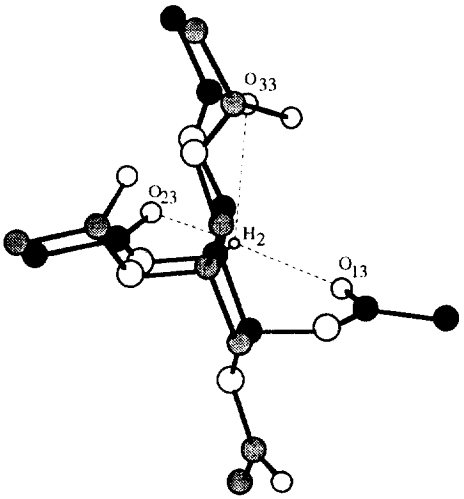

Fig. 3. Superposition of the lowest triacetin conformer and lowest hydrated triacetin (in black). The dotted lines mark the 5 and 6 membered ring-like closures in the triacetin-water cluster.

There are on the average 3.6 water-water contacts per water molecule (hydrogen bonding) for the triacetin · $15H_2O$ complex. The global minimum triacetin-water cluster shows one water molecule (w_5) that succeeds to hydrogen-bond (see fig. 2) all three carbonyl oxygens with four hydrogen bonds; two water molecules (w_{11} and w_{14}) achieve three hydrogen bonds with two of the carbonyl oxygens. It is interesting that these three water molecules stay in position, supporting the triglyceride's structure even after removing the rest twelve water molecules and minimizing the energy. An ab initio 4-31G geometry optimization performed on this three water-triacetin complex confirmed the validity of the water arrangement around triacetin. It follows that these three water molecules are tightly bound to the triacetin molecule, though overall there are twelve water molecules in direct involvement to create the first solvation sphere.

CONCLUSIONS

1) Whithin the MM2 framework triacetin is a highly flexible molecule in gas phase and by extrapolation to non-polar solvents. There are 109 conformers at room temperature; 30 of them with over 1% occurrence. Among the low-lying conformers there is a significant number of ring-like close contacts between the carbonyl oxygens and the triglyceride backbone hydrogens. The 1, 5 O···H non-bonded close contacts are more frequent than the 1, 6 O···H ones between the carbonyl oxygens and the propane-skeleton hydrogens.

2) The water presence around triacetin produces a quite rigid structure since the lowest conformer has a Boltzmann distribution of 86%; the intramolecular (carbonyl oxygen - H_2) close contacts are shorter and there is a simultaneous formation of three intramolecular ring-like arrangements, two 1, 6 O···H and one 1, 5 O···H. Three water molecules are tightly bound to the carbonyl oxygens and by building a hydrogen bond network inside triacetin produce a compact overall structure. Hydration restricts triacetin's conformational flexibility which is an unusual situation for small polar molecules. The stability of the lowest in vacuo and the trihydrated triacetin has been validated by use of ab initio optimizations.

3) The method employed in this work is appropriate for searching the conformational space accessible by a flexible molecule in a particular solvent. Its applicability is demonstrated on the triacetin-water system at room temperature. It is especially useful in circumstances requiring the restriction of the available space for the solvent, in order to study the first solvation sphere. The method seems to be capable of treating systems of mixed solvents, a subject that is currently under investigation.

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