A full conformational space analysis of bilirubin

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ABSTRACT

Ab initio methods were utilized in a gas-phase systematic conformational search of bilirubin conformers. The whole molecule was divided into four fragments. Most stable conformers of them were employed to build 196 conformers of the complete bilirubin molecule. Initial geometries were optimized using HF/3-21G level of theory and the minimum energy conformers were then reoptimized at B3LYP/6-31G(d) level. Ridge-tile conformer was the most stable one, in perfect agreement with X-ray data. We found that while tetrapyrrole backbone shows some flexibility, propionic acid side chains have a greater influence in bilirubin conformation because they can interact through different hydrogen bond patterns with the backbone and between them.

1. Introduction

Bilirubin (isomer 4Z, 15Z bilirubin IXα, Fig. 1), the yellow-or-ange cytotoxic pigment of jaundice, is a linear tetrapyrrole that is formed during the process of heme degradation [1]. In plasma it binds to albumin [2] due to its very low water solubility. Although bilirubin contains hydrophilic functional groups, the crystal structure showed that the molecule adopt a "ridge-tile" (RT) conformation in which the two propionic acid groups make three hydrogen bonds with the opposite dipyrrinone ring system so they are unavailable for interaction with water [3]. This ridge-tile conformation occurs in solution too, although the bilirubin molecule can flip between two enantiomeric conformers (P or M) both of which maintain the internal hydrogen bond network [4]. Between these conformations, various intermediate structures exist due to the conformational flexibility of the tetrapyrrole backbone [5] and propionic acid side chains.

A number of drugs as well as their metabolites can compete with bilirubin for binding to albumin and disrupt the equilibrium between bilirubin and albumin [6–8], increasing the bilirubin free concentration which may cause irreversible brain damage [9]. One of the neurotoxicity mechanisms proposed is the change of membranes structure and properties where bilirubin may aggregate and precipitate. However, studies of Stocker et al. [10,11] introduced the concept that bilirubin has a beneficial role at low, "physiological" plasma concentrations by acting as a potent antioxidant that scavenges peroxyl radicals as efficiently as α-tocopherol. Indeed, bilirubin, and also biliverdin, contain an extended system of conjugated double bonds [12] and a reactive hydrogen atom and thus could possess antioxidant properties, acting as a powerful biological chain-breaking antioxidant.

Due to the above, its conformational preferences have a noticeable medical and biological interest. In the present study, we report a systematic conformational search of most stable gas-phase bilirubin's conformers giving special attention to propionic acid side chains.

2. Computational details

Fig. 1 shows bilirubin molecule and dihedral definitions employed in the text. Due to the fact that bilirubin is a large compound with more than 10 rotatable bonds, it was studied through a conformational analysis of four of its main fragments as depicted in Fig. 2. **P1** is propionic acid on ring D and **P2** propionic acid on ring C. Conformers' notation type will have the following form: $\theta_3 \langle \theta_1 \ \theta_2 \rangle \ \theta_4 \ [\phi_1(\phi_2 \ \phi_3)] \ \{\phi'_1(\phi'_2 \ \phi'_3)\}$ where the first four dihedrals correspond to the tetrapyrrole backbone; the term between square brackets, **P1** dihedrals; and the term

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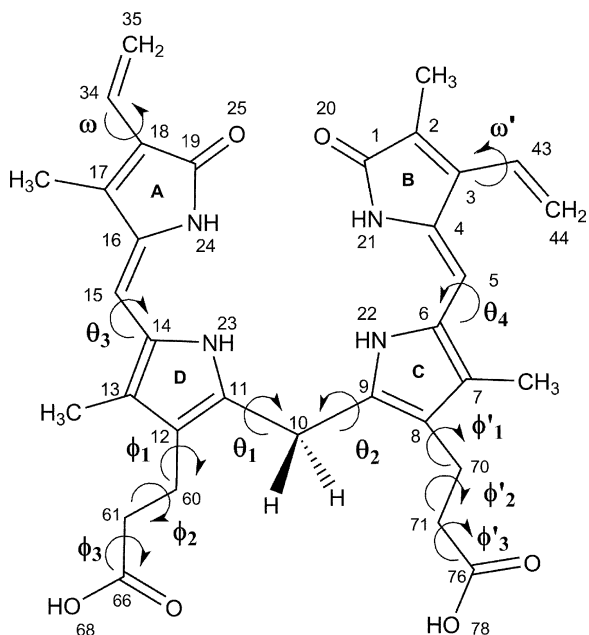
Fig. 1. Full bilirubin molecule dihedral angles definitions: $\omega = (19,18,34,35)$, $\omega' = (2,3,43,44)$, $\theta_1 = (23,11,10,9)$, $\theta_2 = (11,10,9,22)$, $\theta_3 = (16,15,14,23)$, $\theta_4 = (4,5,6,22)$, $\phi_1 = (11,12,60,61)$, $\phi_2 = (12,60,61,66)$, $\phi_3 = (60,61,66,68)$, $\phi_1' = (9,8,70,71)$, $\phi_2' = (8,70,71,76)$ and $\phi_3' = (70,71,76,78)$.

between braces, **P2** dihedrals. A short notation that consists on Roman numbers referring propionic acid conformers will also be employed.

Relaxed potential energy curves and surfaces were performed at HF/3-21G [13] level of theory. Geometry optimizations were carried out at HF/3-21G level and the minimum energy conformers were then optimized at B3LYP/6-31G(d) [14–17] level. All calculations were carried out in the gas-phase using Gaussian 03 [18] program.
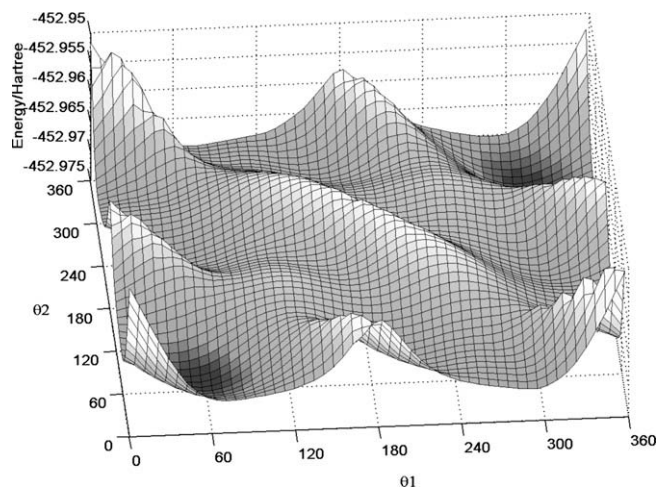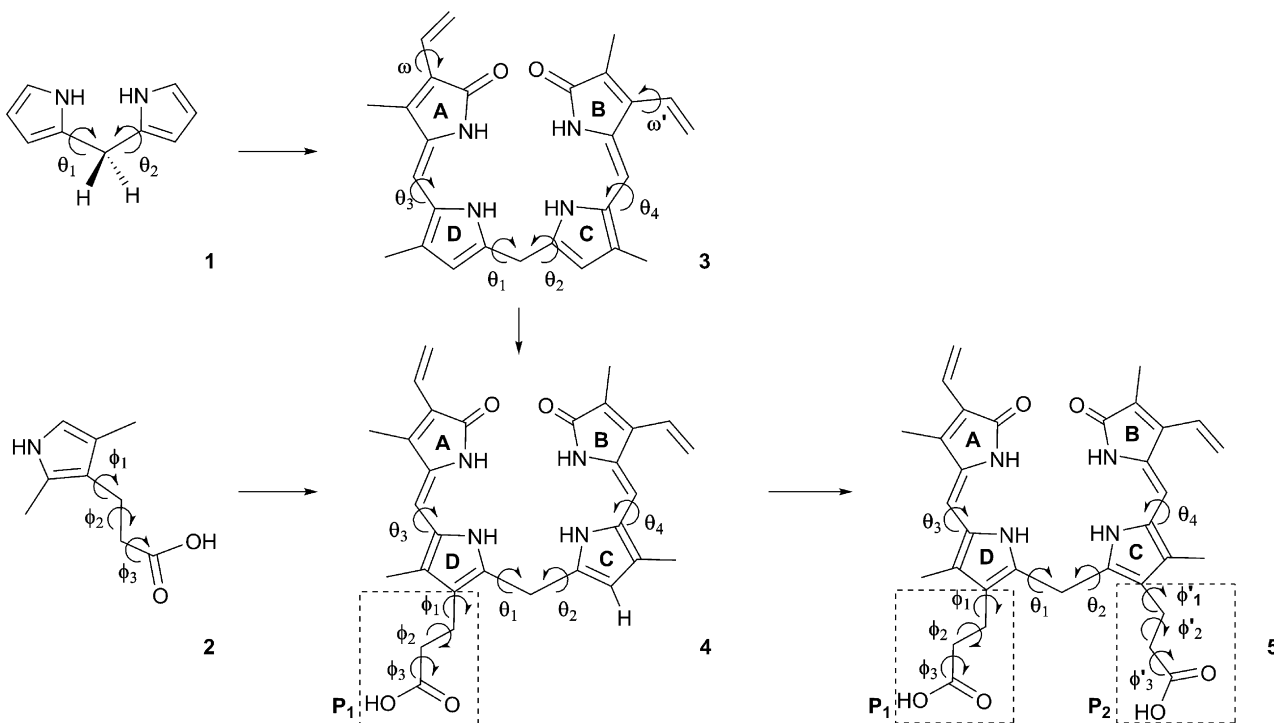

Fig. 3. PES of structure **1** computed at HF/3-21G level of theory.

3. Results and discussion

Bilirubin is a large and very flexible (more than 10 rotatable single bonds) molecule which conformational analyze is a difficult task. Previous theoretical studies on bilirubin conformation have employed graphical interactive model building [19] or a molecular mechanics screening [20]. Our approach involved the construction of several bilirubin conformers through the study of four fragments of it.

3.1. Fragment 1

Fig. 3 shows the PES, $E = f(\theta_1, \theta_2)$, of fragment **1**. The symmetry of this molecule results in a symmetric shape of the surface. The optimized stable geometries are shown with their characteristic θ_1 and θ_2 dihedral angles in Table 1. Minimum energy conformers (**g+g+** and **g−g−**) show θ_1 and θ_2 values next to the final angles

Fig. 2. Sequence of the study of the different fragments (labelled with Arabic numbers) of bilirubin.

Table 1
Relative stability (kcal mol^{-1}) of minimum energy conformers of structure **1** calculated at HF/3-21G level found in PES. Reference value for conformers **g+g+** and **g−g−**: $E_{SCF} = -452.973072$ E_h.
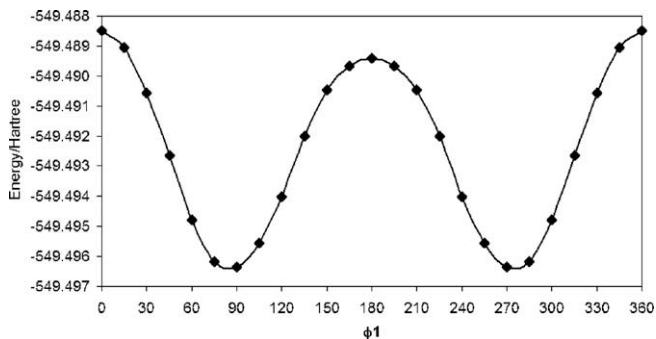
Conformer	θ_1	θ_2	ΔE_{SCF}
g+g+	53.08	52.93	0.00
g+a	48.18	−155.95	2.38
ag−	156.01	−48.29	2.38
ag+	−155.95	48.18	2.38
g−g−	−53.08	−52.93	0.00
g−a	−48.29	156.01	2.38

Table 2
Relative stability (kcal mol^{-1}) of minimum energy conformers of structure **2** found in PESs of both ϕ_1 values. Reference values for conformers **VII** and **II′**: $E_{SCF} = -549.4985999$ E_h.

Conformer	[ϕ_1	(ϕ_2	ϕ_3)]	ΔE_{SCF}
$\phi_1 = 90$				
I	[g+(g+g+)]	94.62	75.22	60.75	3.49
II	[g+(g+a)]	72.63	73.46	−158.07	1.33
III	[g+(aa)]	93.82	179.79	−178.45	1.35
IV	[g+(ag+)]	94.29	179.02	53.11	2.19
V	[g+(ag−)]	90.86	179.60	−53.74	2.28
VI	[g+(g−g−)]	102.43	−65.49	−54.13	1.20
VII	[g+(g−a)]	104.03	−77.13	162.60	0.00
$\phi_1 = -90$				
I′	{g−(g+g+)}	−102.44	65.44	54.14	1.20
II′	{g−(g+a)}	−104.03	77.18	−162.49	0.00
III′	{g−(aa)}	−93.84	−179.77	178.48	1.35
IV′	{g−(ag+)}	−90.86	−179.58	53.78	2.28
V′	{g−(ag−)}	−94.20	−179.00	−53.08	2.19
VI′	{g−(g−g−)}	−71.15	−59.61	−56.52	1.95
VII′	{g−(g−a)}	−109.72	−112.92	178.23	1.33

Fig. 4. Potential energy curve of dihedral angle ϕ_1 of fragment 2 computed at HF/3-21G level of theory.
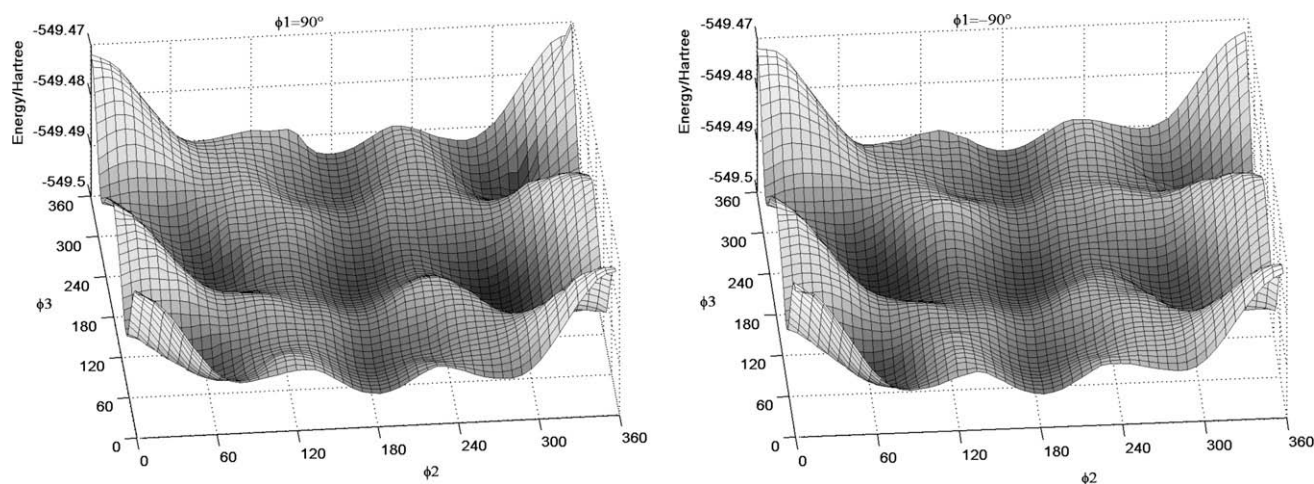
values in bilirubin most stable conformer. Conformers **g+g+**, **g+a** and **ag−** were selected to continue the analysis.
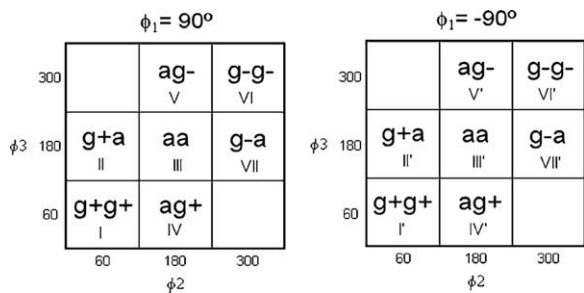
3.2. Fragment 2

Propionic acid substituents were studied in compound **2**. The PEC, of the type $E = f(\phi_1)$ reveals two minimum values for dihedral angles ϕ_1, 90° and −90° (Fig. 4). Fixing ϕ_1 value, two PESs, of the type $E = f(\phi_2, \phi_3)$, were performed and shown in Fig. 5. Seven minima were obtained for each surface and the values of the conformer's dihedrals and energies are collected in Table 2.

3.3. Fragment 3

Structure **3** was build from the most stable selected conformers of structure **1** adding the uppermost pyrrole rings (A and B) in anti

Table 3
Relative stability (kcal mol^{-1}) of minimum energy conformers of structure **3** (tetrapyrrole backbone). Data was computed at HF/3-21G and B3LYP/6-31G(d) levels with the latter in bold. Reference values for conformer **g−⟨g+g+⟩g+** at both levels of calculation: $E_{SCF, HF/3-21G} = -1398.4063$ E_h and $E_{SCF, B3LYP/6-31G(d)} = -1415.17558$ E_h.

| Conformer	θ_3	⟨θ_1	θ_2⟩	θ_4	ΔE_{SCF}
a⟨g+a⟩a	130.41	48.58	−156.29	−129.97	7.72
	145.56	**39.49**	**−148.43**	**−144.86**	**6.04**
a⟨g+a⟩g+	130.56	51.57	−158.78	29.89	6.97
	145.82	**45.36**	**−151.87**	**17.14**	**3.87**
g−⟨g+a⟩g+	−30.78	32.75	−135.67	28.25	4.51
	−16.98	**16.98**	**−122.80**	**16.77**	**0.75**
g+⟨g+a⟩g+	29.83	52.75	−158.81	28.87	6.15
	16.35	**47.37**	**−150.97**	**16.53**	**1.41**
a⟨ag−⟩a	130.15	156.66	−48.86	−129.41	8.81
	145.65	**150.14**	**−43.64**	**−143.42**	**7.47**
a⟨g+g−⟩g+	128.02	95.53	−4.02	24.75	5.71
	143.63	**95.05**	**15.25**	**13.56**	**3.28**
g−⟨ag−⟩a	−29.52	159.21	−52.28	−129.6	7.84
	−16.26	**152.65**	**−46.48**	**−143.18**	**5.07**
g−⟨ag−⟩g+	−27.68	135.36	−33.59	31.21	4.46
	−16.55	**128.59**	**−22.51**	**18.14**	**0.66**
a⟨g+g+⟩a	130.26	54.06	53.92	−128.51	5.71
	145.85	**52.54**	**56.01**	**−143.90**	**4.63**
a⟨g+g+⟩g+	130.14	54.52	55.2	30.59	4.83
	145.57	**52.00**	**57.71**	**17.88**	**2.54**
g−⟨g+g+⟩a	−30.41	50.11	55.85	127.78	4.96
	−17.08	**51.15**	**54.90**	**142.78**	**2.80**
g−⟨g+g+⟩g+	−30.57	25.28	58.3	39.02	0.00
	−16.87	**50.60**	**61.24**	**17.88**	**0.00**

Fig. 5. PES calculated at HF/3-21G level of dihedral ϕ_2 and ϕ_3 of fragment 2 fixing ϕ_1 value to either 90° or −90°.

Fig. 6. Map of stable conformers of structure **4** calculated at HF/3-21G level of theory. Roman numbers refers to propionic acid side chain conformation of structure **2**.

(~180°) or gauche (~0°) conformation, optimized at HF/3-21G level, and reoptimized at B3LYP/6-31 g(d) level of theory. Note from Table 3 that: (i) the conformation with the minimum electronic energy (the ground state) is the same at the two levels; (ii) the relative order of energies is different in the two cases; (iii) the difference between the ground state energy and the "next lowest energy" is smaller in the B3LYP case than in the HF case.

The vinyl group on ring A is planar (dihedral $\omega = 0°$) stabilized by a hydrogen bond with the oxygen atom in the same pyrrole ring (data not shown). Dihedral ω' (vinyl group on ring B) study showed that this group is tilted. There are two possible positions separated by a low energy barrier (data not shown). This agrees with previous data obtained from X-ray [21] and semiempirical calculations [22].
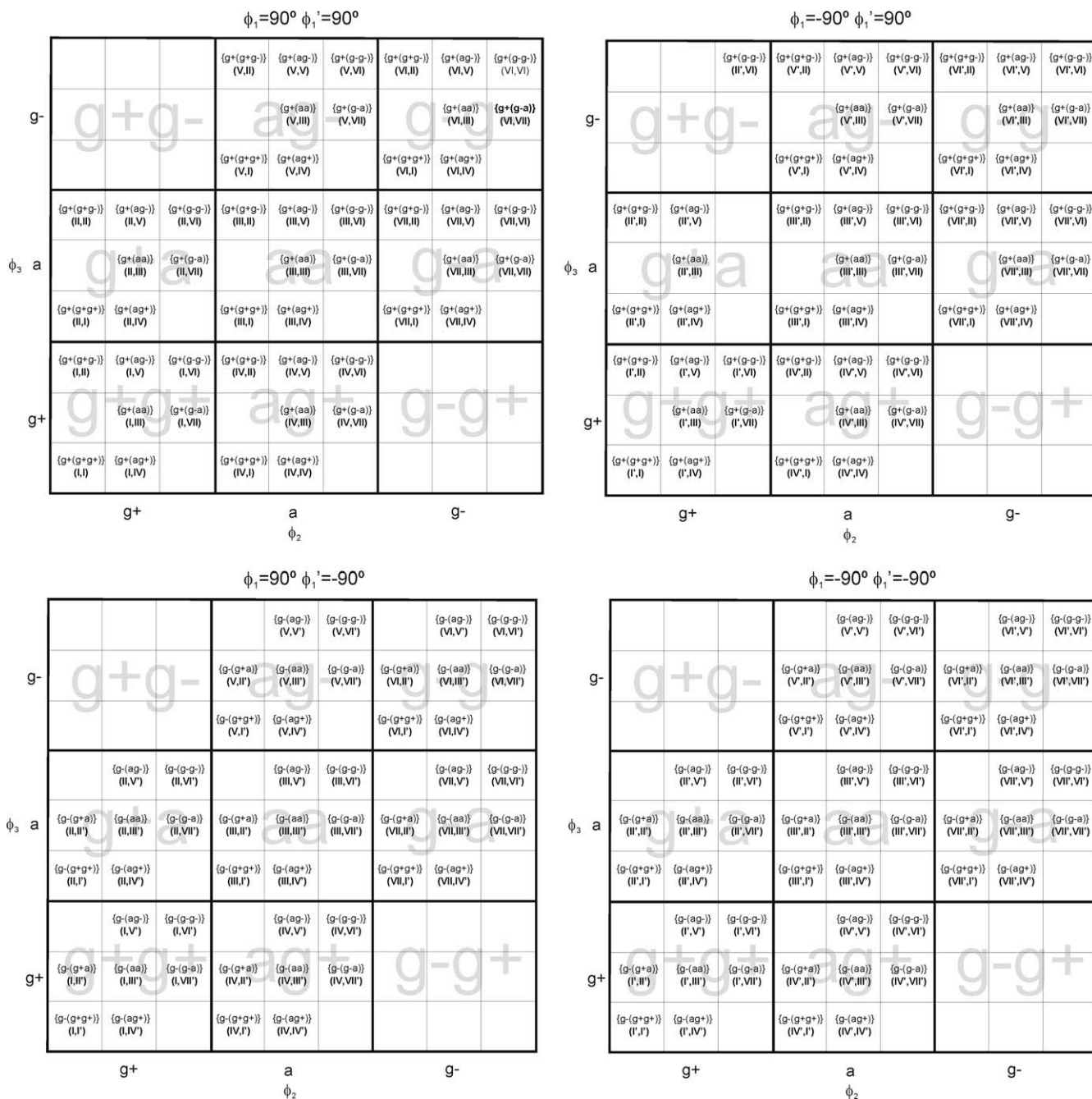
Fig. 7. Maps of stable conformers of bilirubin (structure **5**) computed at HF/3-21G level.

Table 4
Relative stability (kcal mol^{-1}) of 15 selected minimum energy conformers of bilirubin (structure **5**). Calculations were performed at B3LYP/6-31G(d) level. Reference values for conformers (**VII, I**), (**VII, VI**) and (**VII, VII**): E_{SCF} = −1949,53177 E_h.

| Conformer		θ_3	$\langle\theta_1$	$\theta_2\rangle$	θ_4	$[\phi_1$	$(\phi_2$	$\phi_3)]$	$\{\phi'_1$	$(\phi'_2$	$\phi'_3)\}$	ΔE_{SCF}
VII	**I**	3.77	60.92	61.21	4.25	117.56	−68.80	168.25	118.00	−68.30	167.47	0.00
VII	**II**	−13.47	58.65	53.94	7.94	119.17	−65.66	165.49	72.30	57.04	−122.08	11.94
VII	**VI**	3.79	60.93	61.23	4.25	117.56	−68.80	168.23	117.98	−68.35	167.52	0.00
VII	**VII**	3.77	60.92	61.23	4.25	117.54	−68.81	168.26	118.00	−68.33	167.49	0.00
I′	**VII**	−14.30	8.14	64.05	23.77	−104.10	50.05	73.58	86.80	−84.33	129.04	25.94
II′	**VI**	−13.79	8.09	61.91	24.50	−103.50	51.44	−99.56	85.94	−80.15	−52.23	27.36
VII	**I′**	−17.23	57.72	61.80	3.28	116.35	−68.69	168.51	−104.93	62.54	50.35	20.57
VII	**II′**	−17.51	57.37	61.88	2.58	116.43	−67.27	166.71	−101.83	71.84	−156.26	18.86
VII	**III′**	−18.38	57.70	59.14	4.90	116.34	−67.74	167.71	−100.98	176.39	170.93	19.90
VII	**IV′**	−17.45	57.84	59.84	4.62	116.42	−67.73	167.58	−97.48	179.20	72.05	20.77
VII	**V′**	−17.61	57.70	59.90	4.55	116.26	−67.81	167.67	−99.11	−179.45	−62.51	20.80
VII	**VI′**	−19.57	49.70	66.34	−2.64	116.56	−64.79	167.19	−72.31	−60.50	−56.87	21.87
VII	**VII′**	−17.22	58.56	57.90	5.15	116.87	−67.64	166.90	−71.96	−70.27	151.96	20.28
I′	**II′**	−9.76	−16.34	87.14	24.53	−93.70	61.46	69.48	−102.50	68.70	−134.33	33.41
II′	**I′**	−13.12	−3.82	80.99	23.98	−91.91	69.68	−104.18	−84.39	71.60	74.98	33.85

3.4. Fragment 4

The minimum energy conformer of fragment **3** was substituted on ring D with the 14 conformations obtained previously for propionic acid resulting in fourteen structures (Fig. 6) which optimized parameters are collected in Table S1 of supplementary material. Although dihedral ϕ_1 and ϕ_2 values are different from those obtained in fragment **2**, they belong to the same space region as can be seen comparing Figs. 5 and 6. The conformation with the minimum energy (conformer **VII** or **g−⟨g+g+⟩g+[g+(g−a)]**) is stabilized by three intramolecular hydrogen bonds. Conformers **VI** and **I** have two and one hydrogen bonds respectively which are reflected in their lower energy values than the rest of the conformers optimized.

3.5. Bilirubin (structure **5**)

Finally, 196 (14 × 7 × 2) bilirubin conformers were built from the fourteen fragment **4** conformers substituting on ring C each propionic acid conformation (7) for both ϕ_1 values (2). Dihedral angle values and energy values are listed in Table S2–S5. Fig. 7

shows maps of stables conformers founded. Big squares consist on **P1** conformations and little squares show **P2** conformations. Almost all the conformers maintain **P1** conformations so they appear in the same region of the map founded for structure **4**. Only one conformer, the (**II′, VI**) mismatch. **P2** follow the same trend except for propionic acid conformer **II** on uppermost maps. Note that there is one conformer (**II′, VII**) missing because ϕ_1 value has an anti conformation.

We choose a cutoff value of 5 kcal/mol as a criterion to select those conformers of bilirubin (structure **5**) for further calculations at the B3LYP/6-31g(d) level of theory. Fifteen conformers were selected which dihedrals values are listed in Table 4. Three of them: (**VII, I**), (**VII, VI**) and (**VII, VII**) adopted the same minimum energy conformation, the ridge-tile conformation, stabilized by six intramolecular hydrogen bonds (Fig. 8, left). Conformer (**VII, II**) adopted a distorted ridge-tile conformation because of formation of a different hydrogen bond pattern between **P2** and the opposite dipyrrinone (Fig. 8, right). Conformers (**I′, VII**), (**II′, VI**), (**I′, II′**) and (**II′, I′**) are very particular due to the hydrogen bonds which are established between both propionic acid side chains **P1** and **P2** (Fig. 9). The rest of the conformers adopt a half-folded
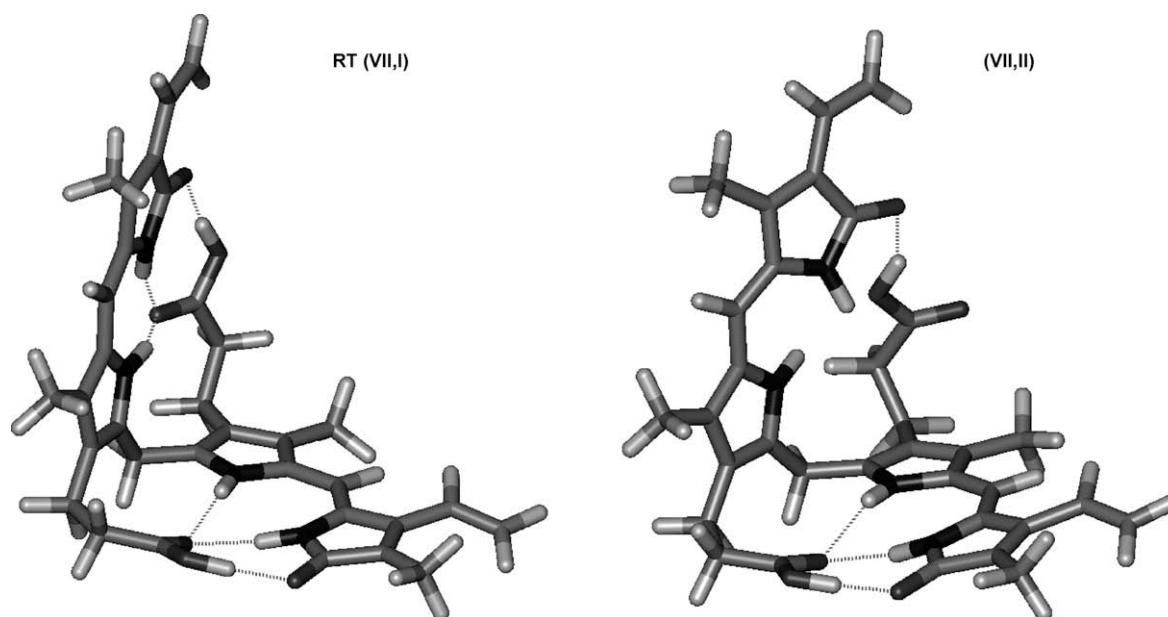
Fig. 8. RT (**VII, I**) conformer shows the 6 H-bonds stabilizing interactions and (**VII, II**) conformer shows the different pattern found in **P2**.
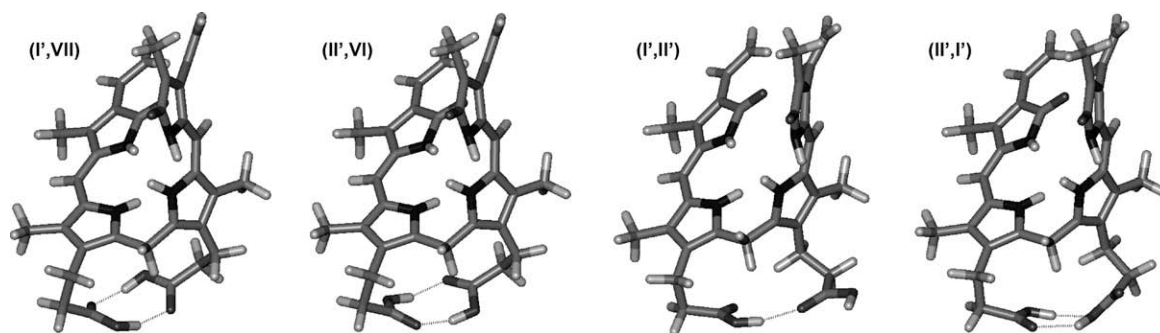
Fig. 9. Bilirubin conformers showing H-bonds established between propionic acid side chain.
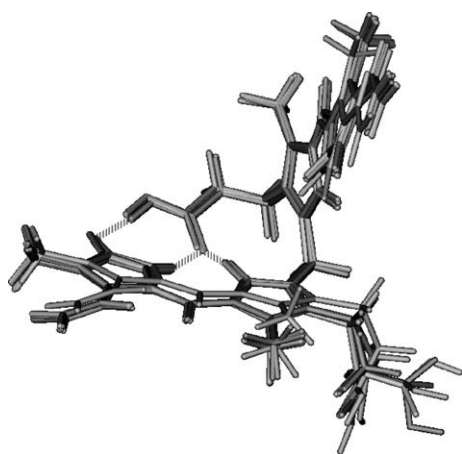

Fig. 10. Superimposition of conformers (**VII, I′**) to (**VII, VII′**) using as template conformer (**VII, II′**).

ridge-tile conformation where only one propionic acid chain (**P1**) make hydrogen bonds with the opposite dipyrrinone and the other one (**P2**) presents different orientations (Fig. 10).

4. Conclusions

Our findings are consistent with previous bilirubin conformational analysis reported, for bilirubin without methyl groups and at a minor level of theory [19,20]. The method we employed covers widely the conformational space of the complete molecule at DFT level taking account of the correlation energy. RT conformation is the most stable one, almost identical to X-ray resolved crystal structure [21]. Nevertheless, the other stable conformers obtained may be important in albumin binding, biological membranes alterations and antioxidant activity.

Although the tetrapyrrole backbone is flexible, we found that propionic acid side chains have a greater influence in bilirubin conformation. As it can be seen in stable conformers maps (Fig. 7), propionic acid side chains conformation directs largely the whole molecule conformation preference. However, the stability is given by hydrogen bond formation which seems to be crucial in conformational changes and properties such us its very low solubility [3] in water media (serum). It has been proposed that hydrogen bond also affect the acid dissociation constants (K_a) because they stabilize the –COOH group with respect to the –COO$^-$ anion [23]. They are so important that they are disrupted when it is conjugated [24] to a water soluble form before it is excreted from the body.

Despite only gas-phase results were obtained, which cannot be employed to extract the solution conformation, founded conformers may be used as starting geometries for further studies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.theochem.2009.06.039.

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