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Computational Study on the Conformations of Gambogic Acid

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Abstract

The conformations of gambogic acid were studied using force fields, MM3*, AMBER*, MMFFs and OPLS2005, and B3LYP methods. In a model molecule, only the MM3* and AMBER* methods produced the same number of conformers as B3LYP, generating two conformations for rings 1 and 2, and a single conformation for rings 3 and 4. The preferred conformations of these rings are maintained in a conformer of the actual gambogic acid generated using the AMBER* and B3LYP methods. Although this calculated conformer matches well with the crystal structure, it shows that H43, C25=C26 and C30=C31 bonds may be misassigned in the crystal structure.

Keywords

Force field; conformation; gambogic acid; ring system

1. Introduction

Gambogic acid (Figure 1) is a natural product isolated from gamboge, the resin from the *Garcinia hanburyi* tree in Southeast Asia. Recently, it has been found that this molecule has potent anti-cancer activities both *in vitro* and *in vivo* [1-7]. In addition, the derivatives of gambogic acid, such as gambogic amide, may also have a potential role in treating neurodegenerative disease [8]. Due to its important biological functions, there has been interest in elucidating the chemical properties and structure of gambogic acid.

Gambogic acid is composed of several rings including an intriguing polycyclic 4-oxatricyclo-[4.3.1.0]decan-2-one system, which was also found in other products isolated from *Garcinia sp* [9,10]. The total synthesis of several compounds that contain such a ring system was reported by Nicolaou and co-workers [11-14]. The structural analysis of gambogic acid was first reported in 1965 using NMR spectroscopy [15]. Later, in 2001, the crystal structure of gambogic acid in the form of a pyridine salt was published [16], which provides detailed three dimensional information on this molecule. Although these studies aid our understanding of the properties and structure of gambogic acid, its conformational flexibility, in particular that of the ring systems, is not clear. Recently, a study on the

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conformations of a similar compound, morellic acid (Figure 1), using the MMFF94 force field and the B3LYP method was reported [17]. However, the conformations of the rings were not discussed. In this study, we have performed a detailed conformational search on gambogic acid, taking care to establish which force field method was best for this task. Selected conformers were further optimized using density functional theory (DFT) and compared with the reported X-ray crystal structure.

2. Computational methods

The force field and DFT calculations were performed using the MacroModel [18] and Gaussian 09 [19] programs, respectively. For the conformational search, four force fields, MM3*, AMBER*, MMFFs and OPLS2005, as implemented in MacroModel, were used. Selected conformers generated from these force fields were further optimized using the B3LYP hybrid density functional [20–22] with the 6-31G(d,p) basis set. Frequency calculations were performed at the same level to confirm that the obtained geometries are minima on the potential energy surface and to get zero-point vibrational energy (ZPVE) corrections. Relative energies were obtained by performing single point calculations at the B3LYP/6-311+G(2df,p) level of theory based on the above optimized geometries and corrected with ZPVE obtained at the B3LYP/6-31G(d,p) level. For the model compound, geometries were also optimized using the cc-pvtz basis set [23,24]. Due to its larger size, on the actual gambogic acid molecule only the 6-31G(d,p) basis set was used for geometry optimization.

3. Results and discussion

3.1. The conformations of a model molecule

Gambogic acid (Figure 1) has several exocyclic groups which contain a number of carbon–carbon single bonds. The free rotation of these bonds results in a large number of conformers. For simplicity, we first substituted all the alkyl exocyclic groups by methyls (Figure 1). For this simplified model, the OPLS2005 force field yields eight conformers, while both MM3* and AMBER* yield four conformers and only two conformers were found using the MMFFs force field. In addition, some of the conformers lie very close in energy. All the conformers and their relative energies are summarized in Table 1. The minimized structures of the eight conformers obtained from the OPLS2005 field (**1–8**) are shown in Figure 2. The conformations of each of the rings are analyzed below.

3.1.1. Ring 1—Ring 1 can be viewed as a 2*H*-pyran derivative. Two possible conformations with C13 located on different sides of the C12–C11–C2–C3–O14 plane (Figure 3) were found with the MM3*, AMBER* and OPLS2005 force fields. Specifically, in conformation **A** C13 is below this plane while it is above the plane in **B**. We note that this also results in a different orientation of the two exocyclic methyl groups which are connected to C13 (see Figure 3). The MMFFs force field, however, yields only conformation **A**. We further verified these two conformations using the DFT method. Both of them were retained during DFT geometry optimization. Since the MMFFs force field failed to predict the two conformations for this ring, we judged it to be the least suitable of the methods we tested for describing the conformation of gambogic acid.

It should be noted that the energy difference between the two conformations of ring 1, **A** and **B**, is quite small. For example, conformer **1** lies only marginally lower in energy than **2** by 0.15–0.22 kJ mol^{−1} using the three force fields which predicted two conformations of ring 1 (Table 1). Furthermore, the energy difference between **1** and **2** further decreases to only 0.09 kJ mol^{−1} with an interconversion barrier of 1.1 kJ mol^{−1} at the B3LYP/6-311+G(2df,p)//B3LYP/6-31G(d,p)+ZPVE level of theory. Using a larger cc-pvtz basis set for geometry

optimization, the energy difference between **1** and **2** slightly increases to 0.24 kJ mol⁻¹ with an interconversion barrier of 0.9 kJ mol⁻¹. Hence, these two conformers lie very close in energy and should be able easily to interconvert.

3.1.2. Ring 2—Because ring 2 is aromatic, it always remains planar in the conformational searches. However, the hydrogen of the exocyclic –OH group may rotate toward ring 3 (conformation **C**, see Figure 3) or ring 1 (conformation **D**, see Figure 3). The former results in the formation of an intramolecular hydrogen bond between the exocyclic –OH and O16 and is, therefore, lower in energy. All the force fields yield these two conformations. However, the obtained relative energies are quite different. In the MM3*, AMBER* and OPLS2005 force fields, conformer **1** is 16.11–29.52 kJ mol⁻¹ lower in energy than **6**. However, the difference increases to 53.3 kJ mol⁻¹ in the MMFFs force field. Using the DFT method at the B3LYP/6-311+G(2df,p)//B3LYP/6-31G(d,p)+ZPVE level, the difference further increases to 61.6 kJ mol⁻¹. Hence, only the MMFFs force field gives a difference close to the DFT level. The other force fields significantly underestimate the importance of the intramolecular O–H···O hydrogen bond.

3.1.3. Ring 3—Ring 3 can be viewed as a ketone derivative of tetrahydropyran. Only the OPLS2005 force field yields two conformations while all the other force fields as well as the DFT method predict only one conformation. In the two conformations generated from the OPLS2005 force field, O12 and O16 are found above the aromatic plane of ring 2 (conformation **E**) or below that plane (conformation **F**), as shown in Figure 3. The former conformation lies ~3 kJ mol⁻¹ lower in energy than the latter. However, only **E** was found using all the other force fields and the DFT method. Hence, the OPLS2005 force field failed to predict correctly the single conformation of this ring, but did find **E** to be the lowest energy conformation. It should be noted that at the B3LYP/6-31G(d,p) level, the C9–C8a–C10a–O10 and C4a–C9a–C9–O16 dihedral angles are 2.2° and 179°, respectively. Thus, ring 3 is almost planar.

3.1.4 Ring 4—For simplicity, we break ring 4 into three sub-rings: C10a–C8a–C8–C7–C6–C5, C10a–C8a–C8–C7–C17–C18 and C10a–C18–C19–O20–C5. All the force fields as well as DFT agree on the conformations of these three sub-rings. The two six-membered rings C10a–C8a–C8–C7–C6–C5 and C10a–C8a–C8–C7–C17–C18 can be viewed as cyclohexene derivatives and are both in boat conformations. The five-membered ring C10a–C18–C19–O20–C5 is a tetrahydrofuran derivative with the four atoms C18–C19–O20–C5 all in the same plane. The remaining carbon C10a lies above this plane, i.e., on the same side as the exocyclic methyl carbon C21.

It is necessary to compare the overall behavior of the four force fields on the conformational search of the model compound. The MM3* and AMBER* yield the same number of conformers as the DFT method. The MMFFs and OPLS2005 force fields, however, predict either fewer or more conformers. Thus, the conformational search for the actual gambogic acid molecule was performed using the MM3* and AMBER* force fields.

3.2. The conformation of gambogic acid

The carboxylic group of gambogic acid was chosen to be ionized as –COO⁻ to match physiological conditions. Because this molecule contains several flexible alkyl groups, many conformers were found. For example, within a relative energy of 100 kJ mol⁻¹, 930 and 856 conformers were found using the AMBER* and MM3* force fields, respectively. Most of them correspond to the free rotation of exocyclic alkyl groups. It is not necessary to discuss all of these conformers. Only the lowest energy conformers and selected higher energy ones (Figure 4) are discussed in this paper.

Notably, the AMBER* and MM3* force fields predict different lowest energy conformers. Specifically, the lowest energy AMBER* conformer (**A1**) has the –OH group of ring 2 hydrogen bonded with the side chain carboxylic acid group, while the lowest energy MM3* conformer (**M1**) has that –OH group hydrogen bonded with the carbonyl oxygen of ring 3. At the AMBER* level, the lowest energy conformer with the same hydrogen bond pattern as **M1** is **A2** (cf. Figure 4), which lies 2.3 kJ mol⁻¹ higher in energy than **A1**. However, upon optimization of both **A1** and **A2** using B3LYP, the former lies 29.7 kJ mol⁻¹ higher in energy than the latter. Hence, the AMBER* force field is not able to produce the correct order of the energies. It should be noted that though **A2** and **M1** have the same O15–H43···O16 hydrogen bond and overall their bond lengths and bond angles are virtually identical (within 0.01 Å and 1°, respectively) at the B3LYP/6-31G(d,p) level, they have different orientations of the exocyclic groups (cf. Figure 4 and Table 2). **A2** and **M1** have the same conformations of the four rings as the model compound conformer **1** at the DFT level. For example, the conformation of ring 1 corresponds to conformation **A**, as discussed above. In addition, ring 3 is planar.

In the model molecule, ring 1 is also able to adopt a different conformation, with C13 above the C12–C11–C2–C3–O14 plane (conformation **B**). In fact, such a conformation was found for gambogic acid in **A1**. However, upon optimization using the DFT method, ring 1 of **A1** converts to conformation **A**. Hence, in the actual gambogic acid molecule, ring 1 may only exist in conformation **A**. Another possible conformation in the model molecule is one with the hydrogen of the –OH group in ring 2 pointing toward ring 1 (conformation **D**). In **A3** such an arrangement is observed. At the DFT level, **A3** lies much higher in energy than **A2** by 73.5 kJ mol⁻¹, so this demonstrates the important contribution of the hydrogen bond between the –OH of ring 2 and the carbonyl oxygen of ring 3.

3.3. Comparison with the crystal structure

Upon optimization using the DFT method, the orientations of the exocyclic groups of **A2** match very well, better than those of **M1**, with the X-ray crystal structure of the pyridine salt of gambogic acid [16] (Figure 5). Table 2 lists the key dihedral angles involving the exocyclic groups of **A2**, **M1** and the crystal structure. Most of the dihedral angles in **A2** agree much better than those of **M1** with the crystal structure. Two of them, C4–C29–C30–C31 and C29–C30–C31–C32, which represent the orientation of the exocyclic group of ring 2, have the opposite sign in **A2** compared to the crystal structure. However, the overall orientation of this exocyclic group still matches well with the crystal structure (cf. Figure 5). Table 3 lists other key geometrical parameters of **A2** at both AMBER* and B3LYP/6-31G(d,p) levels as well as those from the crystal structure. Those calculated structural parameters are very well consistent with experiment. However, some disagreements should be noted. First, the –OH group of ring 2 in the crystal structure does not form an intramolecular hydrogen bond with the carbonyl oxygen of ring 3. Instead, it points towards ring 1 (see the C9a–C1–O45–H43 dihedral angle in Table 3). In the above studies on both the model molecule and the actual gambogic acid, the –OH group of ring 2 prefers to form a hydrogen bond with the carbonyl oxygen of ring 3. In order to investigate the disagreement, we took the coordinates from the crystal structure and optimized them. At the DFT level, the structure with the intramolecular hydrogen bond is 63.3 kJ mol⁻¹ lower in energy than the one without. In addition, the O15–H43 bond in the crystal structure is unphysically short at 0.67 Å (Table 3). Hence, the hydrogen may not be assigned correctly in the crystal structure. Furthermore, three bond lengths differ significantly between the X-ray structure and the calculated methods. The first two are the carbon–carbon double bonds, C25=C26 and C30=C31, which have unusually short lengths in the crystal structure (1.19 Å and 1.22 Å, respectively), largely deviating from those found with the AMBER* (both 1.35 Å) and DFT methods (both 1.34 Å). The experimentally-assigned lengths are too short relative to the

known lengths of carbon-carbon double bonds, which are ~ 1.3 Å [25] and are in fact similar to the typical carbon-carbon triple bond lengths [25]. It is likely that these two bonds were not assigned properly in the crystal structure. Finally, the C24–C25 bond length is too long in the X-ray structure, by 0.16 Å compared to DFT, and considerably longer than the typical C–C single bond of 1.54 Å [25].

4. Conclusions

In this study, we have performed a conformational search on gambogic acid using molecular mechanics and quantum mechanics methods. We started by considering a model molecule with all the exocyclic alkyl groups replaced by methyls. Four force fields, MM3*, AMBER*, MMFFs and OPLS2005, were used. Only the MM3* and AMBER* methods produce the same number of conformers as the B3LYP method. Two possible conformers were found for ring 1 with C13 lying either above or below the C12–C11–C2–C3–O14 plane. These two conformers, however, lie very close in energy. For ring 2, we also found two conformers. That with an intramolecular hydrogen bond O15–H43···O16 lies much lower in energy than the one without this hydrogen bond. Ring 3 can essentially be considered as planar as shown from the C9–C8a–C10a–O10 and C4a–C9a–C9–O16 dihedral angles. The polycyclic ring 4 is composed of several sub-rings. The six-membered rings C10a–C8a–C8–C7–C6–C5 and C10a–C8a–C8–C7–C17–C18 exist in boat conformations. In the five-membered ring C10a–C18–C19–O20–C5, four atoms, C18, C19, O20 and C5, are in the same plane while C10a lies above this plane.

For the actual gambogic acid molecule, the MM3* and AMBER* force fields generate a large number of conformers, with most of them corresponding to the free rotation of the exocyclic groups. The –OH group of ring 2 orients differently in the lowest energy conformer found with the AMBER* (**A1**) force field compared to that found with the MM3* (**M1**) force field. Specifically, it hydrogen bonds with the carboxylic group in **A1** but with the carbonyl oxygen of ring 3 in **M1**. However, when the **A1** and **M1** conformers were reoptimized using the DFT method the hydrogen bonding pattern in **A1** yielded a higher energy conformer than **M1**. In a different higher energy conformer predicted by the AMBER* force field and reoptimized with DFT, **A2**, the orientations of the exocyclic groups match well with the X-ray crystal structure and the conformations of the four rings all match with the model molecule. The lowest energy calculated conformers of gambogic acid contained an O15–H43···O16 hydrogen bond, but in the crystal structure, H43 does not form a hydrogen bond with O16, suggesting that the H43 location has been misassigned. A corresponding calculated conformer **A3** that lacks the intramolecular hydrogen bond lies much higher in energy. Some bond length discrepancies between the computed structures and the X-ray structure were also noted. It is likely that they were not assigned correctly and that the B3LYP/6-31G(d,p) geometry of gambogic acid presented herein will be the best starting point for further calculations.

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Highlights

- ▶ First assessment of computational methods to study the conformation of gambogic acid.
- ▶ The calculated conformer overall matches very well with the X-ray crystal structure.
- ▶ Calculations suggest that some aspects of the crystal structure should be reexamined.

Figure 1.

A schematic illustration of the structures of gambogic acid, morellic acid and a simplified model of gambogic acid.

Figure 2.
The minimized structures of the conformations of the simplified gambogic acid model (see Figure 1) using the OPLS2005 force field.

Figure 3.
A schematic illustration of two possible conformations of ring 1 (**A** and **B**), ring 2 (**C** and **D**) and ring 3 (**E** and **F**).

Figure 4. The minimized structures of the lowest energy conformations of gambogic acid using the AMBER* (**A1**) and MM3* (**M1**) force fields, and two selected conformations generated from the AMBER* (**A2** and **A3**) force field.

Figure 5.
The structures of (a) the X-ray crystal structure of the pyridine salt of gambogic acid and (b) the DFT optimized structure of **A2**.

Table 1

All the possible conformations and their relative energies (kJ mol^{-1}) for the simplified gambogic acid model, using the MM3*, AMBER*, MMFFs, OPLS2005 force fields and DFT.

	MM3*	AMBER*	MMFFs	OPLS2005	DFT ^a	DFT ^b
<div>1</div>	0.0	0.0	0.0	0.0	0.0	0.0
<div>2</div>	0.19	0.15	n/a ^c	0.22	0.09	0.24
<div>3</div>	n/a ^c	n/a ^c	n/a ^c	3.34	n/a ^c	n/a ^c
<div>4</div>	n/a ^c	n/a ^c	n/a ^c	3.68	n/a ^c	n/a ^c

	MM3*	AMBER*	MMFFs	OPLS2005	DFT ^a	DFT ^b
16.37	29.45	n/a ^c	21.34	62.27	61.95
5
16.11	29.52	53.33	21.43	61.62	61.39
6
n/a ^c	n/a ^c	n/a ^c	24.79	n/a ^c	n/a ^c
7
n/a ^c	n/a ^c	n/a ^c	25.75	n/a ^c	n/a ^c
8						

^aB3LYP/6-311+G(2df,p)/B3LYP/6-31G(d,p)+ZPVE

^bB3LYP/cc-pvtz + ZPVE

^cThese conformers were not observed using the corresponding force field or B3LYP method.

Table 2

The key dihedral angles (°) of **A2** and **M1** at the B3LYP/6-31G(d,p) level, and those from the X-ray crystal structure of the pyridine salt of gambogic acid.

	A2	M1	Ref. 16
C11–C12–C13–C22	95	94	119
C11–C12–C13–C23	–140	–141	–120
C12–C13–C23–C24	59	60	62
C13–C23–C24–C25	–177	–82	–167
C23–C24–C25–C26	–111	123	–108
C24–C25–C26–C27	180	180	177
C24–C25–C26–C28	0	–2	–7
C2–C3–C4–C29	177	177	179
C3–C4–C29–C30	88	83	100
C4–C29–C30–C31	165	–126	–154
C29–C30–C31–C32	179	–179	–172
C29–C30–C31–C33	0	1	–4
C8a–C10a–C5–C21	75	80	74
C10a–C5–C21–C34	91	–64	80
C5–C21–C34–C35	–123	126	–138
C21–C34–C35–C36	–174	178	–179
C21–C34–C35–C37	8	–5	5

Table 3

The key bond lengths (Å), bond angles and dihedral angles (°) of **A2** at the AMBER* and B3LYP/6-31G(d,p) (in parentheses) levels, and those from the X-ray crystal structure of the pyridine salt of gambogic acid.

	This work	Ref. 16 ^a	This work	Ref. 16 ^a
C12-C11	1.33 (1.34)	1.298(13)	C21-C34	1.53 (1.51)
C11-C2	1.48 (1.45)	1.475(14)	C34-C35	1.35 (1.35)
C2-C3	1.41 (1.41)	1.380(12)	C35-C36	1.52 (1.51)
C3-O14	1.38 (1.35)	1.346(10)	C35-C37	1.49 (1.55)
O14-C13	1.44 (1.47)	1.453(13)	C37-O38 ^b	1.25 (1.26)
C13-C12	1.53 (1.51)	1.498(15)	C37-O39 ^b	1.25 (1.26)
C2-C1	1.41 (1.40)	1.404(12)	C19-C41	1.54 (1.53)
C1-C9a	1.41 (1.42)	1.434(12)	C19-C42	1.54 (1.54)
C9a-C4a	1.41 (1.42)	1.441(11)	O14-C13-C12	116 (111)
C4a-C4	1.41 (1.39)	1.370(11)	C13-C12-C11	121 (121)
C4-C3	1.41 (1.41)	1.412(12)	C12-C11-C2	120 (120)
C9a-C9	1.48 (1.43)	1.405(11)	C11-C2-C3	119 (119)
C9-C8a	1.47 (1.47)	1.468(12)	C2-C3-O14	122 (120)
C8a-C10a	1.51 (1.51)	1.491(11)	C3-O14-C13	121 (120)
O10-C10a	1.44 (1.43)	1.439(10)	C3-C2-C1	120 (118)
				116(1)

	This work		Ref. 16 ^a		This work		Ref. 16 ^a	
C4a-O10	1.38	(1.36)	1.374(10)		C2-C1-C9a	120	(120)	123(1)
C9-O16	1.23	(1.26)	1.24		C1-C9a-C4a	120	(119)	114.6(9)
C8a-C8	1.34	(1.34)	1.323(10)		C9a-C4a-C4	120	(122)	125(1)
C7-C8	1.52	(1.50)	1.472(13)		C4a-C4-C3	120	(117)	115.9(9)
C6-C7	1.53	(1.54)	1.492(13)		C4-C3-C2	120	(124)	125(1)
C5-C6	1.54	(1.57)	1.555(12)		O10-C4a-C9a	123	(122)	120.2(9)
C5-C10a	1.55	(1.57)	1.526(12)		C4a-C9a-C9	120	(121)	124(1)
C6-O40	1.22	(1.21)	1.20		C9a-C9-C8a	117	(116)	115(1)
C7-C17	1.54	(1.56)	1.547(12)		C9-C8a-C10a	122	(122)	121.5(9)
C17-C18	1.56	(1.55)	1.551(12)		C8a-C10a-O10	115	(115)	116.7(8)
C10a-C18	1.55	(1.58)	1.580(12)		C10a-O10-C4a	123	(123)	120.8(7)
C18-C19	1.55	(1.57)	1.540(13)		O10-C4a-C9a	123	(122)	120.2(9)
C19-O20	1.41	(1.45)	1.480(11)		C5-C10a-C8a	112	(110)	109.7(8)
C5-O20	1.43	(1.44)	1.415(10)		C10a-C8a-C8	115	(116)	115.0(9)
C13-C22	1.53	(1.54)	1.548(15)		C8a-C8-C7	115	(114)	114.9(9)
C13-C23	1.54	(1.54)	1.51		C8-C7-C6	107	(104)	103.9(9)
C23-C24	1.54	(1.54)	1.55		C7-C6-C5	115	(114)	113.0(9)
C24-C25	1.53	(1.50)	1.66		C6-C5-C10a	107	(106)	106.3(9)

	This work	Ref. 16 ^a	This work	Ref. 16 ^a
C25–C26	1.35 (1.34)	1.19	C8–C7–C17	103 (106)
C26–C27	1.53 (1.51)	1.49	C7–C17–C18	109 (109)
C26–C28	1.53 (1.51)	1.42	C17–C18–C10a	109 (108)
O15–H43	0.97 (1.00)	0.67	C18–C10a–C8a	112 (113)
C4–C29	1.53 (1.51)	1.52	C5–C10a–C18	96 (97)
C29–C30	1.53 (1.52)	1.49	C10a–C18–C19	103 (103)
C30–C31	1.35 (1.34)	1.22	C18–C19–O20	106 (105)
C31–C32	1.53 (1.51)	1.54	C19–O20–C5	108 (111)
C31–C33	1.53 (1.51)	1.47	O20–C5–C10a	104 (105)
C5–C21	1.54 (1.54)	1.55	C9a–C1–O15–H43	–11 (–3)
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^aExperimental uncertainties are listed, if available from Ref. 16.

^bO38 and O39 are illustrated in Figure 5.