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Theoretical Studies of the Solvent Effect on the Stability of the Ascorbic Acid Tautomers

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AM1 calculations on three conformers of each of the four tautomeric forms of ascorbic acid are reported. According to the results for isolated molecules, tautomer *3* should be lowest in energy, whereas experimentally only tautomer *1* is observed in the crystal as well as in solution. Solvent effects on the relative stability of the various structures are assessed by applying a combined quantum chemical and force field approach. It turned out to be very important to fully optimize both the water environment and the solute, which was achieved iteratively by means of a suitably modified AM1 Hamiltonian taking into account the charge polarization due to the solvent. Inclusion of the solvent effects reduces appreciably the energy difference between tautomers *1* and *3*, but does not change their energetic ordering. CI calculations indicate that the AM1 method tends to underestimate the bond delocalization effects in the enone substructure of *1* so that correlation effects are important to describe the relative stability of *1* and *3*.

INTRODUCTION

Ascorbic acid (AA) or vitamin C plays a vital role in many processes taking place in all living beings. It is omnipresent both in the animal and the plant word. Although the physiologic role of AA is not quite understood, it has a number of beneficial effects.[1a] AA is the most important orthomolecular substance, as it has been convincingly shown by Linus Pauling – the founder and champion of the orthomolecular medicine.[1b]

A detailed knowledge of the structure of the AA molecule forms the necessary basis for all structure-activity discussions. In general, four tautomers *1–4* shown in Figure 1

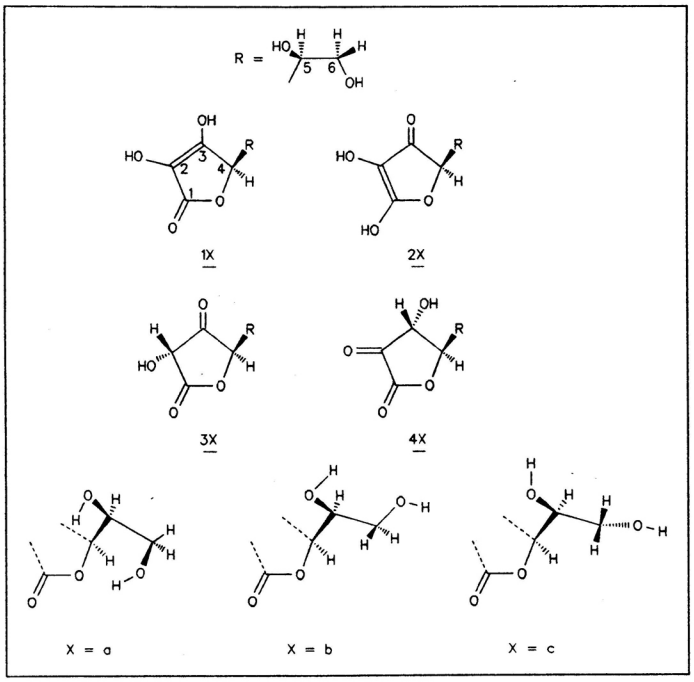

Figure 1. Tautomer *1–4* of ascorbic acid and conformations *a–c* of the side-chain of each of the tautomers. The conformations are characterized by two, one and no hydrogen bonds, respectively (see text).

are discussed. Due to the flexibility of the side chain, different conformers are possible for each of the tautomers. From X-ray data[2] it is known that the most stable tautomer in the solid state is *1*. This is also true in aqueous solution, where ^{13}C-NMR data[3] as well as ^{17}O-NMR[4] data are consistent with tautomer *1* only; ^{1}H-NMR data indicate roughly a 1:1 equilibrium between conformers *a* and *b* (see Figure 1) of tautomer *1*.[5]

Theoretical investigations of the isolated AA molecule are fairly sparse. On the *ab initio* level, a separate geometry optimization of the five-membered ring (α-hydroxy-tetronic acid) and the side chain (1,2-dihydroxyethane) on the STO-3G level[6] as well as an STO-3G optimization of the 9 possible side-chain conformations of tautomer *1* have been described.[7] In a very recent *ab initio* study,[8] the bicyclic form of AA (3-keto-gulonolactone<3,6>-cyclo-hemiketal) was found to be substantially more stable than *1*, if geometry optimization was carried out on the STO-3G level, whereas in 6-31G calculations at these geometries the energy difference for these two tautomers is only about 1 kcal/mol. So far, the bicyclic form has not been detected experimentally either in the solid state or in solutions.

Quantum chemical calculations at the semiempirical level were performed using the MINDO/3 and MNDO methods.[9] The relative stability of the four AA tautomers *1–4* (and their radical forms) was discussed and it was found that the energetically most stable form is *1*, although it should be pointed out that the energy difference between tautomer *1* and *3* was very small. A high flexibility was found for the side chain,

with three shallow local minima for each of the four tautomers. As hydrogen bonds, which are not well described in the MNDO scheme, may be important in determining the various AA conformers, we repeated these calculations using the AM1 scheme. The AM1 parametrization is known to be much more reliable than other semiempirical methods in reproducing features of intramolecular hydrogen bonding.[10] According to the AM1 results, tautomer *3* should be considerably more stable than tautomer *1* in contrast to experimental evidence.

As the experimental data are available only for the solid state and for solutions, whereas all theoretical results are for the isolated molecule, it seems to be of primary interest to study solvent effects on the relative stabilities of the various AA tautomers and conformers. In the present paper, we report the AM1 results for three conformers of each of the four tautomers of AA shown in Figure 1. An optimized water environment is modelled by a molecular mechanics treatment based on an extended AMBER force field.[11] The resultant solute-solvent interaction energies allow for a first estimate of the solvent effects on the structure of AA in water.

QUANTUM CHEMICAL CALCULATIONS ON AA

AM1 calculations were performed for three conformers of each of the four tautomers *1–4* of AA shown in Figure 1. These three conformers were obtained by rotating the terminal CH_2OH group of the side chain, *i.e.* by variation of the dihedral angle C4–C5–C6–O6 with the C3–C4–C5–C6 dihedral angle fixed to the value that is found

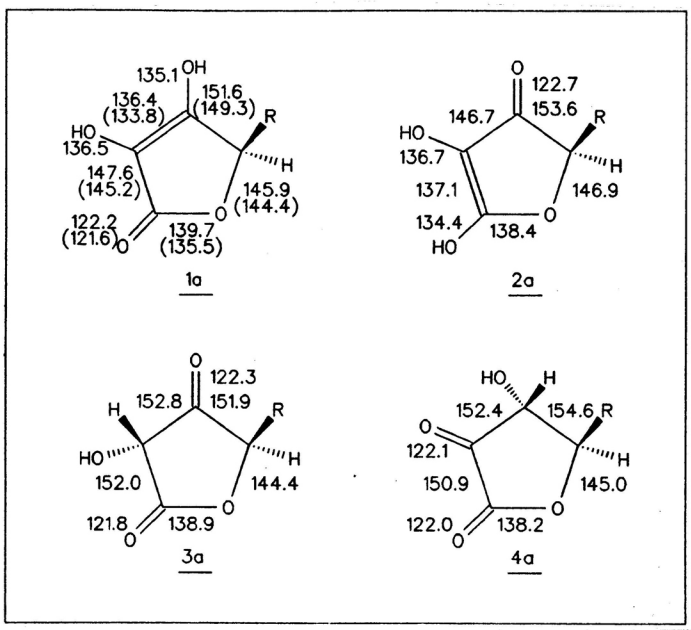

Figure 2. Bond distances (in A) of tautomer *1–4* (conformation *a*) of ascorbic acid, as calculated by AM1 for the isolated molecules. For tautomer *1*, the corresponding X-ray data[2] are given in parentheses.

experimentally in the solid as well as in solution. Conformer *a* is characterized by hydrogen bonds between OH on C5 and C6 and between OH on C6 and the lactone oxygen of the ring, respectively. Conformer *b* exhibits only the hydrogen bond between the side chain OH groups while in conformer *c* no intramolecular hydrogen bond can occur due to the *anti* orientation of the OH groups.

The resultant twelve structures were optimized using an extended AMBER force field to generate starting geometries for the quantum chemical calculations. The AM1 optimization using the SCAMP program[12] converged to a gradient norm smaller than 0.5. Essential geometry parameters of the final structures are shown in Figure 2 together with the X-ray data on tautomer *1*.

The first column of Table I summarizes the resultant heats of formation. Obviously, conformers *a* are the most stable species for all four tautomers *1–4*. Conformers *b* and *c* are less stable by 3.5–4.5 kcal/mol and by 5.5–6 kcal/mol, respectively. An exception is found for the *4c* structure which is only about 4.4 kcal/mol above *4a*. While the AM1 optimized structures for all other molecules resemble the starting geometry very closely, the terminal CH_2OH group of *4c* rotates back into the direction of the five membered ring during the AM1 optimization. Thus, the results for *4c* do not correspond to that of the three conformers described above.

The structure *3a* corresponds to the global minimum of the twelve structures. If we compare the corresponding conformations, the energy ordering of the tautomers is such that *4* is higher in energy than *3*, and tautomer *1*, which according to experiment is most stable, is ranked third.

GENERATION OF A SOLVENT ENVIRONMENT

In order to consider solvent effects on the stability of the tautomers, the following procedure was applied to generate a solvent environment. First, a regular cubic grid of oxygen atoms spaced by 3.5 Å was built and partial charges of –0.5 e were assigned to the atoms. This value of the partial charge was chosen as a compromise between the AM1 value of –0.39 e and the *ab initio* value of –0.8 e in order to obtain a balanced description of intra- and intermolecular electrostatic interactions. Hydrogen atoms were added in a random orientation. The tautomer was placed in the center of this cube and all water molecules within a distance of 1.5 Å from the solute were removed. All water molecules outside a 7 Å radius to any atom of the tautomer were removed, keeping 95 solvent molecules for each system. An extended AMBER forcefield[11] was used to optimize the water structure, leaving the centers of the tautomer fixed in space. A gradient method was applied to yield a geometry with RMS gradient below 0.1 kcal/mol · Å. From the resultant structures, the interaction energy of the tautomer with the water environment was calculated by subtracting the force field energy of the tautomer from the energy of the tautomer and its non bonded interaction with the nearest 75 water molecules. This interaction energy was added to the original heats of formation to yield the entries in column 2 of Table I.

Although the interaction energies are in the range of 40–50 kcal/mol, no significant stabilization of tautomer *1* with respect to the other three tautomers is found. Especially, structure *1b* is even less stabilized as compared to the energy differences of the gas phase AM1 calculations. Only the conformers of tautomer *4* are less favourable and their energies are roughly equal to the energy of *1a*. The most stable structure is now *3b*, which is still about 6 kcal/mol lower in energy than *1a*.

TABLE I

Relative heats of formation (in kcal/mol) of different ascorbic acid structures

Str.[a]	$\Delta(\Delta H_f)$ AM1[b]	$\Delta(\Delta H_f)$ AM1 + MM[c]	$\Delta(\Delta H_f)$ AM1 + $H_{QC,MM}$[d]	$\Delta(\Delta H_f)$ (AM1 + $H_{QC,MM})^e_{opt}$	$\Delta(\Delta H_f)$ AM1-CI + $H_{QC,MM}$[f]
1a	0	0	0	0	0
1b	4.44	10.70	12.60	1.15	2.28
1c	5.97	9.22	9.91	6.51	6.54
2a	6.32	7.29	8.74	7.14	8.28
2b	10.39	16.21	16.00	13.52	15.13
2c	11.90	15.03	12.51	7.28	8.74
3a	-9.85	-2.70	-5.05	-4.03	2.35
3b	-6.18	-6.16	-5.30	-8.10	-1.27
3c	-4.44	-0.07	-1.04	-4.58	2.14
4a	-7.73	-1.00	-0.11	-4.33	2.10
4b	-4.09	-0.25	0.03	-2.74	3.67
4c	-5.43	0.83	-1.24	-4.04	2.47

[a] For identification of the structures see Figure 1.
[b] AM1 calculation on isolated molecules.
[c] AM1 results plus force field interaction with solvent.
[d] Solvent effects included in the AM1 Hamiltonian.
[e] Combined AM1 and force field optimization.
[f] Single point (AM1-CI + $H_{QC,MM}$) calculation at the (AM1 + $H_{QC,MM})_{opt}$ geometry.

COUPLING OF QUANTUM MECHANICS AND MOLECULAR MECHANICS

A modification of the Hamiltonian for the AM1 calculations of the tautomers was introduced to recalculate the electron distribution in the field of the solvent. Various approaches to combined quantum mechanics and force field treatment have been suggested by several groups, differing in the coupling term and also in the non bonding contributions which have to be calculated according to the force field applied.[13] In our calculations the term

$$H_{QC,MM} = -\sum_{i,x}\frac{q_x}{r_{ix}} + \sum_{\alpha,x}\frac{Z_\alpha q_x}{R_{\alpha x}} + \sum_{\alpha,x}\frac{A_{\alpha x}}{R_{\alpha x}^{12}} - \frac{B_{\alpha x}}{R_{\alpha x}^{6}} + \sum_{H,A}\frac{C_{HA}}{R_{HA}^{12}} - \frac{D_{HA}}{R_{HA}^{10}}$$

$$= V_C(\text{Solvent, Electron}) + V_C(\text{Solvent, Core}) + V_{VdW} + V_{H-bond}$$

$$= H'_{elec} + V_{nuc}$$

was added to the AM1 Hamiltonian.

The first term represents the coulomb interaction of an electron and the partial charges q_x of the solvent molecules and is applied to the one-center one-electron integrals

$$-\langle \chi_\mu \left| \frac{q_x}{r_{ix}} \right| \chi_\mu \rangle$$

only. The remaining three terms add to the nuclear repulsion energy of the quantum chemical calculations. The second term describes the coulomb interaction of the core charge of a tautomer center with the partial charges of the solvent. The last two terms include the van der Waals interaction between the solute and solvent centers and the hydrogen bonding term specific to the AMBER force field for the interaction of acidic hydrogen atoms H with hydrogen bond acceptor atoms A, respectively. The hydrogen bonding term is calculated for every suitable pair of centers of the solute and solvent.

Results of the AM1 calculation, including the water, are given in column 3 of Table I. Since the energy terms for the interaction of AA with the water molecules are identical to those used in the force field optimizations, the differences between the second and third columns of Table I are only due to the adjustment of the electron distribution within the tautomers. The general trends of the energies are the same but the differences between *3a* and *3b* are washed out.

The interaction energy between the surrounding water molecules and the AA molecule reduces the energy difference between *3a* and *1a*, but does not change the ordering. A striking inconsistency is seen from the relative stabilization energies for the different species. These energies are about 50 kcal/mol for all structures but the stabilization for conformer *1b* is found to be about 8 kcal/mol less than for conformer *1a*. This causes the large energy difference between these two conformers.

The gradient optimizer always tends to find the nearest energy minimum. When starting from a specific configuration, only one of the large number of possible minimum structures is reached. Thus, the optimized geometries may not be equally well adjusted for all tautomers.

ITERATIVE PROCEDURE TO OPTIMIZE THE SOLUTE-SOLVENT SYSTEM

In order to remove the inconsistency of the water shell, we applied an iterative procedure of optimizing the geometry of ascorbic acid and its solvent environment. The solvent shell was set up from the geometries of the preceding optimization by retaining only the nearest 30 water molecules for every tautomer. The reduced number of water molecules included in the subsequent optimization cycles leads to more flexibility in the hydration shell and, therefore, allows a better adjustment to the solute-solvent interactions.

The iterative procedure consists of two optimization steps:

a) Force field optimization of the solvent

b) AM1 optimization of the ascorbic acid with coupling to the solvent.

These steps are repeated until the resultant total energies calculated by the quantum chemical method do not change.

The relative heats of formation for the optimized systems are summarized in column 4 of Table I. Structure *3b* is still found to be the most favourable but the large energy difference between conformers *1a* and *1b* is reduced from 12.5 to 1.15 kcal/mol. The reason for this stabilization of *1b* can be seen from Figure 3. In the final geometry, twice as many hydrogen bonds are formed between the solute and the solvent as in the starting geometry.

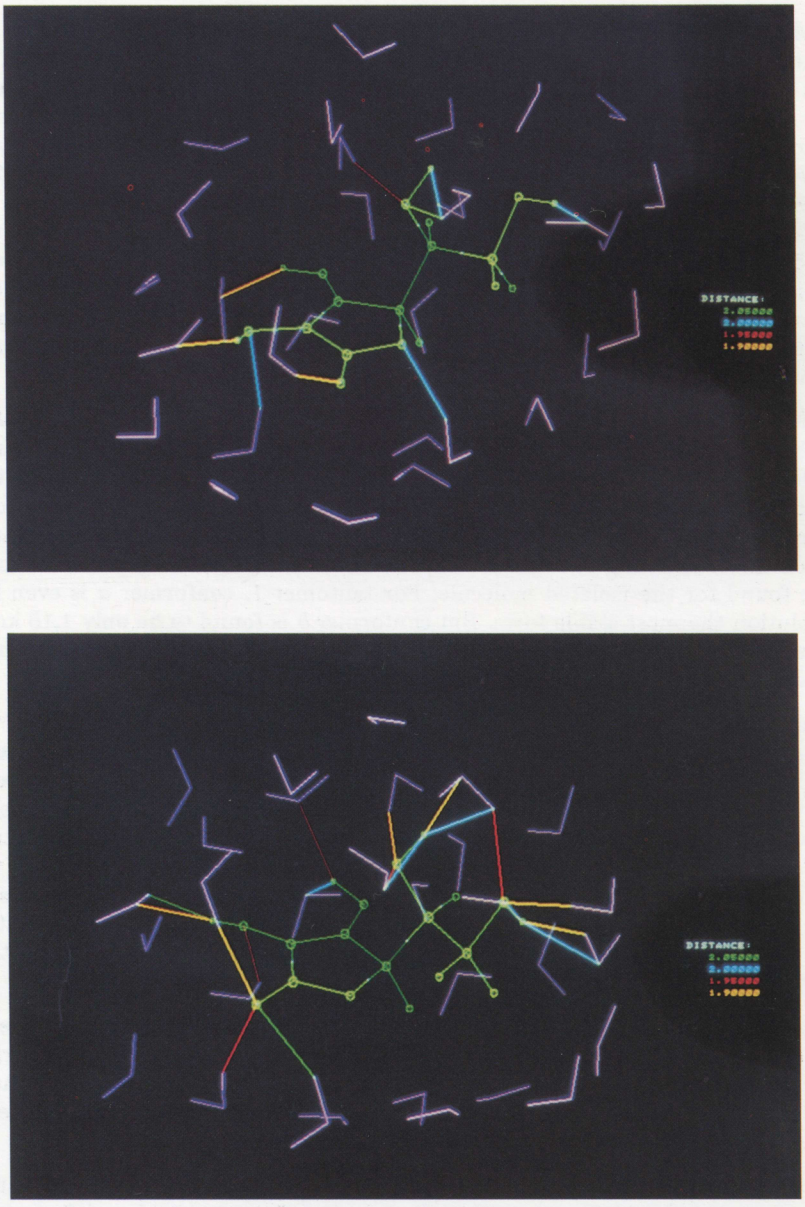

Figure 3. Geometries of the *1b*-solvent system. The hydrogen bonds between the *1b* tautomer and the water molecules are colored according to a distance scale from 2.05 Å (green) to less than 1.90 Å (yellow). a) Starting structure of the iterative optimization. b) Optimized structure of the *1b*-solvent system.

DISCUSSION

AM1 is the only semiempirical method that is parametrized to account for (at least intramolecular) hydrogen bonding, which is essential for a reliable description of AA. This is reflected in the energy ordering of conformers *a* to *c*: For each tautomer, conformer *a* is the most stable species due to the two hydrogen bonds, and conformer *c* with no hydrogen bonds is found to be highest in energy. The AM1 geometry of the five-membered ring in tautomer *1* is closer to the X-ray structure (*cf.* Figure 2) than the MNDO geometry.[9] This holds in particular for the crucial length of the C1–C2 bond (X-ray: 1.452 Å, MNDO: 1.502 Å, AM1: 1.478 Å), which is usually too long in semiempirical calculations because delocalization tends to be underestimated.[14] In terms of AM1 energies tautomers *3* and *4*, which do not show the enone substructure, are significantly more stable, favouring structure *3a* by ~10 kcal/mol over the experimentally most stable structure *1a*. Thus, the larger extent of delocalization in tautomers *1* and *2* is not reflected in the heats of formation.

The entries for the fully optimized solute-solvent systems given in column 4 of Table I show how the solvent affects the energetic ordering of the configurations. Since the side chain OH groups of conformers *b* and *c* can form hydrogen bonds to the solvent, these conformers are expected to be more strongly stabilized by interaction with the solvent than conformer *a*. This is actually found for tautomers *2* to *4* where the solvation effect is strong enough to compensate for the differences in the heats of formation found for the isolated molecule. For tautomer *1*, conformer *a* is even in aqueous solution the most stable form. But conformer *b* is found to be only 1.15 kcal/mol less favourable. This is important because there is experimental evidence from NMR spectra that conformer *1b* predominates in aqueous solution.

The effect of the solvent on the properties and the charge distribution of the ascorbic acid tautomers is shown in Tables II–IV. Table II summarizes the change in the dipole moments from the isolated to the solvated molecules. An increase of about 1 D is found if the solvent is included in the AM1 calculations. The changes in the electron distribution due to polarization by the water molecules are exemplified for structure *1a* in Table III. The presence of positively charge hydrogen atoms close to the oxygen atoms of AA leads to a more pronounced polarization of the O–H bonds. This observation is again reflected in the list of selected geometry parameters of the isolated and solvated tautomer *1a* (Table IV) which show an increase of the O–H bond length upon interaction with the water environment.

Since the electronic ground state of tautomers *1* and *2*, which exhibit the enone substructure, will be strongly affected by configuration interaction (CI), we performed additional AM1-CI calculations on all twelve molecules. The active space for the generation of singly and doubly excited configurations was restricted to the highest four occupied and the lowest four unoccupied orbitals.

The resultant correlation energies and heats of formation are summarized in Table V. As one would expect, the ground state energies of tautomers *1* and *2* are lowered by 6–8 kcal/mol whereas the energies of tautomers *3* and *4* are changed only by about 1 kcal/mol. Thus, the correlation effect in the isolated molecules reduces the energy difference between *1a* and *3a* from about 10 kcal/mol to 3 kcal/mol. Buemi *et al.*[15] reported similar results of AM1-CI calculations for acetylacetone, where the diketo structure is favoured by about 1.7 kcal/mol over the corresponding enol structure.

TABLE II

Calculated dipole moments (in D) for ascorbic acid[a]

structure	μ AM1	μ (AM1 $+H_{QC,MM})_{opt}$
1a	3.34	4.25
1b	2.64	4.29
1c	2.95	4.30
2a	1.32	1.66
2b	2.83	4.14
2c	3.14	3.53
3a	2.94	3.88
3b	3.11	4.09
3c	3.59	4.26
4a	3.69	4.90
4b	3.88	5.31
4c	2.22	2.73

[a] *Cf.* Table I for explanations.

TABLE III

Partial charges of heavy atoms and acidic hydrogen atoms for tautomer 1a *of ascorbic acid*[a]

atom	AM1	(AM1 + $H_{QC,MM})_{opt}$
C1	0.337	0.349
C2	−0.144	−0.168
C3	0.038	0.044
C4	0.025	0.033
C5	−0.004	−0.003
C6	−0.051	−0.044
O1	−0.277	−0.283
O2	−0.226	−0.335
O3	−0.209	−0.244
O4	−0.269	−0.230
O5	−0.308	−0.410
O6	−0.322	−0.477
H2	0.242	0.273
H3	0.249	0.291
H5	0.224	0.275
H6	0.216	0.266

[a] *Cf.* Table I for explanations.

In order to get a first estimate of the effect of CI on the energy ordering of the solvated AA species, we performed single point AMI-CI calculations with the modified Hamiltonian at the geometries determined by the iterative optimization procedure described above. The last column in Table I summarizes the relative heats of formation for the twelve structures. These data show that without additional optimization tautomer *1* and tautomer *3* are comparable in energy.

TABLE IV

Selected bond lengths (in pm) for the optimized structure of isolated and solvated tautomer 1a[a]

bond	AM1	(AM1 + $H_{QC,MM})_{opt}$
C1–C2	147.6	147.2
C2–C3	136.4	136.6
C3–C4	151.6	151.4
C4–C5	153.5	153.0
C5–C6	153.4	153.1
O1–C1	122.2	122.6
O2–C2	136.5	136.1
O3–C3	135.1	134.6
O4–C4	145.9	146.1
O5–C5	141.0	141.3
O6–C6	141.1	141.4
H2–O2	97.2	98.0
H3–O3	97.4	98.5
H5–O5	97.0	97.8
H6–O6	96.8	97.5

[a] *Cf.* Table I for explanations.

TABLE V

Energy lowering by CI and relative heats of formation (in kcal/mol) of different ascorbic acid structures[a]

structure	$E_{SCF}-E_{CI}$	$\Delta(\Delta H_f)_{AM1-CI}$
1a	–7.55	0
1b	–7.48	4.51
1c	–8.14	5.38
2a	–6.13	7.73
2b	–6.09	11.85
2c	–6.09	13.36
3a	–0.76	–3.06
3b	–0.77	0.59
3c	–0.88	2.23
4a	–0.75	–0.94
4b	–1.33	2.13
4c	–0.94	1.17

[a] *Cf.* Table I for explanations.

CONCLUSION

The combined quantum chemical and force field calculations show clearly that structure *1a* of ascorbic acid is stabilized most efficiently by interaction with the surrounding water molecules. Nevertheless, tautomer *1* is still about 8 kcal/mol higher in energy than tautomer *3*. The results of CI calculations suggest that the reason why tautomer *3* is favoured may be sought in the AM1 scheme itself, which apparently does not describe correctly delocalization effects in α,β-unsaturated carbonyl compounds.

From the results of the single point AM1-CI calculation for the solvated species we expect that a determination of the structure of AA and its interaction with the surrounding water molecules on the higher level of the quantum chemical method will lead to results consistent with the experimental data. Such calculations are presently under way.

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SAŽETAK

Teorijsko istraživanje utjecaja otapala na stabilnost tautomera askorbinske kiseline

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Prikazani su rezultati AM1 računa tri konformera za svaki od četiriju tautomera askorbinske kiseline (AA). Zatim je procijenjen utjecaj otapala (vode) na njihovu stabilnost kombiniranom primjenom metode AM1 i molekulske mehanike. Pokazano je da se raspored molekula otapala (H_2O) mora potpuno optimirati kao i molekule otopljene tvari (tautomeri AA) što je postignuto primjenom modificiranog AM1 hamiltonijana, koji uzima u obzir polarizaciju naboja uzrokovanu molekulama otapala.