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Author's Review

Vitamin C and Its Radicals: Tautomerism, Electronic Structure and Properties*

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The biological importance and activity of ascorbic acid and its radicals are briefly reviewed. The quantum mechanical calculations performed on these remarkable compounds are presented in some detail. Particular attention is devoted to structural and electronic features offered by the semiempirical MINDO/3 and MNDO schemes. By making use of the self-consistent charge (SCC-MO) method, the ESCA spectra of the ascorbic acid tautomers are predicted. It is found that the radical anion is more stable than each of the four AA tautomers. This is of importance because the unusual biological protective property of ascorbate against free radical damage is most likely related to the stability of its radical. Origins of the enhanced stability of the radical anion are analyzed by the energy partitioning technique.

I strongly believe that a wider use of ascorbic acid could greately improve our vital statistics and also cut down on cancer.

Albert Szent-Györgyi

1. INTRODUCTION

L-ascorbic acid*** or vitamin C was isolated by Szent-Györgyi[1] from the adrenal cortex of the ox, green pepper and from orange juice in 1928. Structural features were recognized by Herbert *et al.*[2] and confirmed by detailed

* Dedicated to Professor Mihailo Lj. Mihailović on the occassion of his 60th birthday.

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*** IUPAC name: 1-threo-2,4,5,6-pentohexane-2-carboxylic acid lactone, shortened notation AA will be used hereafter.

analyses of X-ray and neutron diffraction results by Hvoslef[3]. AA is a relatively small molecule possessing remarkable properties. Its widespread appearance in plant and animal worlds confirms its biological versatility. AA exhibits a broad pattern of biochemical activities which are still not quite understood. It is, however, beyond doubt that AA plays a number of important roles. According to Szent-Györgyi the development of AA is one of the mile-stones of evolution, which can be compared with the appearance of light and oxygen.[4] Most animals, except primates, some birds, fishes and guinea pig, produce AA either in the kidneys or liver. Humans, as well as the aforementioned animal species, have to insure the AA intake by food. The AA free diet leads invariably to scurvy, which was cured by juice of fresh citrus fruits and described in detail by the British physician Lind.[5] Protection against scurvy is one of the very few biochemical functions of AA which is beyond controversy. Its influence on health is much more than that as will be discussed below. As it will appear, acute or chronic AA tissue desaturation occurs in many pathophysiological states and *vice versa*. We shall first give a brief review of the most important aspects of the AA biochemical activity and then proceed with the presentation of quantum mechanical calculations of the electronic structure of AA and its radicals. As pointed out by Szent-Györgyi, the solution of the AA activity and the cancer problem should be sought at the electronic level.[4,6]

2. BIOLOGICAL IMPORTANCE OF ASCORBIC ACID

AA is omnipresent in blood and tissues but the highest concentrations are found in the adrenal and pituitary glands, liver, spleen and brain. Chronical AA deficiency leads to typical symptoms of scurvy: tissue fragility, capillary hemorrhage and defective wound healing. If AA is not present in a diet, the whole body disintegrates. As Lind writes in an autopsy: ... »all parts were so mixed up and blended together to form one mass or lump that individual organs could not be identified.«.[5] This is a consequence of the failure in the synthesis of collagen, a protein of a triplet-helix structure, which provides strength to the cellular matrix.[7–9] Lack of AA causes depolymerization of collagen. Administration of AA, on the other hand, leads to a relatively quick tissue recovery in a *deus ex machina* manner. It is noteworthy that an analogy in scurvy and uncontrolled cell proliferation is observed[9] (*vide infra*). The human need for AA in wound healing and in the postsurgical maintenance of strong scar tissue has been well documented by now.[10]

AA is also known as one of the most important reducing substances occurring naturally in living tissues. When AA is oxydized, giving dehydroascorbic acid (DHAA), it is generally assumed that this is accomplished by the Michaelis concept of a two-step oxidation involving a free radical intermediate.[11] Since AA represents a powerful antioxidant, it is used as an additive to bread, meat, beer and wine. AA acts synergistically with other food antioxidants and small amounts of AA yield a great gain in efficiency. AA reacts with O_2^- and OH and may provide some protection against other free radicals.[12] Importantly, AA blocks the formation of *N*-nitroso compounds, like *e. g.* nitrosamines and nitrosamides, which are produced by the reaction of

nitrite with nitrogen compounds. This is a remarkable finding because the N—NO fragment is highly carcinogenic. Populations exposed to appreciable levels of nitrate *via* food or pollution appear to show a high incidence of gastric and liver cancer.[13] Additionally, a number of drugs administered in large doses yield NO-derivatives. It is comforting that AA is quite efficient in preventing formation of N—NO compounds in food and *in vivo*.[14]

Cholesterol is an ingredient of cell membranes of vital importance for the cell growth. However, its excessive presence leads to sedimentation on the walls of artheries and consequently to atherosclerosis. It has been well established by now that AA is necessary for cholesterol transformation into bile acids. Chronic marginal AA deficiency leads to cholesterol accumulation in the liver and finally to hipercholesterolemia.[15,16] An inverse correlation exists between the aorta AA and cholesterol concentrations, as revealed by post-mortem biopsies.[16] Increased AA intake leads to a drop in the plasma cholesterol concentration.[17] However, administration of supplementary AA does not suffice. After a longer period of time cholesterol tends to achieve a higher level again. This undesirable effect can be circumvented by a simultaneous administration of pectin and AA.[17] The former binds bile acids in the intestine, thus constantly shifting the equilibrium between cholesterol and bile acids to the latter. Since pectin occurs in fruits and vegetables, just like AA, an appropriate natural diet could control, to a large extent, some types of arteriosclerosis.[17]

It seems that AA has a beneficial effect on immunocompetence. The leucocyte AA level drops slowly during AA deficiency.[18] An adequate concentration of AA is necessary for leukocytes to maintain effecient phagocytosis.[19] It is interesting to mention that the AA uptake by leucocytes is considerably enhanced by aspirin during viral infections. The opposite is the case during good health.[20] The production of interferon is dependent, at least to some extent, on the AA action.[21] A three-fold increase in the serum interferon was observed in cultures exposed to various viruses if AA was present.[20] It is also noteworthy that the number of leukemic cells in culture was reduced by addition of AA.[22] Another important role of AA is its inactivation of excessive histamine concentrations in a variety of stress situations, such as viral attacks in colds, atopic allergies *etc.*[20,23,24]

The influence of AA on the common cold is a subject matter of heated debates. It is beyond doubt, however, that proper administration of AA at least ameliorates cold symptoms, diminishing their severity and duration.[25] It is well documented that AA reduces catarrhal symptoms and increases pulmonary volume.[26] This can be ascribed to antihistaminic[20] and nonspecific antibacterial and antiviral effects of AA.[27,28] It should be pointed out that during a cold a considerable depletion of AA in blood and tissues is observed. Therefore, AA saturation can be achieved only by its proper intake in a diet, as discussed by Pauling.[25]

The relation of AA to cancer is of general interest. This topic is considered in depth by Cameron, Pauling and Leibovitz.[9] They have conclusively shown that the host resistance to the invading neoplastic cells depends on a number of factors all of which are influenced by AA. They underline the importance of a strong intercellular matrix in the process of encapsulation of

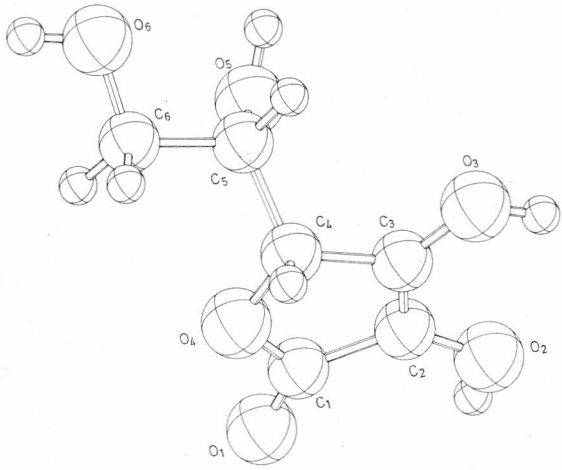


Figure 1. The calculated ground state conformation of the most stable ascorbic acid tautomer.

cells exhibiting undifferentiated proliferations. This can be quite efficient in slow-growing tumors. Strong intercellular matrix is formed by means of collagen, the synthesis of which depends on the availability of AA, as mentioned earlier. Desintegration of collagen in the vicinity of malignant proliferating cells resembles rather closely the process of scurvy. AA is obviously helpful in this respect. There is indeed some epidemiological evidence indicating that cancer incidence in large population groups is inversely related to the average daily intake of AA.[9] It is known that nitrosamines cause cancer in the alimentary tract, liver, lung and urinary bladder.[29-31] AA provides protection against nitrosamines (*vide supra*), as well as against some aromatic hydrocarbons.[9] Cameron, Pauling and Leibovitz conclude that supplemental high doses of AA do not only increase the well-being but also produce a statistically significant increase in the survival times of advanced cancer patients.[9] A recent study by Pauling *et al.*[32] has clearly shown that the rate of appearance of the first spontaneous mammary tumor decreases significantly with an increase in the amount of AA in the food. The beneficial effect of appreciable administration of AA in cancer resistance is understandable if the relation between this vitamin and the increase in immunocompetence is taken into account.[33] Care has to be taken to avoid the rebound effects.[34]

To summarize, increase of immunocompetence and interferon production, antihistaminic effects, nitrosamine blocking, radical quenching and decrease of cholesterol represent AA activities which are of paramount biological importance. These effects are sometimes enhanced by synergetic activity with drugs. There is also persuasive evidence that AA is associated with the respirative process in plants[35] and animals.[36] All these mechanisms have yet to be elucidated. We feel that quantum chemical methods will prove very useful in this respect. Some electronic structure calculations performed so far are described in the next section.

3. ELECTRONIC STRUCTURE OF ASCORBIC ACID AND ITS RADICALS

3.1 *Early Calculations of the Electronic Structure*

The first MO calculation on AA was performed by Pullman and Pullman.[37] Flood and Skancke[38] examined the structure and UV spectrum of AA acid and AA^- anion by the semiempirical PPP approach. The effect of the chain on the ring was studied by the CNDO/2 method. It was found that the ring is little affected by the presence of the chain. The PPP results gave UV absorption bands close to observed values, but geometric parameters were not in good agreement with experiment. It was found also that the protolytic proton is that bound to O(3) oxygen in accordance with experience. This finding can be rationalized in terms of the change in π-electron delocalization energy along the ene-diol fragment.

The conformations of $AA^{-\cdot}$ and $DHAA^{-\cdot}$ radical anion were examined by the *ab initio* minimum basis STO-3G scheme.[39] The experimental bond distances were used as input and only two dihedral (torsional) angles determining the conformation of the side chain and its relative orientation to the ring were varied. The computed conformations were in accordance with the measured ones. The effect of ionization was discussed in terms of bond orders.

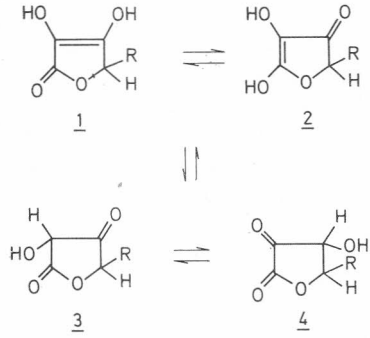
Charge transfer complexes of AA with formamide and glyoxal molecules were studied by using the STO-3G basis set and employing the supermolecule concept.[40] The aim of these calculation was to test the donor-acceptor properties of AA. Instead of AA, a model compound without the side chain (α-hydroxytetronic acid) was considered because of computational limitations. It was argued that the side chain affects very little the electronic structure of the ring. This is probably true, but the adequacy of α-hydroxytetronic acid in mimicking the biological properties of AA is questionable. It is well known that α-hydroxytetronic acid has no antiscorbutic properties.[41] Furthermore, the mere fact that most animals produce AA and not α-hydroxytetronic acid is not insignificant. Hence, the side chain has an important biological role presumably due to the chiral carbon atom(s), which has to be elucidated. Another drawback of this *ab initio* study is the use of the very modest STO-3G basis set and lack of geometry optimization. It was found that charge transfer is very sensitive to precise geometrical parameters. The AA ring can act as electron acceptor in the complex with formamide and in an in-plane complex with glyoxal. However, in the stacked configuration the AA ring donates electrons to glyoxal. These calculations were refined later by including geometry optimization of the model compound(s) and extended to various radicals of the α-hydroxytetronic acid.[42] Full geometry variations of supermolecules were not performed for practical reasons, but a large number of configurations were examined. The main outcome of these calculations is the conjecture that the AA anion and anion radical can act as electron donors to glyoxal. Such donation could subsequently enhance the electron charge transfer from protein to ketoaldehydes, as observed by Szent-Györgyi.[4,43] This finding lends some support to the hypothesis that proteins interacting with electron accepting molecules may behave as semiconductors, being thus operative in the healthy cell living process.[4]

Isotropic hyperfine coupling constants of several possible forms of AA radical were investigated by Thomson[44] applying INDO and *ab initio* STO-3G methods. The geometry of radicals was not optimized and the basic structural

parameters of the ring were adopted from the experimentally determined crystal structure of AA itself.[3] STO-3G calculations were carried out on the model compounds derived from α-hydroxytetronic acid whilst the semiempirical INDO method was employed on both model molecules and actual AA radicals. The computed hyperfine coupling constants are in rather poor numerical accordance with the observed data. However, they strongly support the conjecture that AA radical exists as the anion[45,46] possessing a strongly conjugated tricarbonyl system. This is of importance in view of the possible biological role of the AA radical[9] and the controversy regarding its structure. Some researchers interpreted the ESR spectrum in terms of a neutral species.[47,48]

3.2 *MINDO/3 and MNDO Calculations of Structure and Stability*

Semiempirical MINDO/3 and MNDO exploration of the stability and structure of AA[49] and AA radicals[50] deserves separate comments. These methods[51,52] automatically optimize all geometric parameters, being thus free of any bias introduced by the use of »standard« bond distances and bond angles, by employment of experimental structural parameters which in fact differ from the equilibrium ones, or by replacement of the actual molecule by a model. MINDO/3 and MNDO are of course very approximate methods and their limitations are known,[51,52] but if they are consistently applied in a set of closely related molecules, it is not unreasonable to assume that relative errors will be cancelled out to a large extent. With this idea in mind we performed MINDO/3 + MNDO calculations of the AA tautomers (Figure 2). The radical species were examined by the unrestricted MINDO/3 SCF formalism.[53] As mentioned above, the ordering of stability of the AA tautomers is expected to be quite reliable. Indeed, MINDO/3 and MNDO gave different estimates of their enthalpies of formation but the hierarchy of tautomers was the same.[49] The most stable tautomer *1* is that found in the crystal structure[3] and in solutions, as revealed by ^{13}C-NMR measurements.[54] The structural features of *1* are of interest and will be discussed in more detail. The calculated values are compared with the measured data in Table I. A survey of the results shows that the agreement between theoretical and experimental structural parameters is moderate. There are several reasons for discrepancies. In the first place, MINDO/3 and MNDO methods are very



R = CH(OH) CH_2(OH)

Figure 2. Tautomeric equilibria of ascorbic acid.

TABLE I

Comparison with Experiment of Some Selected Structural Parameters of the Most Stable AA Tautomer 1 as Calculated by MINDO/3 and MINDO Methods for the Conformation Found in Crystal (Bond Distances are in Å, Angles in Degrees)*

Parameter	MINDO/3	MNDO	EXPTL.
C(1)—C(2)	1.493	1.502	1.452
C(2)—C(3)	1.374	1.375	1.338
C(3)—C(4)	1.532	1.547	1.493
C(4)—O(4)	1.387	1.423	1.444
C(1)—O(4)	1.361	1.375	1.355
C(1)—O(1)	1.210	1.220	1.216
C(2)—O(2)	1.339	1.349	1.361
C(3)—O(3)	1.321	1.341	1.326
C(4)—C(5)	1.549	1.582	1.521
C(5)—O(5)	1.368	1.398	1.427
C(5)—C(6)	1.537	1.575	1.521
C(6)—O(6)	1.354	1.397	1.431
O(2)—H	0.952	0.949	0.981
O(3)—H	0.952	0.950	0.966
O(5)—H	0.950	0.947	1.012
O(6)—H	0.951	0.946	0.960
C(4)—H	1.139	1.123	1.073
C(5)—H	1.141	1.126	1.117
C(6)—H	1.129	1.123	1.105
C(4)—O(4)—C(1)	118.0	112.0	109.1
O(4)—C(4)—C(3)	100.7	103.7	104.0
C(2)—C(3)—C(4)	109.3	108.9	109.5
O(4)—C(1)—O(1)	124.6	119.7	121.4
C(2)—C(3)—O(3)	134.5	131.6	133.5
O(2)—C(2)—C(3)	123.8	125.7	127.5
C(3)—C(4)—C(5)	124.2	116.2	114.8
C(4)—C(5)—C(6)	121.1	114.4	112.7
O(5)—C(5)—C(6)	104.8	107.0	106.9
C(5)—C(6)—O(6)	108.6	109.2	108.0
C(3)—C(4)—H	106.1	108.8	112.9
C(5)—C(6)—H	113.3	111.1	108.1
C(2)—O(2)—H	111.7	112.5	110.1
C(3)—O(3)—H	100.7	103.7	104.0
C(5)—O(5)—H	113.0	111.9	114.0
C(6)—O(6)—H	110.9	111.1	104.3
C(6)—C(5)—C(4)—H	69.7	60.6	
C(4)—C(5)—O(5)—H	90.3	91.0	
O(6)—C(6)—C(5)—C(4)	—61.3	—64.8	
C(5)—C(6)—O(6)—H	176.3	174.8	

* Dihedral angle C_A—C_B—C_C—C_D is defined in the following way. The first atom C_A is the closest to the observer. Watching along the C_B—C_C bond, projection of the C_A—C_B bond is rotated around the C_B—C_C axis until a coincidence with the projection C_C—C_D is achieved. Torsional angle is positive or negative if the rotation is counterclockwise or clockwise, respectively.

approximate SCF schemes which provide only fair estimates of the molecular shape and size. Most of the errors are systematic in nature and can be remedied by additional empirical adjustments. We shall not dwell on that because structural features will be discussed at the qualitative level. It suffices to say that each of these two methods defines its own geometry

scale and that the trend of the changes of particular bonds is usually well reproduced. Secondly, the experimental data and theoretical results are, strictly speaking, not comparable. Perusal of the measured values[3] shows that there are two different AA molecules in a crystal unit. Some of the corresponding structural parameters differ markedly in the two molecules because of the difference in crystal environment. Their average values are given in Table I. Furthermore, crystal effects involving electrostatic and polarization interactions, as well as strong hydrogen bonds, appreciably influence the experimental geometry. Finally, theoretical bond distances and angles correspond to a minimum on the Born-Oppenheimer potential surface, whilst the experimental data include effects of thermal motion *etc.* These points should be kept in mind when comparing theoretical and experimental sets of structural data. One has to mention also that the side chain is very flexible and that rotation barriers around various bonds are low. We found three local minima and the experimental conformation appearing in a crystal is one of them. This, however, is not the most stable conformation of the free molecule according to our calculations (*vide infra*). We found that the five-membered ring is very closely planar in accordance with the experimental finding.[3] More pronounced nonplanarity is predicted for other tautomers. Bond angles are significantly better reproduced by the MNDO method, being in reasonable agreement with observed values. Bond distances will be discussed in terms of hybridization s-characters and π-bond orders which are displayed in Table II. The intimate relation between the variation of

TABLE II

s-Character of the Local Hybrid Orbitals (in %) and π-electron Bond Orders in the Most Stable AA Tautomer, as Estimated by the MINDO/3 and MNDO Methods*

	s-character			π-bond orders	
Bond	MINDO/3	MNDO	Bond	MINDO/3	MNDO
C(1)—O(4)	24.5—18.1	27.4—11.9	C(3)—C(4)	0.17	0.14
O(4)—C(4)	14.7—18.2	10.8—12.8	C(4)—O(4)	0.18	0.15
C(3)—C(4)	34.3—28.2	33.8—24.9	C(1)—O(1)	0.75	0.86
C(3)—C(2)	40.0—37.5	38.6—38.4	C(1)—C(2)	0.28	0.22
C(1)—C(2)	41.9—35.3	40.9—33.7	C(2)—C(3)	0.87	0.86
C(1)—O(1)	34.5—14.9	31.1—10.7	C(3)—O(3)	0.31	0.33
C(2)—O(2)	25.5—14.8	26.5—12.0	C(2)—O(2)	0.22	0.26
C(3)—O(3)	24.8—16.5	26.0—12.4	C(1)—O(4)	0.38	0.37
C(4)—C(5)	32.0—29.5	29.9—27.9			
C(5)—C(6)	31.3—31.5	26.8—25.1			
C(5)—O(5)	19.0—14.2	17.9—10.2			
C(6)—O(6)	21.0—14.1	17.7— 9.1			
O(3)—H	13.7	13.5			
O(2)—H	13.3	13.5			
O(5)—H	13.4	12.4			

* The s-characters of the hybrids belonging to the A—B bond are written as s_{AB}—s_{BA}. The first entry corresponds to a hybrid placed on atom A pointing toward atom B.

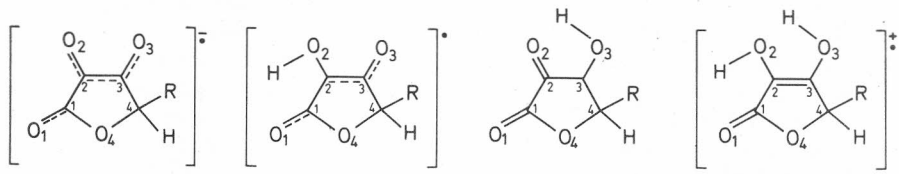
hybridization and changes in bond distances is well documented.[55-57] The semiempirical MO methods used in this work are not based on hybrid orbitals. Nevertheless, the hybrid s-characters can be deduced from the charge--density bond-order matrix.[58,59] One observes that MINDO/3 and MNDO schemes give somewhat different values for the same bond. However, each of

these methods defines again its own scale and a fairly consistent picture is obtained within the same framework. It should be pointed out that thus obtained s-characters deviate from »ideal« canonical states for two reasons: (a) Symmetry of local molecular environments is, as a rule, different from those corresponding to T_d, D_{3h} and D_{och} point groups, (b) populations of s-orbitals are usually higher than unity. Concomitantly, s-characters are shifted to higher values. A useful rule dictating the s-character composition of a particular bond was found.[56] It says that hybrids forming a given bond tend to have similar s-characters. This is compatible with the electronegativity equalization principle. They are, however, never the same due to the s-content requirements of the neighbouring bonds emanating from the atoms forming a bond in question, unless, they are determined by symmetry conditions. This is observed *e. g.* in CO bonds. Oxygen has a 2s orbital of a low energy being, consequently, very suitable for accommodation of electrons. Its population is close to two and, concomitantly, participation in covalent bonding is small. s-characters of oxygen are typically between 10% and 15%. There is a pronounced tendency of carbon hybrids in CO bonds to match this low percentage as close as possible. Hence, the s-character of carbon in CO bonds is always lower than in the corresponding CC bond. Another factor influencing bond distances is the bond order of mobile π-electrons. For this purpose we need a reference point, which is provided by C(3)—C(4) and C(4)—O(4) bonds. They are considered as localized in the ordinary sense. Therefore, their bond orders define zero level. One observes that C(1)=O(1) and C(2)=C(3) behave as typical double bonds. However, some delocalization is present in C(1)—O(4), C(1)—C(2) and C(3)—O(3) bonds representing a sort of branching resonance. Interestingly, the C(2)—O(2) bond does not exhibit π-electron delocalization. Now, the difference in bond distances of C(1)—O(4) and C(4)—O(4) bonds can be ascribed to a higher s-content and larger π-bond order of the former. If we compare O(2)—C(2) and O(3)—C(3) bonds, it is noticed that their average s-character is practically the same. Hence, the smaller C(3)—O(3) distance is presumably a consequence of the higher π-bond order. A short C(1)—C(2) bond, which is formally a single one, arises due to the high s-orbital participation and appreciable π-bond order. All these findings are corroborated by the energy partitioning[60] analysis.* The experimental C(4)—C(5) and C(5)—C(6) distances are shorter than *e. g.* in ethane. Therefore, one could expect an increase in the s-content. Whilst the MINDO/3 s-characters are in line with this conjecture, MNDO values are obviously too low, thus contributing to unrealistic bond distances (1.578 Å and 1.575 Å, respectively). The O(2)—H and O(3)—H bond distances are of interest in view of the question of the protolytic proton. One observes that O—H bonds have almost the same s-characters indicating closely similar bond energies. This conclusion is in accordance with the results of energy partitioning, which yields — 14 eV for the O—H two-center term. The effective charges of hydrogens in hydroxyl groups are also practically the same (0.26 | e | and 0.20 | e | for MINDO/3 and MNDO, respectively). Hence, the site of the proton abstraction is obviously determined by the properties of the final (anion) state (*vide infra*). The difference in O(2)—H and O(3)—H bond distances is apparently due to the packing forces in crystal. The side chain is of particular

* Results of energy partitioning analyses are available on request.

interest. C(3) and C(6) atoms are *trans* relative to the central C(4)—C(5) bond. All bonds of the chain are essentially in staggered positions. An exception is the O(5)—H bond where the hydrogen is pushed away by the C(2)=C(3) double bond. The conformation described in Table I has the following MINDO/3 (MNDO) ΔH_f values: —1175.4 (—1048.7) kJ mol^{-1}. A MNDO study[61] of the rotation barrier of C(6)H_2O(6)H moiety around the C(5)—C(6) bond revealed that the most stable conformer is that with O(6) atom gauche to the O(5) position (O(6)—C(6)—C(5)—O(5) angle is more specifically 66°) possessing ΔH_f = = —1055 kJ mol^{-1}. It is noteworthy that rotation has very little influence on the ring. Another remarkable finding is that the conformation with the torsional angle of 315° is only 16 kJ mol^{-1} above the absolute minimum (ΔH_f = = —1039 kJ mol^{-1}). This conformation could be of great significance because the distribution of six oxygens forms a half-open cavity suitable for accommodating a sizeable cation. Hence, 4 kcal mol^{-1} can be easily overcompensated by chelating stabilization. This result probably gives the simplest explanation of the AA reducing power exerted on the transition metal ions like Cu^{2+}, Ca^{2+} *etc.*[62] The same applies also to radical anion complexes with divalent metals M^{2+}. Incidentally, AA is so effective in lowering the valence state of metals that it is used in analytical chemistry.[63] It seems that flexibility of the side chain is at least partly responsible for the biological versatility of AA. It should be pointed out that relative stabilities of AA tautomers are determined by a rather delicate balance of various energy terms (vide Appendix).

Species possessing unpaired electrons are of particular interest due to their pronounced chemical and biological activity. There are four different radicals derived from AA (Figure 3). Our MINDO/3 calculations[50] indicate that the $AA^{\cdot -}$ anion radical is by far the most stable form (ΔH_f = —1225.5 kJ mol^{-1}). This is in line with earlier theoretical[44] and experimental investigations.[45,46] Increased $AA^{\cdot -}$ stability is probably related to an unusually pronounced protective ability of ascorbate against free radical damage. Origin of this stability is analyzed in some detail in Appendix. The structural MINDO/3 data and bonding parameters, like s-character and π-electron bond orders, are presented in Tables III and IV, respectively. We shall only discuss the differences relative to the parent AA molecule. In the $AA^{\cdot -}$ anion radical the protons attached to O(2) and O(3) in AA are removed. This affects the



R = CH(OH)CH_2(OH)

Figure 3. Radical species derived from ascorbic acid.

ring structure mostly through the mobile π-system. There is also a redistribution of s-characters. The main feature is the substantial increase of the π-electron delocalization over the O(1)—C(1)—C(2)—C(3)—O(3) fragment including now C(2)—O(2) branch in addition. Delocalization in C(1)—O(4) bond is still not negligible but it is lower than in AA. This is evidenced by the changes in bond distances and bond orders (Tables III and IV). The C(1)—O(1) bond is slightly increased due to a small decrease in the π-electron bond density and average s-content. C(2)—O(2) and C(3)—O(3) bond distances are

TABLE III

MINDO/3 Structural Parameters of $AA^{-\cdot}$ Anion Radical (Distances in Å, Angles in Degrees)

Parameter	MINDO/3	Parameter	MINDO/3
C(1)—C(2)	1.492	C(1)—O(4)—C(4)	117.5
C(2)—C(3)	1.466	O(4)—C(4)—C(3)	103.2
C(3)—C(4)	1.552	C(4)—C(3)—C(2)	106.4
C(4)—O(4)	1.381	O(1)—C(1)—O(4)	120.8
C(1)—O(4)	1.381	O(3)—C(3)—C(2)	131.7
C(1)—O(1)	1.218	O(2)—C(2)—C(3)	128.4
C(2)—O(2)	1.251	C(5)—C(4)—C(3)	124.1
C(3)—O(3)	1.227	C(6)—C(5)—C(4)	122.3
C(4)—C(5)	1.546	C(5)—O(5)—H	105.4
C(5)—O(5)	1.368	O(6)—C(6)—C(5)	101.8
C(5)—C(6)	1.537	C(5)—O(5)—H	112.5
C(6)—O(6)	1.357	C(5)—C(6)—O(6)	109.5
O(5)—H	0.951	C(6)—C(5)—C(3)—H	68.9
O(6)—H	0.951	O(5)—C(5)—C(6)—C(4)	—129.7
C(4)—H	1.144	C(6)—C(5)—O(5)—H	7.1
C(5)—H	1.133	O(6)—C(6)—C(5)—C(4)	—63.2
C(6)—H	1.133		

TABLE IV

s-Characters of the Local Hybrid Orbitals (in %) and π-electron Bond Orders in $AA^{-\cdot}$ Anion Radical, as Estimated by the MINDO/3 Method

Bond	s_A — s_B	Bond	π-bond order
C(1)—O(4)	20.2—18.5	C(1)—O(4)	0.36
C(4)—O(4)	15.4—17.9	C(4)—O(4)	0.20
C(3)—C(4)	32.1—28.6	C(1)—O(1)	0.73
C(2)—C(3)	39.2—35.2	C(1)—C(2)	0.30
C(1)—C(2)	46.1—32.4	C(2)—C(3)	0.45
C(1)—O(1)	30.7—15.0	C(3)—O(3)	0.73
C(3)—O(3)	29.4—15.4	C(2)—O(2)	0.56
C(2)—O(2)	31.1—13.5	C(3)—C(4)	0.15
C(4)—C(5)	27.8—32.6		
C(5)—C(6)	28.8—34.0		
C(5)—O(5)	19.8—13.8		
C(6)—O(6)	20.2—14.9		
C(4)—H	18.7		
C(5)—H	18.0		
O(5)—H	13.0		
O(6)—H	12.5		
C(6)—H	21.7		

considerably shorter due to the parallel relatively large and relatively small increase in π-bond orders and s-character, respectively. The opposite is the case with the C(2)—C(3) bond but its lengthening is almost solely a consequence of the significant depletion of the π-electron density. It is rather peculiar that the C(1)—C(2) distance did not change at all. Inspection of the data given in Table IV shows that the increase in the π-bond order is rather small and the average s-character is practically constant. Other structural properties are very similar to those found in AA. The ring is planar and the side chain is little affected by the abstraction of the two ring protons and an electron. The only difference worth mentioning is a decrease of the C(4)—C(5)—O(5)—H torsional angle from 90° to 57°.

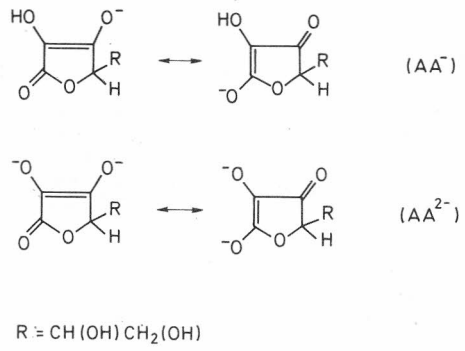


Figure 4. VB structures of AA^- and AA^{2-}.

The structure of AA^- anion (Figure 4) is of some interest because it can provide evidence of the possible site of deprotonation. The MINDO/3 geometry is compared with the experimental data of sodium ascorbate[64] crystal in Table V. Taking into account that the theoretical values are compared with experimental results for the AA^- anion in the presence of the Na^+ ion in crystal lattice, one can say that the overall agreement is satisfactory. The protolytic proton belongs to the O(3)—H bond. The main contribution to the stability of this form of AA^- comes from the increased delocalization energy of the π-electron system, as discussed first by Flood and Skancke.[38] The structure of the AA^- anion was discussed recently in terms of the measured spin-spin coupling constants.[65,66] It was concluded that the valence bond structure involving the C(1)—$O(1)^-$ single bond and C(3)—O(3) double bond gives the largest contribution to the ground state wavefunction. This is not corroborated by the present calculations and the estimated bond distances. The MINDO/3 C(1)O(1) and C(3)O(3) distances are similar in magnitude and comparable to a value characteristic of a C=O double bond. It is interesting to note that atomic charges of the O(1) and O(3) atoms are — 0.63 and — 0.68, respectively. They are considerably larger than the charge of the O(2) atom of — 0.42 | e |. Hence, the negative charge is not localized but distributed over oxygens instead. We also give in Table V the MINDO/3 structure of the AA^{2-} dianion because of recent experimental interest in this species.[66] Berger concludes that two VB structures, the first including C(2)—$O(2)^-$ and C(3)—$O(3)^-$

TABLE V

Comparison of the Structural Parameters of AA^- Anion, as Obtained by the MINDO/3 Method with the Experimental Data. MINDO/3 Estimate of the Geometry of AA^{2-} Dianion. (Bond Distances in Å, Bond Angles in Degrees)

Parameter	AA^- MINDO/3	AA^- Exptl.	AA^{-2} MINDO/3
C(1)—O(4)	1.387	1.358	1.411
O(4)—C(4)	1.384	1.448	1.374
C(3)—C(4)	1.556	1.516	1.566
C(2)—C(3)	1.418	1.372	1.409
C(1)—C(2)	1.444	1.416	1.471
C(1)—O(1)	1.226	1.233	1.237
C(2)—O(2)	1.364	1.284	1.304
C(3)—O(3)	1.239	1.287	1.269
C(4)—C(5)	1.546	1.536	1.556
C(5)—C(6)	1.536	1.503	1.539
C(5)—O(5)	1.374	1.410	1.381
C(6)—O(6)	1.357	1.423	1.361
O(2)—H	0.952	—	
C(4)—H	1.144	—	1.157
O(5)—H	0.951	—	0.951
O(6)—H	0.951	—	0.951
C(6)—H	1.132	—	1.136
C(1)—O(4)—C(4)	116.4	108.0	113.6
O(4)—C(4)—C(3)	103.6	105.2	104.2
C(2)—C(3)—C(4)	103.9	105.8	106.8
O(4)—C(1)—O(1)	120.6	120.4	117.1
O(2)—C(2)—C(3)	129.5	128.7	130.4
C(2)—C(3)—O(3)	122.1	131.3	135.5
C(3)—C(4)—C(5)	123.8	116.1	124.7
C(4)—C(5)—C(6)	122.1	110.1	123.4
O(5)—C(5)—C(6)	102.1	108.5	100.4
C(5)—C(6)—O(6)	109.7	108.5	110.8
C(5)—O(5)—H	111.9	110.0	110.4
C(6)—O(6)—H	110.9	112.0	111.1
C(6)—C(5)—C(4)—H	70.6		70.7
C(4)—C(5)—O(5)—H	69.8		42.2
O(6)—C(6)—C(5)—C(4)	—63.8		—69.3
C(5)—C(6)—O(6)—H	172.0		169.8

single bonds, and the second possesing C(1)—O(1)$^-$ and C(2)—O(2)$^-$ distribution of essentially single bonds, equally contribute to the ground state wavefunction. Our calculations indicate that the former VB structure is more important than the latter. These findings, however, should be taken with a grain of caution in view of the approximate nature of the semiempirical schemes applied.

3.3 *Semiempirical Estimate of the ESCA Chemical Shifts*

ESCA chemical shifts give a useful insight into the charge distribution in molecules.[67,68] This fact stems from the finding that changes in inner-shell binding energies parallel those of electrostatic potentials exerted on the nuclei of atoms undergoing ionization.[69] The potential on the nucleus can be rather accurately calculated within the atomic monopole approximation,[70] yielding a basis for the atomic monopole electrostatic potential (AMEP) model.[57] This, in turn, is a special case of the more general modified atom in a mole-

cule (MAM) model.[57] The AMEP model proved particularly helpful in rationalizing the ESCA shifts if the atomic monopoles were derived from the semiempirical self-consistent charge (SCC-MO) wavefunctions.[70-72] The simplest formula relates the inner-core energy shifts ΔBE_A to the electron density placed on the host nucleus A:

$$\Delta BE_A = k_1 Q_A + k_4 \tag{1}$$

Although formula (1) is quite useful, it does not include the reorganization effect accompanying the ionization process. This can be taken into account by the transition potential model[73] or by the equivalent core concept[74] which, translated into the AMEP language, yield the formulae (2) and (3), respectively:

$$\Delta BE_A = k_1 Q_A{}^{TP} + k_3 M_A{}^{TP} + k_4 \tag{2}$$

and

$$\Delta BE_A = k_1 (\zeta_{2sA} Q_{2sA} + \zeta_{2pA} Q_{2pA}) + k_2 (\tilde{\zeta}_{2asA} \tilde{Q}_{2sA} + \tilde{\zeta}_{2pA} \tilde{Q}_{2pA}) + k_3 (M_A + M_{\tilde{A}}) + k_4 \tag{3}$$

Here TP refers to the transition potential and tilde denotes the equivalent core. M_A signifies the Madelung term, whilst Q_{2s} and Q_{2p} are populations of the 2s orbital and 2p-subshell, respectively. The nonlinear parameters of AOs are given by and taken from the Clementi-Raimondi paper.[75] The SCC-MO calculations were carried out on tetronic acid and AA tautomers by using MNDO geometries.[76] The error introduced by approximate geometric structures is not highly pronounced for the $1/r$ operator.[57] The representative results for the tetronic acid and the most stable AA tautomer are given in Table VI. The estimated ESCA shifts obtained by the eqns. (2) and (3) are to be preferred because they explicitly involve the relaxation effect. It is interesting to observe the TP and EC formalism yield practically the same results. Further, comparison of the data obtained for α-hydroxy tetronic acid and AA indicates that the side chain has a negligible influence on the ΔBE_A values of the ring atoms. However, different AA tautomers can be clearly distinguished by considering the ESCA shifts.[76] Finally, the SCC-AMEP results presented in Table VI should be helpful in interpretation and assignment of experimental data which will appear in the future.

Of further interest is the relaxation energy because it can be measured in principle[77-80] although the experimental estimates so far are sparse. The reorganization effect can be sensibly broken down into three contributions:

$$E_A{}^r = E_A{}^r(contr) + E_A{}^r(flow) + E_A{}^r(mix) \tag{4}$$

where particular components have a simple physical meaning.[57] The first appears because of the contraction of the valence orbitals of the host atom, as a consequence of the increase in effective positive charge of the nucleus in question. The second arises from the electron flow toward the created positive hole whilst the last term is the combined effect exhibited by the ionized atom. Some characteristic values offered by the equivalent core model are displayed in Table VII. One observes that the relaxation energy depends strongly on the nature of the ionized atom. Secondly, the $E_A{}^r(contr)$ and $E_A{}^r(flow)$ terms are fairly constant. The mixed effect of the charge drift and contraction, described by the $E_A{}^r(mix)$ term, is responsible for the variation in reorganization energy. The relaxation of the ring atoms is little effected

TABLE VI

Solid State ESCA Chemical Shifts in α-Hydroxytetronic Acid and AA as Estimated by the SCC-MO Method Employing the AMEP Model (in ev)

Compound	Atom	Eq (1)	Eq (2)	Eq (3)
α-hydroxy-	C_1	— 1.1	— 0.7	— 0.7
tetronic acid	C_2	— 2.2	— 2.6	— 2.6
	C_3	— 2.0	— 2.7	— 2.8
	C_4	— 2.1	— 2.0	— 2.0
	O_1	— 10.0	— 10.0	— 9.9
	O_2	— 8.0	— 7.4	— 7.9
	O_3	— 7.0	— 7.1	— 7.3
	O_4	— 7.9	— 8.3	— 8.2
ascorbic acid	C_1	— 1.1	— 0.8	— 0.8
(most stable	C_2	— 2.2	— 2.7	— 2.7
tautomer)	C_3	— 1.9	— 2.8	— 2.8
	C_4	— 1.7	— 2.1	— 2.1
	C_5	— 2.0	— 2.2	— 2.2
	C_6	— 2.6	— 2.2	— 2.2
	O_1	— 10.1	— 10.0	— 9.9
	O_2	— 8.0	— 7.4	— 7.9
	O_3	— 7.0	— 7.2	— 7.4
	O_4	— 7.9	— 8.2	— 8.1
	O_5	— 9.3	— 8.4	— 9.1
	O_6	— 9.4	— 8.1	— 8.9

TABLE VII

Relaxation Energies upon the Inner-Core Ionization in α-Hydroxytetronic Acid and the Most Stable AA Tautomer, as Estimated by the SCC-AMEP Model Employing the Equivalent Core Concept (in ev)

Molecule	Atom	E_A^r (*contr*)	E_A^r (*flow*)	E_A^r (*mix*)	E_A^r
α-hydroxy	C_1	8.7	— 3.8	12.3	17.2
tetronic acid	C_2	8.8	— 3.9	12.6	17.5
	C_3	8.8	— 3.9	13.0	17.9
	C_4	8.8	— 4.0	11.4	16.3
	O_1	14.0	— 2.4	13.3	24.9
	O_2	13.8	— 2.7	10.1	21.3
	O_3	13.7	— 2.7	10.7	21.8
	O_4	13.8	— 2.7	12.2	23.3
ascorbic acid	C_1	8.7	— 3.7	12.5	17.5
(the most	C_2	8.8	— 3.8	12.7	17.7
stable tautomer)	C_3	8.8	— 3.8	13.1	18.1
	C_4	8.7	— 3.7	12.3	17.3
	C_5	8.8	— 3.9	12.1	17.0
	C_6	8.9	— 4.2	11.2	15.9
	O_1	14.0	— 2.3	13.4	25.1
	O_2	13.8	— 2.7	10.2	21.3
	O_3	13.7	— 2.6	10.7	21.8
	O_4	13.8	— 2.6	12.3	23.5
	O_5	13.9	— 2.8	9.5	20.7
	O_6	14.0	— 2.9	9.3	20.4

by the presence of the side chain except for the site of substitution (C_4 atom), where the chain contributes to the increase in E_A^r by 1 eV. The most stabilized oxygen atom in the final ionic state is found at the O_1 position. It is interesting to mention that the extent of the charge reorganization effect can be correlated with proton affinity.[81,82]

4. FINAL REMARKS

Ascorbic acid and its anions play a vital role in plants, animals and humans. AA is of considerable importance in a large number of healing processes. In spite of intensive scientific efforts for over 50 years, its biological activity is not fully understood. A detailed insight into the electronic biochemical aspect of AA can be provided only by quantum mechanical computations. We feel that the results presented in this paper, albeit very approximative, represent a step in this direction. Most of the theoretical results are in agreement with experimental findings. A lot of work should be done in future. For example, the strain in the five-membered ring, indicated by the off-line electron density maxima[3] which imply appearance of bent bonds, should be better examined. Another point of interest is the question of the bicyclic ring formation in the oxydation of AA — a contention which was recently challenged by Fleming *et al.*[83] We plan to examine the structure of dehydroascorbic acid (DHAA), complexes of AA with metal cations and the hydration phenomenon. Additional experimental work is desirable too.

APPENDIX

Relative stabilities of the AA tautomers and $AA^{\cdot-}$ radical for the conformation of the side chain found in the AA crystal[3] will be analyzed in terms of MINDO/3 values by using the energy partitioning technique. A thorough discussion of the latter is available elsewhere[50]. Briefly, the total SCF energy in the ZDO approximation can be written as a sum of three terms:

$$E_t = E_1 + E_2 + E_3 \tag{5}$$

where $E_1 = \sum_A E_A$ is a one-center contribution, $E_2 = \sum_{A-B} E(A—B)$ is a sum of stabilizing interactions of directly bonded atoms and $E_3 = (1/2) \sum_A E_A^{nb}$ is a total molecular nonbonded repulsion term expressed conveniently as a sum over all atoms. Perusal of the results displayed in Table VIII reveals that the relative stabilities of the tautomers are determined by a delicate balance of the E_i ($i = 1, 2, 3$) terms. The E_1 and E_3 terms decrease along the series *1*—*4*. The absolute value of the

TABLE VIII

Energy Decomposition and Relative Stabilities of the AA Tautomers and $AA^{\cdot-}$ Radical Anion as obtained by the MINDO/3 Method (in eV Unless Otherwise Stated)

Compound	*1*	*2*	*3*	*4*	*5*
E_1	—2454.9	—2455.5	—2457.4	—2458.0	—2447.6
E_2	—310.4	—309.1	—307.0	—305.5	—288.3
E_3	15.8	15.4	15.3	14.6	15.4
E_t	—2749.5	—2749.1	—2749.2	—2748.9	—2720.5
Δ_1	0	—	—	—	—7.6
Δ_2	0	—	—	—	—5.2
Δ_3	0	—	—	—	0.6
ΔH_f*	—1175.4	—1140.3	—1147.5	—1118.3	—1225.5
$\Delta(\Delta H_f)$*	0	35.1	27.9	57.1	—50.1

* In kJ mol^{-1}.

gradient of the E_3 term is very small. On the contrary, the E_2 term increases sharply along the series, assuming the lowest value for the tautomer *1*. It appears that the E_2 term is responsible for the greatest stability of this tautomer. Closer analysis shows that a favourable bonding pattern between the heavy atoms of the planar fragment in *1* leads to such a low value of E_2. We shall now consider the question of the pronounced stability of the radical anion *5*. Since *5* and the parent compound *1* have different numbers of chemical bonds, the following formula is appropriate:

$$\Delta H_f(5) - \Delta H_f(1) = [\Delta_1 + \Delta_2 + \Delta_3] + \{\alpha + \beta + \gamma\} \quad (6)$$

where $\Delta_1 = E_1(5) - [E_1(1) - E_1(H(O_2)) - E_1(H(O_3))]$, $\Delta_2 = E_2(5) - [E_2(1) - E_2(O_2 - H) - E_2(O_3 - H)]$ and $\Delta_3 = E_3(5) - [E_3(1) - E_3^{nb}(H(O_2)) - E_3^{nb}(H(O_3)) + E_3^{nb}(H(O_2), H(O_3))]$. Here a hydrogen atom attached to oxygen O_i is denoted by $H(O_i)$. A term in square parenthesis represents a perturbation of the AA skeleton upon abstraction of H and H^+ atoms. The expression in curly parenthesis describes energy loss due to missing O—H bonds in *5*. The corresponding formulae read: $\alpha = -[E_1(H(O_2)) + E_1(H(O_3)) + E_2(O_2 - H) + E_2(O_3 - H) + E_3^{nb}(H(O_2)) + E_3^{nb}(H(O_3)) - E_3^{nb}(H(O_2), H(O_3))]$, $\beta = -[E_t^{pr}(5) - E_t^{pr}(1)]$ and $\gamma = \sum_A \Delta H_f^A(5) - \sum_A \Delta H_f^A(1)$ where E_t^{pr} is a sum of the electronic energies of isolated atoms and ΔH_f^A are their heats of formation. It turns out that stabilization of the skeleton in *5* (—12.2 eV) outweighs the bonding energies of the $O_2 - H$ and $O_3 - H$ bonds in *1* (11.7 eV). Hence the $AA^{\cdot-}$ radical anion is more stable than *1* by —0.5 eV. Large contributions to nonbonded repulsion is actually somewhat increased in *5* relative to *1* (Table VIII).

Note added in proof: Although we have tried to cover all of the available basic knowledge on vitamin C and its radicals, some interesting papers escaped our attention, because the literature on the AA is very abundant, and scattered over a large number of journals. We shall try to remedy this at least partly, by mentionign some additional papers. Szent-Györgyi *et al.*[84] have performed ab initio minimal basis set calculation of AA adducts with Schiff bases of lysine side chains. AA was mimicked by the model α-hydroxytetronic acid compound. Further, an important observation was made by Cathcart[85] who found that the so called bowell tolerance of AA intake depends on the physical constitution of a patient and — remarkably — on the nature of a desease. A person tolerating a certain amount of AA in a state of good health might be able to tolerate 10 times more with a severe cold, 20 times more with viral pneumonia or mononucleosis etc. Obviously, our bodily needs for AA increase sharply with the severity of the stress situation. Finally, it is worth mentioning that megadoses of AA provide efficient prophylaxis of viral hepatitis B.[86]

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SAŽETAK

C vitamin i njegovi radikali: tautomerija, elektronska struktura i svojstva

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Razmatrani su ukratko značaj i biološka aktivnost C vitamina i njihovih radikala. S mnogo više detalja prikazani su rezultati kvantno-mehaničkih proračuna ovih važnih spojeva. Posebna pažnja posvećena je strukturnim i elektronskim značajkama dobivenim s pomoću MINDO/3 i MNDO metoda. Predviđeni su ESCA spektri tautomera C vitamina primjenom metode samousklađenoga naboja (SCC-MO). Glavni zaključci teorijskih razmatranja u skladu su s eksperimentalnim rezultatima.