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Tautomeric equilibria for ionized oxamic acid - inhibitor of LDH

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Abstract

Amide-iminol tautomerism was studied for ionized oxamic acid (**OA**$^{+\bullet}$) in the gas phase using the DFT method with the UB3LYP functional and various basis sets {6-31++G(d,p), 6-311+G(d,p), and aug-cc-pVDZ}. Among twenty tautomers-rotamers possible for **OA**$^{+\bullet}$, eleven isomers were found to be thermodynamically stable. Similarly as for the neutral molecule, ionization (**OA** $\rightarrow$ **OA**$^{+\bullet}$ + e) favors the amidization process (amide $\leftarrow$ iminol). Isomerization seems to change solely the conformational preferences. π-Electron delocalization in the NCO and OCO moieties is close to that for n-π conjugated fragments.

Keywords: Oxamic acid; Amide-iminol tautomerism; Ionization; π-Electron delocalization; DFT.

1. Introduction

Intramolecular proton-transfer accompanied by migration of π-electrons is called prototropic tautomerism or simply prototropy [1-3]. It takes place for molecules containing the X=Y group conjugated with the ZH group, X=Y–ZH $\rightleftharpoons$ HX–Y=Z. Prototropy influences the structure of compound, its stability, chemical and biochemical reactivity, and biological activity. The most commonly studied forms of prototropy are keto-enol, amide-iminol, and enamine-imine tautomerism. Generally, the keto, amide, and enamine forms are favored in the gas phase, solution and solid state. Some exceptions are phenols stabilized by resonance or acethylacetone stabilized by intramolecular H-bonds.

Oxamic acid ($H_2NCOCOOH$, **OA**) is a C-carboxyl derivative of formamide (H_2NCOH). Being an inhibitor of lactate dehydrogenase (LDH), it binds specifically with the LDH-NADH complex, and blocks the active center of the enzyme [4-6]. This property opened new possibilities of applications of oxamic acid in biotechnology for selective bioseparation of LDH [7, 8] or in analyses of biomaterials containing LDH [9-11]. To understand interactions of oxamic acid with LDH, it is very important to understand all phenomena that dictate the structure of interacting compound.

Similarly as formamide, oxamic acid exhibits amide-iminol tautomerism (1). Prototropy leads to two tautomeric forms: the amide {H_2N–C(COOH)=O} and iminol {HN=C(COOH)–OH} tautomers. Due to flexibility of the N–C–C–O chain in the amide tautomer, four amide structures are possible (**OA1**–**OA4**, Fig. 1). In the case of the iminol tautomer, rotational isomerism around the single bonds in the O–C–C–O chain and geometrical

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isomerism around the double C=N bond lead to sixteen iminol structures (**OA5**–**OA20**, Fig. 1). These types of isomerism have been already studied for neutral **OA** [12-15].

$H_2N–C(R)=O$ (amide) ⇌ $HN=C(R)–OH$ (iminol) (1)
(R = H or COOH)

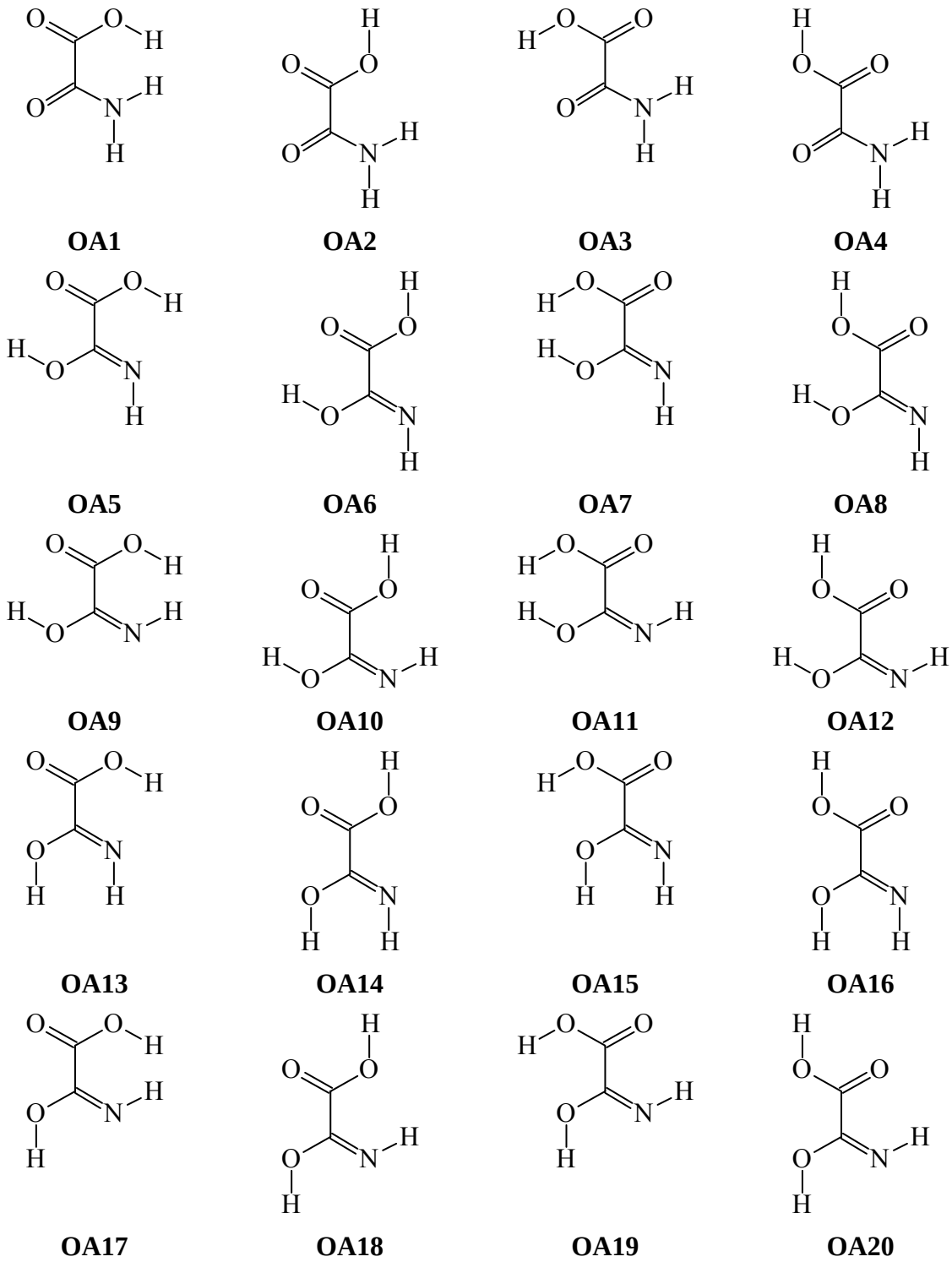

Fig. 1. Possible structures for the amide and iminol tautomers of oxamic acid.

The aim of this paper is to study the effect of ionization (lose of one electron) on amide-iminol conversion for oxamic acid. Since tautomeric equilibria are very difficult or even impossible to investigate by experiment when amounts of less important tautomers are lower than 0.1 %, we applied here quantum-chemical methods. For our investigations, we chose the DFT method [16] with the UB3LYP functional [17] and various basis sets {6-31++G(d,p), 6-311+G(d,p), and aug-cc-pVDZ} [18, 19]. We considered all twenty possible tautomers-rotamers (Fig. 1) for the radical cation of oxamic acid (**OA**$^{+\bullet}$). We analyzed effect of ionization on tautomeric equilibria and on π-electron distribution when going from the neutral to ionized form (**OA** $\rightarrow$ **OA**$^{+\bullet}$ + e). For analysis of π-electron delocalization, we used the HOMED (harmonic oscillator model of electron delocalization [20]) index to geometries optimized at the UB3LYP/6-311+G(d,p) level.

2. Computational details

Geometries of all possible tautomers-rotamers for the radical cation of oxamic acid (Fig. 1) were fully optimized without any symmetry constraints at the DFT(UB3LYP)/6-31++G(d,p) level [16-18]. Two other basis sets {6-311+G(d,p) and aug-cc-pVDZ} [18,19] were also tested for selected isomers. For minima (with real frequencies), the Gibbs free energies ($G = H - TS$) were calculated at 298.15 K using the same levels of theory. The *G* values include changes in the zero-point energy (ZPE) and thermal corrections (vibrational, rotational and translational) to the enthalpy (*H*) and to the entropy (*S*). The tautomeric equilibrium constants (*K*T) for tautomeric conversions were estimated from the Gibbs free energies of the corresponding pairs of tautomers ($1.3643 \cdot pKT = \Delta GT$). The percentage contents of individual tautomers were calculated on the basis of the estimated p*K*T {p*K*T = – log [x/(1-x)]}. Calculations were performed using the Gaussian 03 program [21].

3. Results and discussion

3.1. Thermodynamic stabilities

For neutral oxamic acid, three amide structures (**OA2-OA4**) have been found to be stable at various quantum-chemical levels (HF, MP2, DFT, and G2) [15]. The structure **OA1** is unstable, probably, due to repulsion between the carboxyl OH group and the amide NH group. During optimization **OA1** isomerizes to **OA3**, which has the lowest energy. The Gibbs free energies of **OA2** and **OA4** are larger than that of **OA3** by 2-5 kcal mol^{-1}. Among sixteen iminol isomers possible for neutral oxamic acid in the gas phase, solely two structures (**OA7** and **OA11**) are found to be unstable at the DFT(B3LYP)/6-31++G(d,p) level, probably due to repulsion between the OH groups [15]. During optimization, they isomerize to **OA5** and **OA19**, respectively. All stable iminol isomers have larger Gibbs free energies than that of the most stable amide structure (**OA3**) by more than 10-20 kcal mol^{-1}. **OA5** is the most stable iminol isomer. The use of different methods and different basis sets have no important influence on geometrical and thermodynamic parameters of the neutral amide and iminol isomers. Therefore, we selected the DFT method with the UB3LYP functional and the 6-31++G(d,p) basis set for search of thermodynamically stable isomers of ionized oxamic acid.

DFT calculations showed evidently that ionization of oxamic acid isomers changes their stability. For the amide tautomer, only two structures (**OA3**$^{+\bullet}$ and **OA4**$^{+\bullet}$) are found to be stable at the UB3LYP/6-31++G(d,p) level. The **OA1**$^{+\bullet}$ and **OA2**$^{+\bullet}$ isomers are unstable. During optimization they isomerize to **OA3**$^{+\bullet}$ and **OA4**$^{+\bullet}$, respectively. The amide isomer **OA4**$^{+\bullet}$ has lower Gibbs free energy than **OA3**$^{+\bullet}$ by 2 kcal mol^{-1}. For the iminol tautomer, only nine isomers are stable at the UB3LYP/6-31++G(d,p) level: **OA5**$^{+\bullet}$, **OA9**$^{+\bullet}$, **OA10**$^{+\bullet}$, **OA12**$^{+\bullet}$,

OA13$^{+\bullet}$, **OA15**$^{+\bullet}$, **OA16**$^{+\bullet}$, **OA18**$^{+\bullet}$, and **OA19**$^{+\bullet}$. Other isomers, **OA6**$^{+\bullet}$, **OA7**$^{+\bullet}$, **OA8**$^{+\bullet}$, **OA11**$^{+\bullet}$, **OA14**$^{+\bullet}$, **OA17**$^{+\bullet}$, and **OA20**$^{+\bullet}$, are unstable and during optimization isomerize to **OA10**$^{+\bullet}$, **OA5**$^{+\bullet}$, **OA12**$^{+\bullet}$, **OA9**$^{+\bullet}$, **OA16**$^{+\bullet}$, **OA19**$^{+\bullet}$, and **OA18**$^{+\bullet}$, respectively. **OA10**$^{+\bullet}$ is the most stable iminol isomer for ionized oxamic acid. However, its Gibbs free energy is larger than that of **OA4**$^{+\bullet}$ by 15 kcal mol^{-1}, and it may be considered as rare form in the tautomeric mixture of oxamic acid, similarly as other iminol isomers.

The relative Gibbs free energies calculated at the UB3LYP/6-31++G(d,p) level for all stable ionized isomers of oxamic acid are listed in Table 1. For comparison, the relative Gibbs free energies for the corresponding stable neutral isomers are also given in this Table. The comparison shows that the order of stabilities for the ionized isomers (**OA4**$^{+\bullet}$ > **OA3**$^{+\bullet}$ > **OA10**$^{+\bullet}$ > **OA18**$^{+\bullet}$ > **OA12**$^{+\bullet}$ > **OA19**$^{+\bullet}$ > **OA5**$^{+\bullet}$ > **OA9**$^{+\bullet}$ > **OA16**$^{+\bullet}$ ≈ **OA13**$^{+\bullet}$ > **OA15**$^{+\bullet}$) is not the same as that for the neutral ones (**OA3** > **OA4** > **OA5** > **OA19** > **OA18** > **OA10** > **OA12** > **OA13** > **OA16** > **OA9** > **OA15**). However, ionization favors the amidization process, similarly as for the neutral molecule. Ionization seems to change solely conformational preferences: from **OA3** for the neutral amide tautomer to **OA4**$^{+\bullet}$ for the ionized amide tautomer, and from **OA5** for the neutral iminol tautomer to **OA10**$^{+\bullet}$ for the ionized iminol tautomer. Differences in the relative Gibbs free energies between the most stable neutral iminol and amide tautomers (14.0 kcal mol^{-1}) and between the most stable ionized iminol and amide tautomers (15.0 kcal mol^{-1}) are almost the same. Ionization seems to have no significant effect on tautomeric amide/iminol equilibria. This may suggest that one electron in the most stable amide and iminol radical cations is taken from the COOH group. When one electron is taken from the amide group in the parent system (formamide/formamidic acid), difference in the relative Gibbs free energy between the iminol and amide tautomers is lower than for oxamic acid [22] similarly as for the neutral molecules [23]. For neutral molecule, this difference is six times greater (11.5 kcal mol^{-1} at the G2 level [23]).

Table 1
Comparison of the relative Gibbs energies (ΔG in kcal mol^{-1}) for neutral and ionized isomers of oxamic acid.

Isomer	ΔG[a]	Isomer	ΔG[b]	Isomer	ΔG[a]	Isomer	ΔG[b]
OA3	0.0	**OA3**$^{+\bullet}$	2.0	**OA13**	21.2	**OA13**$^{+\bullet}$	27.3
OA4	3.1	**OA4**$^{+\bullet}$	0.0	**OA15**	25.5	**OA15**$^{+\bullet}$	32.4
OA5	14.0	**OA5**$^{+\bullet}$	25.7	**OA16**	23.8	**OA16**$^{+\bullet}$	27.2
OA9	25.0	**OA9**$^{+\bullet}$	26.3	**OA18**	18.4	**OA18**$^{+\bullet}$	16.9
OA10	19.0	**OA10**$^{+\bullet}$	15.0	**OA19**	17.8	**OA19**$^{+\bullet}$	25.1
OA12	20.3	**OA12**$^{+\bullet}$	22.0				

[a] At the B3LYP/6-31++G(d,p) level, taken from ref. [15].
[b] At the UB3LYP/6-31++G(d,p) level.

3.2. Geometrical parameters

Selected geometrical parameters for all stable amide and iminol isomers of ionized oxamic acid are summarized in Table 2. Generally, rotational and geometrical isomerism, and consequently, intramolecular interactions slightly influence the covalent bond lengths and angles. Some exception is only the C–C bond. The ranges of variations for the C–O, C=O, C–C, C–N, and C=N bond lengths are as follows: 1.28-1.32, 1.19-1.24, 1.50-1.70, 1.31, and 1.24-1.28 Å, respectively. The greatest variations are for the single C–C bond (0.2 Å). For comparison, the lengths of the same types of bonds for neutral isomers vary in the following way: 1.33-1.38,

1.20-1.22, 1.52-1.55, 1.34-1.36, and 1.26-1.27 Å, respectively [15]. The ranges of variations for the O=C–O, O–C–αC, O=C–αC, C–αC=O, C–αC–O, C–αC–N, C–αC=N, O=αC–N, and O–αC=N angles are as follows: 120.0-132.7, 110.4-127.0, 109.8-127.1, 114.3-123.6, 108.7-121.5, 115.4-115.6, 106,5-120.9, 120.7-130.5, and 124.6-136.7°, respectively. They slightly differ from those for neutral isomers: 121.5-124.8, 110.2-118.7, 119.7-126.3, 109.8-116.6, 119,2-122.7, 111.3-114.9, 118.3-128.7, 125.1-126.9, and 120.1-130.1°, respectively [15]. The greatest variations are for the O–C–C, C–C=N, and O–C=N angles. They are larger for ionized than for neutral isomers.

Table 2
Selected geometrical parameters for stable isomers of ionized oxamic acid {H_2N6(O5=)C4(O3=)C2O1H} estimated at the UB3LYP/6-31++G(d,p) level.

covalent bond lengths (in Å)

Isomer	C2O1	C2O3	C2C4	C4O5	C4N6
OA3$^{+\bullet}$	1.28	1.20	1.70	1.21	1.31
OA4$^{+\bullet}$	1.30	1.20	1.63	1.23	1.31
OA5$^{+\bullet}$	1.30	1.20	1.62	1.29	1.26
OA9$^{+\bullet}$	1.30	1.20	1.63	1.29	1.26
OA10$^{+\bullet}$	1.31	1.20	1.59	1.29	1.26
OA12$^{+\bullet}$	1.32	1.19	1.59	1.29	1.28
OA13$^{+\bullet}$	1.29	1.21	1.60	1.32	1.24
OA15$^{+\bullet}$	1.29	1.24	1.50	1.31	1.27
OA16$^{+\bullet}$	1.30	1.23	1.50	1.30	1.28
OA18$^{+\bullet}$	1.31	1.19	1.59	1.30	1.25
OA19$^{+\bullet}$	1.30	1.19	1.63	1.31	1.25

angles (in degree)

Isomer	O1C2O3	O1C2C4	O3C2C4	C2C4O5	C2C4N6	O5C4N6	O3C2C4O5
OA3$^{+\bullet}$	120.0	112.9	127.1	123.6	115.6	120.7	180.0
OA4$^{+\bullet}$	132.7	110.5	116.8	114.3	115.4	130.5	180.0
OA5$^{+\bullet}$	127.5	119.4	113.1	114.4	118.1	127.3	8.0
OA9$^{+\bullet}$	127.5	119.7	112.7	114.3	120.9	124.6	17.5
OA10$^{+\bullet}$	131.8	111.7	116.5	116.2	118.8	125.0	0.4
OA12$^{+\bullet}$	131.6	106.3	122.0	119.9	117.0	123.0	160.8
OA13$^{+\bullet}$	125.8	118.3	115.9	109.2	114.1	136.7	0.0
OA15$^{+\bullet}$	123.2	127.0	109.8	119.4	106.5	134.0	180.0
OA16$^{+\bullet}$	128.6	119.7	111.7	121.5	106.8	131.7	180.0
OA18$^{+\bullet}$	131.1	110.4	118.6	111.5	118.9	129.6	0.0
OA19$^{+\bullet}$	128.9	115.3	115.8	108.7	120.7	130.4	125.2

To study the effect of ionization on geometrical parameters of particular isomers of oxamic acid, two stable amide isomers (**OA3** and **OA4**) and two stable iminol isomers (**OA5** and **OA10**) were considered here. The selected geometrical parameters for the neutral [15] and ionized forms, calculated at the same level of theory {(B3LYP)/6-31++G(d,p) and UB3LYP/6-31++G(d,p)}, are given in Table 3. The comparison provides the following information. Generally, ionization lengthens the central C–C bond for both the amide and iminol isomers. Except the C4O5 bond for **OA4$^{+\bullet}$**, ionization shortens the single and double CX bonds. The

shortening effect of the CX bonds is, however, considerably smaller (< 0.5 Å) than the lengthening effect of the C–C bond (0.07-0.15 Å).

Table 3
Comparison of bond lengths (in Å) and angles (in degree) for the same neutral and ionized amide and iminol forms of oxamic acid

amide isomers						
Bond length	**OA3**[a]	**OA3**$^{+\bullet}$[b]	Δ[c]	**OA4**[a]	**OA4**$^{+\bullet}$[b]	Δ[c]
C2O1	1.332	1.285	-0.047	1.334	1.299	-0.035
C2O3	1.209	1.196	-0.013	1.215	1.198	-0.017
C2C4	1.548	1.697	0.149	1.547	1.627	0.080
C4O5	1.230	1.213	-0.017	1.218	1.228	0.010
C4N6	1.343	1.308	-0.035	1.355	1.308	-0.047
Angle						
O1C2C4	111.9	112.9	1.0	112.3	110.5	-1.8
O1C2O3	124.8	120.0	-4.8	124.6	132.7	8.1
O3C2C4	123.2	127.1	3.9	123.0	116.8	-6.2
C2C4O5	119.2	123.6	4.4	122.7	114.3	-8.4
O5C4N6	126.9	120.7	-6.2	126.0	130.5	4.5
C2C4N6	113.9	115.6	1.7	111.3	115.2	3.9
O3C2C4O5	180.0	180.0	0.0	180.0	180.0	0.0
iminol isomers						
Bond length	**OA5**[a]	**OA5**$^{+\bullet}$[b]	Δ[c]	**OA10**[a]	**OA10**$^{+\bullet}$[b]	Δ[c]
C2O1	1.328	1.298	-0.030	1.341	1.310	-0.031
C2O3	1.214	1.201	-0.013	1.215	1.198	-0.017
C2C4	1.524	1.617	0.093	1.523	1.591	0.068
C4O5	1.339	1.291	-0.048	1.345	1.293	-0.052
C4N6	1.270	1.260	-0.010	1.267	1.257	-0.010
Angle						
O1C2C4	120.5	113.1	-7.4	122.2	116.5	-5.7
O1C2O3	124.8	127.5	2.7	124.4	131.8	7.4
O3C2C4	114.7	119.4	4.7	113.4	111.7	-1.7
C2C4O5	114.2	114.4	0.2	112.1	116.2	4.1
O5C4N6	127.5	127.3	-0.2	121.3	125.0	3.7
C2C4N6	118.3	118.1	-0.2	126.6	118.8	-7.8
O3C2C4O5	0.0	8.0	8.0	0.0	0.4	0.4

[a] At the B3LYP/6-31++G(d,p) level as in ref. [15].
[b] At the UB3LYP/6-31++G(d,p) level.
[c] Difference between geometrical parameters of the ionized and neutral forms.

For the amide isomers, the smallest variations occur for the O1C2C4 (**OA3**$^{+\bullet}$ and **OA4**$^{+\bullet}$) and C2C4N6 (**OA3**$^{+\bullet}$) angles. The strongest reduction ionization effect is observed for the O5C4N6 (**OA3**$^{+\bullet}$), O3C2C4 (**OA4**$^{+\bullet}$), and C2C4O5 (**OA4**$^{+\bullet}$) angles. The strongest augmentation ionization effect takes place for the C2C4O5 (**OA3**$^{+\bullet}$), O3C2C4 (**OA3**$^{+\bullet}$), and O1C2O3 (**OA4**$^{+\bullet}$) angles. For the iminol isomers, the smallest effect is found for the O3C2C4 (**OA10**$^{+\bullet}$) angle and for angles around the C4 atom (**OA5**$^{+\bullet}$). The strongest reduction ionization effect occurs for the O1C2C4 (**OA5**$^{+\bullet}$ and **OA10**$^{+\bullet}$) and C2C4N6 (**OA10**$^{+\bullet}$) angles. The

strongest augmentation ionization effect takes place for the $O^3C^2C^4$ (**OA5**$^{+\bullet}$) and $O^1C^2O^3$ (**OA10**$^{+\bullet}$) angles. These variations of angles depend on conformation and intramolecular interactions. Intramolecular interactions possible for oxamic acid are also responsible for planarity of both the neutral and ionized isomers. Hence, the dihedral $O^3C^2C^4O^5$ angle does not vary very much ($\Delta < 10°$).

3.3. π-Electron delocalization

To describe quantitatively π-electron delocalization in various cyclic and acyclic systems, the geometry-based HOMA (Harmonic Oscillator Model of Aromaticity) index was defined more than 30 years ago [24], and reformulated in 1993 [25]. Since different measures were employed for the CX bonds in the reformulated HOMA index [25], its applications to heterocompounds have led to unexpected results [3]. Taking these discrepancies into account, we applied here the geometry-based HOMED index [20]. The HOMED index was defined in this way that it could measure any type of resonance effect, such as σ-π hyperconjugation, n-π conjugation, π-π conjugation, and also aromaticity, possible for π-electron systems [20].

The HOMED index can be estimated according to equation (2), where n is the number of bonds taken into account, R_o is the appropriate optimum bond length, R_i is the real bond length, and α is the normalization constant $\{\alpha = 2\cdot[(R_o - R_s)^2 + (R_o - R_d)^2]^{-1}$ for the even number of bonds, i.e., the same number of single and double bonds; R_s and R_d are the reference single and reference double bond lengths$\}$. The following R_s, R_d, and R_o values (in Å), calculated at the B3LYP/6-311+G(d,p) level were taken here [20]: 1.5300 (ethane), 1.3288 (ethene) and 1.3943 (benzene) for the CC bonds, 1.4658 (methylamine), 1.2670 (methylimine) and 1.3342 (1,3,5-triazine) for the CN bonds, and 1.4238 (methanol), 1.2017 (formaldehyde) and 1.2811 (protonated carbonic acid) for the CO bonds. On the basis of these R values, the normalization α constants equal to 88.09, 91.60, and 75.00 were used for the CC, CN, and CO bonds, respectively.

$$\mathrm{HOMED} = 1 - [\alpha\cdot\Sigma(R_o - R_i)^2]/n \qquad (2)$$

The HOMED indices were estimated for two n-π conjugated fragments of ionized oxamic acid, the carboxylic (OCO) and amide/iminol (OCN) groups. The two most stable amide isomers (**OA3**$^{+\bullet}$ and **OA4**$^{+\bullet}$) and the two most stable iminol isomers (**OA10**$^{+\bullet}$ and **OA18**$^{+\bullet}$) of ionized oxamic acid, optimized at the UB3LYP/6-311+G(d,p) level, were selected for the HOMED estimation. The use of the same level of theory for ionized oxamic acid as for the reference molecules has this advantage that the computational errors may cancel out in the procedure of the HOMED estimation [20]. For the ionized amide **OA3**$^{+\bullet}$ and **OA4**$^{+\bullet}$ isomers, the estimated HOMED indices are equal to 0.6412 and 0.6665 for the carboxylic group, and to 0.0.7125 and 0.8000 for the amide group, respectively. For the ionized iminol **OA10**$^{+\bullet}$ and **OA18**$^{+\bullet}$ isomers, the estimated HOMED indices are equal to 0.6582 for the carboxylic group, and to 0.0.6806 and 0.6399 for the iminol group, respectively. All estimated HOMED values are close to those estimated for the most stable neutral tautomers of oxamic acid (0.6756 and 0.8653 for **OA3**, and 0.7152 and 0.6696 for **OA5**) [15]. They are typical for the n-π conjugated fragments ($0.5 <$ HOMED < 1) [20].

Table 4
DFT thermodynamic parameters (in kcal mol^{-1}) for selected amide→iminol conversions in ionized oxamic acid.

Basis set	Conversion	ΔH_T	$T\Delta S_T$	ΔG_T	K_T
6-31++G(d,p)	**OA3$^{+\bullet}$→OA10$^{+\bullet}$**	14.0	1.0	13.0	$3.0\cdot10^{-10}$
	OA3$^{+\bullet}$→OA18$^{+\bullet}$	15.0	0.2	14.8	$1.4\cdot10^{-11}$
	OA4$^{+\bullet}$→OA10$^{+\bullet}$	16.2	1.2	15.0	$9.6\cdot10^{-12}$
	OA4$^{+\bullet}$→OA18$^{+\bullet}$	17.2	0.3	16.9	$4.3\cdot10^{-13}$
6-311+G(d,p)	**OA3$^{+\bullet}$→OA10$^{+\bullet}$**	14.8	0.4	14.4	$2.9\cdot10^{-11}$
	OA3$^{+\bullet}$→OA18$^{+\bullet}$	15.6	0.0	15.6	$3.8\cdot10^{-12}$
	OA4$^{+\bullet}$→OA10$^{+\bullet}$	16.6	0.6	16.0	$1.8\cdot10^{-12}$
	OA4$^{+\bullet}$→OA18$^{+\bullet}$	17.4	0.2	17.2	$2.4\cdot10^{-13}$
aug-cc-pVDZ	**OA3$^{+\bullet}$→OA10$^{+\bullet}$**	14.3	0.2	14.0	$5.3\cdot10^{-11}$
	OA3$^{+\bullet}$→OA18$^{+\bullet}$	15.6	0.2	15.4	$5.4\cdot10^{-12}$
	OA4$^{+\bullet}$→OA10$^{+\bullet}$	15.9	0.4	15.5	$4.6\cdot10^{-12}$
	OA4$^{+\bullet}$→OA18$^{+\bullet}$	17.2	0.4	16.8	$4.7\cdot10^{-13}$

3.4. Amide-iminol conversion

Four isomers, the two most stable amide structures (**OA3$^{+\bullet}$** and **OA4$^{+\bullet}$**) and the two most stable iminol structures (**OA10$^{+\bullet}$** and **OA18$^{+\bullet}$**), were selected for investigations of amide-iminol tautomerism in ionized oxamic acid. For DFT(UB3LYP) calculations, various basis sets were employed, 6-31++G(d,p), 6-311+G(d,p), and aug-cc-pVDZ. Table 4 summarizes thermodynamic parameters such as the enthalpy of tautomerization (ΔH_T), its entropy term ($T\Delta S_T$), its Gibbs free energy (ΔG_T), and the tautomeric equilibrium constant (K_T) estimated for all possible tautomeric amide→iminol conversions between the selected isomers: **OA3$^{+\bullet}$** → **OA10$^{+\bullet}$**, **OA3$^{+\bullet}$** → **OA18$^{+\bullet}$**, **OA4$^{+\bullet}$** → **OA10$^{+\bullet}$**, and **OA4$^{+\bullet}$** → **OA18$^{+\bullet}$**. Estimations show that the change of the basis set has only a slight influence on the thermodynamic parameters. The amide isomers (**OA3$^{+\bullet}$** and **OA4$^{+\bullet}$**) evidently dominate in the tautomeric mixture of ionized oxamic acid (100%). The percentage contents of the most stable iminol isomers (**OA10$^{+\bullet}$** and **OA18$^{+\bullet}$**) are lower than $1\cdot10^{-9}$%. At each level of theory, the entropy terms are close to zero, indicating that the amide-iminol tautomerization process is isoentropic similarly as other proton-transfer reactions in the gas phase [3,26].

The favored route of amidization from the iminol isomer **OA10$^{+\bullet}$** to the amide isomer **OA4$^{+\bullet}$** may be as follows: **OA10$^{+\bullet}$** → **OA12$^{+\bullet}$** → **OA18$^{+\bullet}$** → {**TS-OA18$^{+\bullet}$/OA4$^{+\bullet}$**} → **OA4$^{+\bullet}$**. The direct tautomeric conversion may take place between the iminol **OA18$^{+\bullet}$** isomer and the amide **OA4$^{+\bullet}$** isomer, where **TS-OA18$^{+\bullet}$/OA4$^{+\bullet}$** is the transition state for this step. The other route of isomerization from **OA10$^{+\bullet}$** to **OA3$^{+\bullet}$** is also possible: **OA10$^{+\bullet}$** → **OA12$^{+\bullet}$** → **OA18$^{+\bullet}$** → **OA19$^{+\bullet}$** → {**TS-OA19$^{+\bullet}$/OA3$^{+\bullet}$**} → **OA3$^{+\bullet}$**. In this case, the direct tautomeric conversion may occur between the amide **OA3$^{+\bullet}$** isomer and the iminol **OA19$^{+\bullet}$** isomer, where **TS-OA19$^{+\bullet}$/OA3$^{+\bullet}$** is the transition state for this step. The relative energies between all these stable isomers (except the transition states) are not larger than 25 kcal mol^{-1}.

Table 5
DFT thermodynamic parameters (in kcal mol-1) for ionization of oxamic acid.

Ionization	ΔE_i [a]	ΔH_i [b]	$T\Delta S_i$ [b]	ΔG_i [b]
OA3 → **OA3**$^{+\bullet}$ + e	231.0	231.2	1.1	230.2
OA4 → **OA4**$^{+\bullet}$ + e	225.5	225.5	0.4	225.1
OA5 → **OA5**$^{+\bullet}$ + e	240.6	241.1	1.3	239.8
OA9 → **OA9**$^{+\bullet}$ + e	230.0	230.4	0.9	229.4
OA10 → **OA10**$^{+\bullet}$ + e	225.6	226.2	2.1	224.1
OA12 → **OA12**$^{+\bullet}$ + e	230.6	225.9	1.1	229.8
OA13 → **OA13**$^{+\bullet}$ + e	235.0	235.4	1.2	234.2
OA15 → **OA15**$^{+\bullet}$ + e	235.0	235.1	0.2	235.0
OA16 → **OA16**$^{+\bullet}$ + e	231.2	231.4	-0.2	231.5
OA18 → **OA18**$^{+\bullet}$ + e	227.1	227.5	0.8	226.6
OA19 → **OA19**$^{+\bullet}$ + e	236.6	237.1	1.7	235.4

[a] At 0 K, ZPE included.
[b] At 298.15 K, thermal corrections included.

3.5. Ionization energies

In the presence of ionizing agents or during positive ionization in the mass spectrometer, neutral oxamic acid may loss one electron. Consequently, it may be transferred to its ionized (oxidized) state (**OA** → **OA**$^{+\bullet}$ + e). For each individual isomer, the thermodynamic parameters (ΔE_i, ΔH_i, $T\Delta S_i$, and ΔG_i) for the ionization process can be estimated as differences between the parameters of the corresponding species, **OA**$^{+\bullet}$ and **OA** (Table 5). The estimations {B3LYP/6-31++G(d,p)} show that the relative thermal corrections for the electron-transfer process of oxamic acid are close to zero, and thus, $\Delta E_i \approx \Delta H_i \approx \Delta G_i$. The entropy terms are also close to zero, indicating that this process is isoentropic in the gas phase similarly as the proton-transfer conversion. Ionization is a very endothermic process and requires more than 200 kcal mol-1. Indeed, the experimental ionization energy for oxamic acid is 242.4 kcal mol-1 [26]. For comparison, the experimental ionization energy for formamide is equal to 234.3 kcal mol-1, and for formic acid is equal to 261.3 kcal mol-1 [26].

4. Conclusions

DFT calculations performed for twenty isomers of ionized oxamic acid showed that the amide isomers dominate in the tautomeric mixture, similarly as for the neutral molecule. Ionization seems to change solely the conformational preference when going from the neutral (**OA3**) to ionized form (**OA4**$^{+\bullet}$). Change of the basis set has no significant effect on thermodynamic parameters of the amide/iminol conversion. Similar change of the conformational preference takes place for the rare iminol isomers when proceeding from the neutral (**OA5**) to ionized form (**OA10**$^{+\bullet}$). There are no structural data for ionized oxamic acid (radical cation) in the literature and no comparison can be made.

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