

Genotoxic halofuranones in water: isomerization and acidity of mucohalic acids

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Among the genotoxic halofuranones formed by chlorination in water are mucochloric acid (MCA, 3,4-dichloro-5-hydroxyfuran-2(5H)-one) and mucobromic acid (MBA, 3,4-dibromo-5-hydroxyfuran-2(5H)-one). These acids are direct genotoxins and potential carcinogens, with the capacity to alkylate the DNA bases. In recent years, they have also attracted attention in the synthesis of furanone derivatives. Mucohalic acids (MXA) exist in solution as an equilibrium between three species; a cyclic lactone-lactol (MXA_{cl}), an open-chain aldehyde-acid (MXA_{op}^o), and the dissociated form of the latter (MXA_{op}^-). The distribution of the three species in the equilibrium has synthetic, toxicological, and environmental implications owing to their different functionalization. The case of the neutral open-chain form is of special interest, since it is expected to be highly reactive. We have experimentally determined the apparent dissociation constant of the cyclic species ($K_a^{app} = [H^+][MXA_{op}^-]/[MXA_{cl}]$). Their values (pK_a^{app} (MCA) = 3.95 and pK_a^{app} (MBA) = 4.27) suggest that at neutral pH MXA are mostly present as the dissociated carboxylate-aldehyde. The dissociation constant of the open-chain neutral species ($K_a = [H^+][MXA_{op}^-]/[MXA_{op}^o]$) and the cyclization equilibrium constant ($K_{iso} = [MXA_{op}^o]/[MXA_{cl}]$) were determined in water and organic solvents, using density functional theory and *ab initio* methods. The results suggest that the undissociated aldehyde is a minor species at any given pH. The structure of MXA in solution and the influence of the level of theory on the calculated geometry are discussed. Copyright © 2011 John Wiley & Sons, Ltd.

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Keywords: cyclization; dissociation constant; equilibrium constant; mucohalic; mucochloric; pK_a

INTRODUCTION

Chlorination of humic substances in water gives rise to a variety of genotoxins such as halofuranones.^[1–4] The considerable contribution of this group of compounds to the observed mutagenicity of chlorinated tap water has received widespread attention.^[5,6] Mucochloric acid (MCA, 3,4-dichloro-5-hydroxyfuran-2(5H)-one) and mucobromic acid (MBA, 3,4-dibromo-5-hydroxyfuran-2(5H)-one) are two halohydroxyfuranones that have been shown to be direct genotoxins and potential carcinogens.^[7–14] DNA adducts of mucohalic acids (MXA) are known, with the isolation of etheno, oxalo etheno, and halopropenal derivatives of guanosine, adenosine, and cytosine.^[15–22]

In recent years, MXA have also attracted much attention in organic synthesis owing to their high functionalization and availability, and they have been used in the synthesis of heterocycles such as gamma-substituted gamma-butenolides and gamma-lactams, and other furanone derivatives.^[23–35]

MXA exist in solution as an equilibrium between two species:^[36–40] the cyclic lactone-lactol form (MXA_{cl}) and the open-chain aldehyde-acid form (MXA_{op}^o). Because the open-chain form is a carboxylic acid, in certain solvents, such as water, it is also in equilibrium with its dissociated form (MXA_{op}^-). MXA_{op}^- and MXA_{op}^o are aldehydes and thus, in aqueous solution both are also in equilibrium with their respective hydrates (MXA_{hyd}^- and MXA_{hyd}^o).

The equilibrium distribution of the species has synthetic, toxicological, and environmental implications, since their functionalization, and hence their reactivity, is very different. From the ecotoxicological point of view, MXA_{op}^- and MXA_{op}^o are of interest,

since the aldehyde group is known to react with DNA. Because the negative charge in MXA_{op}^- reduces the electrophilicity of the electron-deficient carbonyl group, the concentration of the highly reactive neutral MXA_{op}^o in aqueous solution (as under physiological conditions), albeit low, is of great importance.

The increasing interest in these molecules, from both the synthetic and toxicological points of view demands further knowledge of their aqueous reactivity, and to our knowledge the equilibria of MXA in solution have not been addressed in quantitative chemical terms. Thus, here we are prompted to address these issues from both the experimental and computational points of view.

EXPERIMENTAL AND COMPUTATIONAL SECTION

Experimental spectroscopic study

In acidic aqueous solution, the major species is known to be the cyclic halofuranone, which suggests that in the ring-closure

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reaction (K_{iso}) MXA_{cl} is the predominant form. When pH is increased, $\text{MXA}_{\text{op}}^{\circ}$ undergoes deprotonation, forming $\text{MXA}_{\text{op}}^{-}$ (K_{a}), and the global equilibrium is shifted to the right. Thus, $\text{MXA}_{\text{op}}^{\circ}$ and its hydrate, $\text{MXA}_{\text{hyd}}^{\circ}$, are expected to be minor species.

In organic solvents, in which $\text{MXA}_{\text{op}}^{\circ}$ cannot undergo dissociation, only the isomerization equilibrium is observed and MXA_{cl} is always major.

Methodology

An apparent acid dissociation constant exists between protonated and unprotonated species:

$$K_{\text{a}}^{\text{app}} = \frac{([\text{MXA}_{\text{op}}^{-}] + [\text{MXA}_{\text{hyd}}^{-}])[H^{+}]}{([\text{MXA}_{\text{op}}^{\circ}] + [\text{MXA}_{\text{hyd}}^{\circ}] + [\text{MXA}_{\text{cl}}])} \quad (1)$$

This can be rewritten in terms of the four microscopic equilibrium constants depicted in Scheme 1.

$$K_{\text{iso}} = \frac{[\text{MXA}_{\text{op}}^{\circ}]}{[\text{MXA}_{\text{cl}}]} \quad (2)$$

$$K_{\text{a}} = \frac{[\text{MXA}_{\text{op}}^{-}][H^{+}]}{[\text{MXA}_{\text{op}}^{\circ}]} \quad (3)$$

$$K_{\text{hyd}}^{-} = \frac{[\text{MXA}_{\text{hyd}}^{-}]}{[\text{MXA}_{\text{op}}^{-}]} \quad (4)$$

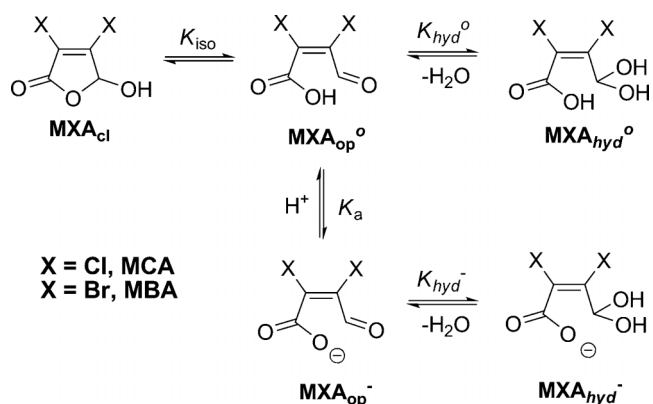
$$K_{\text{hyd}}^{\circ} = \frac{[\text{MXA}_{\text{hyd}}^{\circ}]}{[\text{MXA}_{\text{op}}^{\circ}]} \quad (5)$$

as

$$K_{\text{a}}^{\text{app}} = \frac{K_{\text{a}}(1 + K_{\text{hyd}}^{-})}{(1 + K_{\text{hyd}}^{\circ} + \frac{1}{K_{\text{iso}}})} \quad (6)$$

Since all the equilibria are relatively fast, spectrophotometric titration of MXA and fitting of the spectroscopic data to Eqn (7) affords $K_{\text{a}}^{\text{app}}$.

$$\text{p}K_{\text{a}}^{\text{app}} = \text{pH} - \log \frac{[\varepsilon - \varepsilon_{\text{bas}}]}{[\varepsilon_{\text{acid}} - \varepsilon]} \quad (7)$$



Scheme 1. Forms of mucohalic acids in equilibrium

^1H NMR spectroscopy is more sensitive than UV-Vis spectrophotometry to faster equilibria and thus, the equilibrium constant measured by nuclear magnetic resonance (NMR) titration is that of the faster equilibrium between MXA_{cl} and $\text{MXA}_{\text{op}}^{-}$, excluding the hydrate forms (see below).

$$\text{p}K_{\text{NMR}}^{\text{app}} = -\log \frac{K_{\text{a}}}{(1 + \frac{1}{K_{\text{iso}}})} = \text{pH} - \log \frac{[\delta - \delta_{\text{bas}}]}{[\delta_{\text{acid}} - \delta]} \quad (8)$$

Experimental procedure

MCA (99%) and MBA (99%) were purchased from Aldrich.

UV-Vis spectra measurements were carried out on a Shimadzu UV-2401-PC spectrophotometer equipped with a thermoelectric six-cell holder temperature control system (± 0.1 °C). The temperature of the reaction mixtures was kept constant (± 0.05 °C) with a Lauda Ecoline RE120 thermostat. ^1H NMR spectra were recorded with a Bruker 400 MHz in D_2O .

Spectrophotometric titration of the MXA was carried out by adding concentrated HCl dropwise to 200 cm^3 of MXA at $\sim 1 \times 10^{-4} \text{ M}$, starting at $\text{pH} \approx 7$. Because the total added volume did not exceed 1.0 cm^3 , the effect of dilution was not taken into account. Ionic strength was fixed at 0.10 M with NaNO_3 .

Computational calculation of equilibrium constants

General remarks

The computational calculation of equilibrium constants (K) in solution is very demanding.^[41] It may be seen from the definition of K (Eqn (9)) that an error of 5.7 kJ mol^{-1} in ΔG° results in a deviation of 1 unit in $\log K$.

$$\log K = -\frac{\Delta G^{\circ}}{RT \ln 10} = -\frac{G_{\text{products}} - G_{\text{reactants}}}{RT \ln 10} \quad (9)$$

Gas-phase free energies are usually calculated using high accuracy *ab initio* methods. The largest error lies in solution energies, which are often calculated using Self Consistent Reaction Field (SCRF) methods.

Calculation of K_{iso}

By applying Eqn (9) to the equilibrium constant K_{iso} Eqn (10) is obtained.

$$\text{p}K_{\text{iso}} = -\log K_{\text{iso}} = \frac{\Delta G_{\text{iso}}}{RT \ln 10} = \frac{(G_{\text{MXA}_{\text{op}}^{\circ}} - G_{\text{MXA}_{\text{cl}}})}{RT \ln 10} \quad (10)$$

Calculation of K_{a}

The direct computational calculation of the acid dissociation constants in the $\text{AH} \rightleftharpoons \text{A}^{-} + \text{H}^{+}$ reaction is not easy. The free energy of solvation of the hydronium ion cannot be calculated by simple methods, and PCM approaches underestimate it, such that experimental values must be used. Also, the short-range solvent-solute interactions (hydrogen bonding, ion-dipole, etc), which are important in the case of ionic solutes, are not rigorously modeled and the method therefore is likely to introduce systematic errors.

A common approach to correct these errors is the use of a relative method; $\text{AH} + \text{B}^{-} \rightleftharpoons \text{A}^{-} + \text{BH}$.^[42-50] A proton-exchange reaction with a species (BH) similar to AH, whose $\text{p}K_{\text{a}}$ is well

known experimentally is used, and hence it is not necessary to know the solvation energy of H^+ .

The pK_a (Eqn (3)) is calculated via Eqns (11) and (12), using trichloroacetic acid as a reference, such that $pK_a(BH) = 0.7$.

$$pK_a(AH) = -\log K_a(AH) = pK_a(BH) + \frac{\Delta G^\circ}{RT \ln 10} \quad (11)$$

$$\Delta G^\circ = G(BH) + G(A^-) - G(B^-) - G(AH) \quad (12)$$

Computational details

All calculations were performed using Gaussian 03.^[51] Reaction free energies were obtained at the DFT-B3LYP 6-31++G(d,p),^[52,53] DFT-B3LYP 6-31++G(2df,2pd), MP2 6-31++G(d,p), CBS-QB3, and G3MP2B3 levels of theory and all structures were checked to be true minima (zero imaginary frequencies). The presence of two halogen atoms, containing many electrons, and especially in the case of bromine, hinders the use of larger basis sets or higher levels of theory.

The effect of solvation on structure and energy was computed via the SCRF keyword, using the Gaussian 03 default Integral Equation Formalism for the Polarizable Continuum Model (IEF-PCM).^[54–56]

RESULTS AND DISCUSSION

Experimental study of the open-chain and cyclic forms

Figure 1 shows the spectra of MCA and MBA at various pH. Absorbance values were measured at the wavelengths where MXA_{cl} and MXA_{op} showed maximum absorption; $\lambda = 231.0$ and 263.0 nm for MCA; 245.0 and 276.0 nm for MBA.

Figure 2 shows the excellent fit of the variation in absorbance with pH to Eqn (7). The pK_a^{app} values shown in Table 1 were calculated from the fits at the two wavelengths.

The variation with pH in the 1H NMR spectra of MXA, composed of only one major signal (Fig. 3), reveals a displacement from the chemical shift of the dihalohydroxyfuranone, MXA_{cl} , ($\delta_{MCA} = 6.198$ ppm and $\delta_{MBA} = 6.198$ ppm) to that of the aldehyde, MXA_{op}^- , ($\delta_{MCA} = 9.696$ ppm; $\delta_{MBA} = 9.409$ ppm).

A smaller peak corresponding to the hydrate of the aldehyde group MXA_{hyd}^- ($\delta_{MCA} = 6.110$ ppm; $\delta_{MBA} = 5.715$ ppm) is observed at high pH, where the linear aldehyde is the most abundant species (see below).

The fitting of these results to Eqn (8) affords $pK_{NMR}^{app} = 4.06 \pm 0.10$ for MCA and $pK_{NMR}^{app} = 4.34 \pm 0.10$ for MBA at 25.0 °C.

As has been stated, K_{iso} (see below for a quantitative discussion) is small, so that $1/K_{iso}$ dominates the denominator in both Eqns (6) and (8) (pK_{hyd}^o is estimated^[57] to be close to 0 and thus, negligible).

$$K_a^{app} = \frac{K_a(1 + K_{hyd}^-)}{(1 + K_{hyd}^o + \frac{1}{K_{iso}})} \simeq K_a K_{iso} (1 + K_{hyd}^-) \simeq K_{NMR}^{app} (1 + K_{hyd}^-) \quad (13)$$

K_{hyd}^- has been determined previously to be 0.23 for MCA and 0.17 for MBA.^[58] These values can be combined with pK_{NMR}^{app} to calculate pK_a^{app} using NMR measurements. Results ($pK_a^{app} = 3.97 \pm 0.10$ and 4.27 ± 0.10) are in excellent agreement with the spectrophotometric measurements.

Fitting K_a^{app} values to the van't Hoff equation (Eqn (14)) affords the reaction enthalpies (Table 2), which are positive and small.

$$\frac{\partial \ln K_a^{app}}{\partial (\frac{1}{T})} = \Delta H_{prot} \quad (14)$$

The apparent acid dissociation constants of MXA_{cl} to form MXA_{op}^- , defined as in Eqn (1), are around 10^{-4} . The values for MCA and MBA are very similar and the greater apparent acidity of MCA is consistent with the higher electronegativity of chlorine.

These values suggest that at the approximately neutral pH of tap water or most natural waters MXA are present, by about 10^3 to 1, as MXA_{op}^- rather than MXA_{cl} . In addition, at physiological pH, MXA are mostly in their open-chain dissociated form and can therefore react through the aldehyde group with nucleophilic sites in macromolecules.

The amount of MXA_{op}^o present in the equilibrium cannot be determined experimentally, and was therefore addressed using a computational approach.

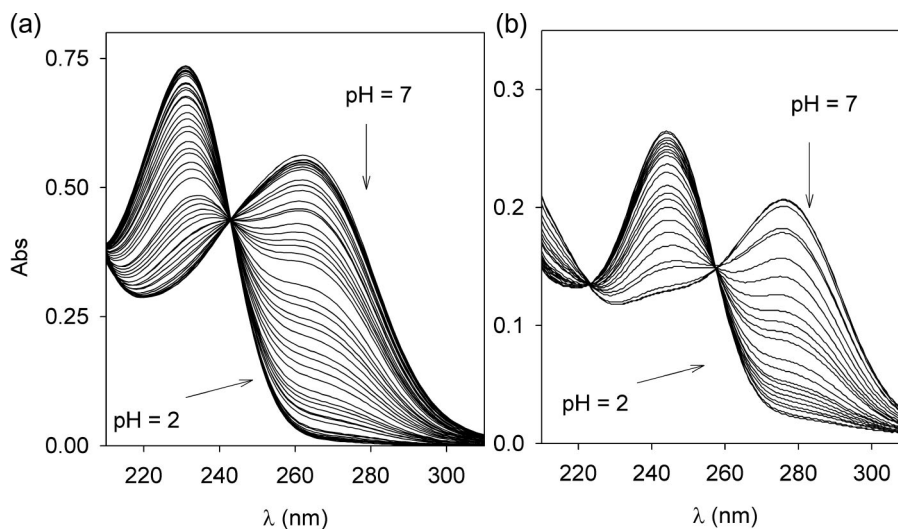


Figure 1. Variation in the UV-Vis spectra of MXA with pH, $T = 25.0$ °C: (a) $X = Cl$, $[MCA] = 1.0 \times 10^{-4}$ M; (b) $X = Br$, $[MBA] = 3.0 \times 10^{-5}$ M

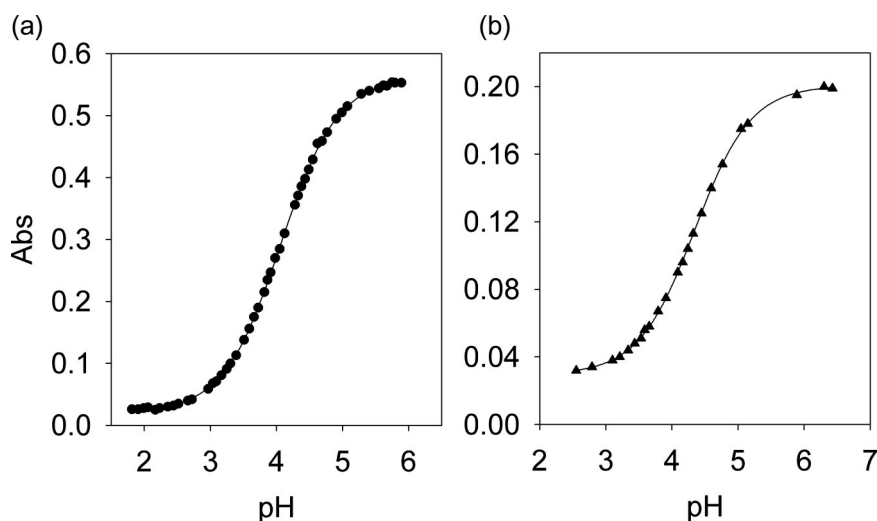


Figure 2. Fit of experimental data to Eqn (7). $T = 25.0\text{ }^{\circ}\text{C}$: (a) $X = \text{Cl}$, $[\text{MCA}] = 1.0 \times 10^{-4}\text{ M}$, $\lambda = 263\text{ nm}$; (b) $X = \text{Br}$, $[\text{MBA}] = 3.0 \times 10^{-5}\text{ M}$, $\lambda = 276\text{ nm}$

Computational study of the open-chain and cyclic forms

Since the separation of K_a^{app} into the two main contributions of K_{iso} and K_a is very interesting but experimentally unattainable, it was approached using computational methods by calculating K_{iso} and K_a separately. To do so, we calculated the optimized

energies and geometries of the compounds at several levels of theory using IEF-PCM for solvation.

Structure of mucohalic acids in solution

MXA_{Cl} were calculated to have planar structures at all levels of theory, in both water and organic solvents (DMSO and heptanes) (Fig. 4). In $\text{MXA}_{\text{op}}^{\circ}$ and $\text{MXA}_{\text{op}}^{-}$, the $\text{CHO}-\text{C}=\text{C}=\text{C}$ atoms lie on a plane due to double bond conjugation, but the carboxylic/carboxylate group is rotated out, because the decrease in π conjugation energy is compensated by a decreased repulsion between the aldehydic hydrogen and the carboxylic oxygen. The dihedral angles are shown in Table 3 and, with one exception (MP2 for $\text{MXA}_{\text{op}}^{-}$), the values for the same compound are consistent within the different methods in the three solvents studied ($\pm 5^{\circ}$).

Interestingly, the calculated dihedral angle ($\text{C}=\text{C}-\text{C}-\text{O}$) in $\text{MXA}_{\text{op}}^{-}$ depends on the level of theory used. When the correlation energy is not taken into account (DFT-B3LYP), the carboxylate group is almost perpendicular to the plane formed by the rest of the molecule, but when MP2 is used a significant deviation from the right angle is observed.

When the dihedral angle is fixed at certain values, a plot like the one shown in Fig. 5 is obtained. It may be observed that, as

Table 1. Experimental values for $\text{p}K_a^{\text{app}}$		
$T\text{ (}^{\circ}\text{C)}$	$\text{p}K_a^{\text{app}}$ ^a	
	MCA	MBA
15.0	4.06 ± 0.01	4.35 ± 0.01
20.0	4.02 ± 0.01	4.31 ± 0.02
25.0	3.95 ± 0.02	4.27 ± 0.02
30.0	3.90 ± 0.02	4.22 ± 0.02
35.0	3.86 ± 0.01	4.19 ± 0.02

^a Values are given with the standard deviation obtained from the fit to Eqn (7).

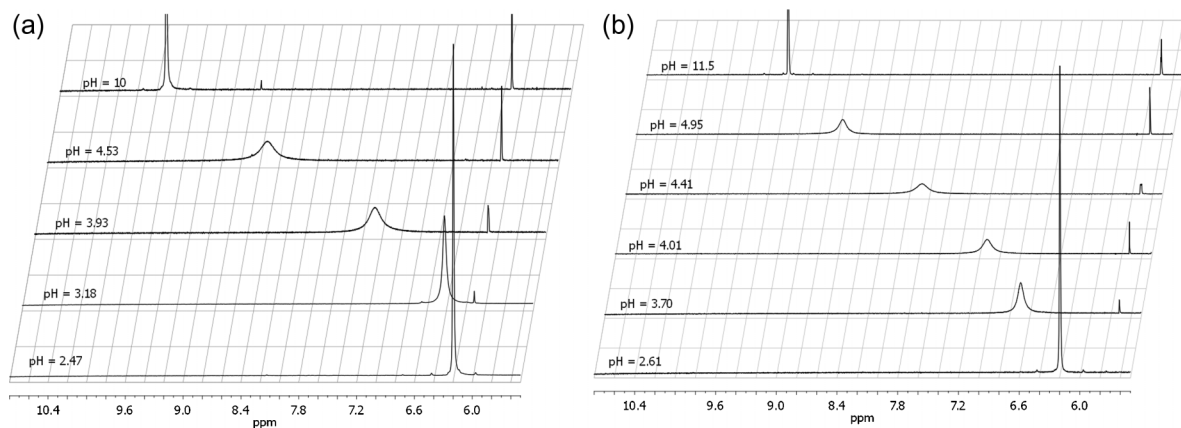


Figure 3. Variation in the ^1H NMR spectra of MXA with pH, $T = 25.0\text{ }^{\circ}\text{C}$: (a) $X = \text{Cl}$, $[\text{MCA}] = 7.0 \times 10^{-2}\text{ M}$; (b) $X = \text{Br}$, $[\text{MBA}] = 7.0 \times 10^{-2}\text{ M}$

Table 2. Reaction enthalpies derived from K_a^{app}

	ΔH_{prot} (kJ mol ⁻¹)
MCA	17.7 ± 0.9
MBA	14.0 ± 0.5

expected, the plots are symmetrical with respect to 90°, and, more importantly, that at the DFT-B3LYP level there is a minimum at 90° (the plot is therefore U shaped), whereas at the MP2 level, minima exist at ~65° and ~115° (and the plot is W shaped).

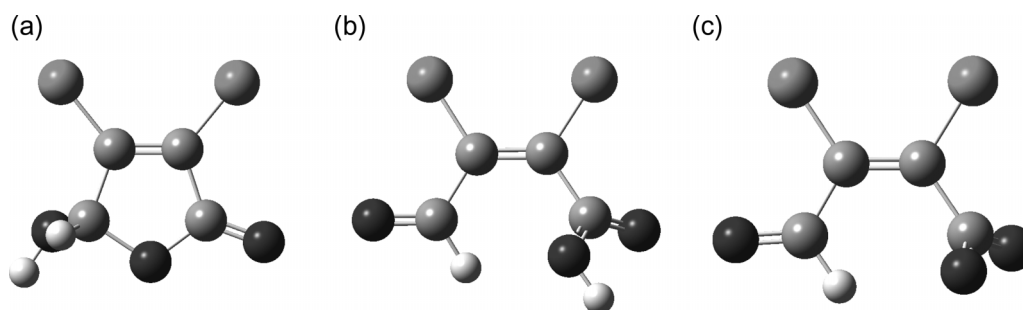
This is possibly caused by the negative charge and the resulting lone pair present in MXA_{op}^- , which may increase the need for correction of the correlation energy. Nevertheless, the energy differences over a wide range of angles were small and non-significant for the purposes of the present work.

Calculation of K_{iso}

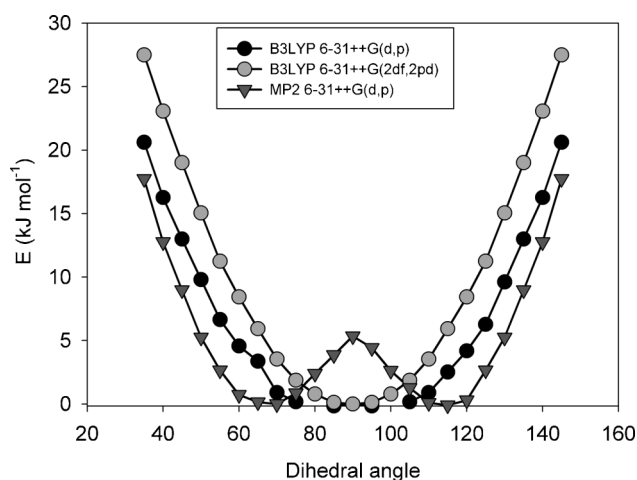
The experimental results suggest that, both in organic solvents and acidic aqueous media, MXA are mainly present in their cyclic form.^[37–40]

Calculation of the free energies in aqueous solution of MXA_{cl} and $\text{MXA}_{\text{op}}^{\ominus}$ at different levels of theory and the use of Eqn (10) afford the values shown in Table 4.

The mean values of $\text{p}K_{\text{iso}}$ are 3.60 for MCA and 3.91 for MBA, which shows that the cyclic forms are more stable than the open-chain isomers. Thus, at any given pH, $\text{MXA}_{\text{op}}^{\ominus}$ are present only at minor proportions with respect to MXA_{cl} (1 to 10^{2.5}–10^{5.7}). Increasing $\text{p}K_{\text{iso}}$ values are observed as the level of theory increases, with more powerful methods affording reaction free energies around 30 kJ mol⁻¹.

**Figure 4.** Structure of mucohalic acids. (a) MCA_{cl} (b) MCA_{op} (c) MCA^- **Table 3.** Dihedral angle C=C—C—O

Method	MCA		MBA	
	$\text{MCA}_{\text{op}}^{\ominus}$	MCA_{op}^-	$\text{MBA}_{\text{op}}^{\ominus}$	MBA_{op}^-
DFT-B3LYP 6-31++G(d,p)	137.1°	83.3°	121.9°	90.5°
DFT-B3LYP 6-31++G(2df,2pd)	132.6°	90.3°	114.5°	90.5°
MP2 6-31++G(d,p)	130.6°	66.4°	121.1°	74.1°

**Figure 5.** Energy of MXA_{op}^- at different levels of theory as a function of the C=C—C—O dihedral angle

The $\text{p}K_{\text{iso}}$ values in organic solvents DMSO and heptane (Table 5) do not differ much from those in water. This shifted equilibrium and the presence of a small amount of aldehyde should be taken into account when carrying out reactions of MXA in organic solvents.

Calculation of K_a

$\text{MXA}_{\text{op}}^{\ominus}$ are acidic and dissociate in aqueous solution yielding MXA_{op}^- . Since the higher the acidity, the lower the concentration of highly reactive and potentially toxic $\text{MXA}_{\text{op}}^{\ominus}$, knowledge of their $\text{p}K_a$ values is important.

As a rule, determination of reaction free energies precise enough for the prediction of equilibrium constants demands very

Table 4. Calculated ΔG_{iso} and pK_{iso} in water

	DFT-B3LYP 6-31++G(d,p)		DFT-B3LYP 6-31++G(2df,2pd)		MP2 6-31++G(d,p)		CBS-QB3		G3MP2B3	
	ΔG_{iso} (kJ mol ⁻¹)	pK_{iso}	ΔG_{iso} (kJ mol ⁻¹)	pK_{iso}	ΔG_{iso} (kJ mol ⁻¹)	pK_{iso}	ΔG_{iso} (kJ mol ⁻¹)	pK_{iso}	ΔG_{iso} (kJ mol ⁻¹)	pK_{iso}
MCA	13.82	2.42	13.81	2.42	19.88	3.48	29.3	5.13	26.03	4.57
MBA	23.65	4.14	18.76	3.29	24.45	4.29	32.7	5.73	—	—

Table 5. Calculated pK_{iso} in organic solvents

	pK_{iso}									
	DFT-B3LYP 6-31++G(d,p)		DFT-B3LYP 6-31++G(2df,2pd)		MP2 6-31++G(d,p)		CBS-QB3		G3MP2B3	
	DMSO	Heptane	DMSO	Heptane	DMSO	Heptane	DMSO	Heptane	DMSO	Heptane
MCA	3.38	3.23	3.17	3.16	3.61	3.56	5.22	5.22	4.61	4.64
MBA	4.17	3.94	3.50	3.28	4.15	3.99	6.05	5.71	—	—

high levels of theory such as CBS and G-n. These methods include empirical corrections and provide gas-phase free energies of reaction within less than 1 kcal mol⁻¹ of the experimental value. Prediction of pK_{a} values is no exception.^[41]

Given the accuracy of gas-phase thermochemistry, the most important source of error in pK_{a} calculations lies in the solvation energies. These are usually computed, as in this work, by PCM methods, which rely on empirical parameters, in particular cavity radii. Carboxylic acids and carboxylate anions have been used extensively in the defining sets for cavity radii, which helps to understand the good results usually obtained with these types of compounds. Occasionally, halogen atoms give poor results in PCM calculations, and the use of experimental values for comparison is encouraged.^[57,59]

PCM methods have proven to work accurately for the determination of acid dissociation constants for many types of molecules, especially when parameters such as cavity radii are optimized for a certain group of compounds, when a relative approach is used to correct for systematic errors in solvation energies or when explicit solvent molecules are included in the calculations.^[41]

The values calculated for pK_{a} using a relative approach (Eqns (11) and (12)) are shown in Table 6. They suggest that

$\text{MXA}_{\text{op}}^{\ominus}$ are very strong carboxylic acids, with pK_{a} values close to 1. This is consistent with the inductive effect of the halogen substituents and the electron delocalization in the α - β insaturation; for instance, pK_{a} (chloroacetic acid) = 2.85 and pK_{a} (acrylic acid) = 4.25 as compared to pK_{a} (acetic acid) = 4.75.

The fact that the predicted pK_{a} values show little variation at the different levels of theory is probably due to favorable error cancellation arising from the use of a relative approach.

The low pK_{a} values suggest that $\text{MXA}_{\text{op}}^{\ominus}$ are present at very low proportions in aqueous solution at neutral or basic pH (accounting for about 1 in 10⁶ at pH = 7). They also explain why, despite the high K_{iso} values, MXA are not in their cyclic forms at neutral or basic pH, but rather in an open-chain (dissociated) form.^[37-40]

Since its concentration is always orders of magnitude lower, the minor presence of the open-chain undissociated aldehyde is expected to limit its impact on environment and health.

The computational values for $pK_{\text{calc}}^{\text{app}} = pK_{\text{a}} + pK_{\text{iso}} - \log(1 + K_{\text{hyd}}^-)$ are also shown in Table 6. The calculated values are around 4 in the case of MCA and 5 in the case of MBA (the averages for the three methods are 3.8 and 5.3 for MCA and MBA, respectively). Computationally estimated equilibrium constants in solution are usually affected by errors of not less than ± 1.0

Table 6. Calculated pK_{a} and $pK_{\text{calc}}^{\text{app}}$

	DFT-B3LYP 6-31++G(d,p)		DFT-B3LYP 6-31++G(2df,2pd)		MP2 6-31++G(d,p)		CBS-QB3		G3MP2B3	
	pK_{a}	$pK_{\text{calc}}^{\text{app}}$	pK_{a}	$pK_{\text{calc}}^{\text{app}}$	pK_{a}	$pK_{\text{calc}}^{\text{app}}$	pK_{a}	$pK_{\text{calc}}^{\text{app}}$	pK_{a}	$pK_{\text{calc}}^{\text{app}}$
MCA	1.07	3.40	0.80	3.13	1.26	4.65	0.57	5.61	1.27	5.75
MBA	1.48	5.55	1.55	4.77	1.10	5.32	0.85	6.51	—	—

logarithm units,^[41] and the $pK_{\text{calc}}^{\text{app}}$ values in this work arise from the combination of two calculated equilibrium constants. The agreement between the experimental (Table 1) and computational (Table 6) results supports the accuracy of the computational results (pK_{a} and pK_{iso}).

CONCLUSIONS

- (i) MXA exist in aqueous solution as a pH-dependent mixture of the cyclic (MXA_{cl}) and the dissociated form (MXA_{op}^-), the open-chain non-dissociated form ($\text{MXA}_{\text{op}}^{\circ}$) always being minor, which limits its possible impact on health or the environment.
- (ii) The apparent dissociation constants of MXA are $pK_{\text{a}}^{\text{app}}(\text{MCA}) = 3.95$ and $pK_{\text{a}}^{\text{app}}(\text{MBA}) = 4.27$ at 25.0 °C.
- (iii) The results suggest that MXA in their cyclic forms are about 15–30 kJ mol⁻¹ more stable than their open-chain isomers, both in water and organic solvents.
- (iv) The calculated $pK_{\text{a}} \sim 1$ value for $\text{MXA}_{\text{op}}^{\circ}$ suggests that they are strong acids in aqueous solution and readily dissociate to MXA_{op}^- , affording very low concentrations of $\text{MXA}_{\text{op}}^{\circ}$.
- (v) The experimental and computational values for $pK_{\text{calc}}^{\text{app}}$ are in good agreement, which further supports the accuracy of the computational approach.

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