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Halogenated 2,5-pyrrolidinediones: synthesis, bacterial mutagenicity in Ames tester strain TA-100 and semi-empirical molecular orbital calculations

Beverly A. Freeman, Robert E. Wilson, Ronald G. Binder, William F. Haddon*

US Department of Agriculture, Western Regional Research Center, Agricultural Research Service, 800 Buchanan Street, Albany, CA 94710, USA

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Abstract

The chloroimide 3,3-dichloro-4-(dichloromethylene)-2,5-pyrrolidinedione, a tetrachloroitaconimide, is the principal mutagen produced by chlorination of simulated poultry chiller water. It is the second most potent mutagenic disinfection by-product of chlorination ever reported. Six of seven new synthetic analogs of this compound are direct-acting mutagens in Ames tester strain TA-100. Computed energies of the lowest unoccupied molecular orbital (E_{LUMO}) and of the radical anion stability ($\Delta H_f^{rad} - \Delta H_f$) from MNDO-PM3 for the chloroimides show a quantitative correlation with the Ames TA-100 bacterial mutagenicity values. The molar mutagenicities of these direct acting mutagenic imides having an exocyclic double bond fit the same linear correlation ($\ln M_m$ vs. E_{LUMO}; $\ln M_m$ vs. $\Delta H_f^{rad} - \Delta H_f$) as the chlorinated 2(5*H*)-furanones, including the potent mutagen MX, 3-chloro-4-(dichloro-methyl)-5-hydroxy-2(5*H*)-furanone, a by-product of water chlorination and paper bleaching with chlorine. Mutagenicity data for related haloimides having endocyclic double bonds are also given. For the same number of chlorine atoms, the imides with endocyclic double bonds have significantly higher Ames mutagenicity compared to their structural analogs with exocyclic double bonds, but do not follow the same E_{LUMO} or $\Delta H_f^{rad} - \Delta H_f$ correlation as the exocyclic chloroimides and the chlorinated 2(5*H*)-furanones. Published by Elsevier Science B.V.

Keywords: Chlorination; Mutagenicity; Pyrrolidinediones; Chloroimides; MX; Computation; Semi-empirical; LUMO

1. Introduction

The chlorine disinfection by-product 3,3-dichloro-4-(dichloromethylene)-2,5-pyrrolidinedione (**1c,** Fig. 1) is the second most potent direct acting Ames mutagen ever reported in studies of chlorination [1]. We determined the structure of the new mutagenic chloroimide (**1c**), in the course of studying the Ames mutagenicity produced by chlorination of chiller water used in poultry processing operations [1–3]; chlorination of poultry chiller water improves the safety of processed foods by reducing the levels of human bacterial pathogens. However, excessive chlorination produces measurable Ames mutagenicity in the processing waters when the levels of chlorination significantly exceed the amounts established for use in poultry processing plants [2]. Recycling of these mutagenic by-products could limit the reuse of water in commercial food processing. The objectives of our study of disinfection by-products from poultry chlorination were to establish the structure of the active compounds and to assess their contribution to Ames mutagenicity.

To elucidate chemical structures of the poultry chiller water mutagens, we carried out measure-

* Corresponding author.

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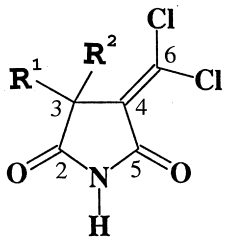
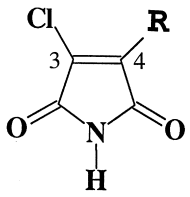
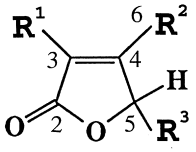


Compound	R^1	R^2
1a	H	H
1b	H	Cl
1c	Cl	Cl



Compound	R
1d	Cl
1e	$CHCl_2$
1f	CH_2Cl
1g	CH_3
1h	CH_2Br



Compound	R^1	R^2	R^3
2a (MX)	Cl	$CHCl_2$	OH
2b	Cl	$CHCl_2$	H
2c	Cl	CH_2Cl	OH
2d	Cl	CH_2Cl	H
2e	H	CH_2Cl	OH
2f	H	CH_2Cl	H
2g	Cl	CH_3	OH
2h	Br	CH_3	OH
2i	Cl	Cl	OH
2j	Cl	Cl	H

Fig. 1. Structures of mutagenic chloroimides (**1a–h**) and chlorofuranones (**2a–j**).

ments on a simulated processing mixture at a high chlorination level. Compound **1c** accounted for a substantial fraction of the observed mutagenicity at 1500 ppm chlorination [1]. This single compound result had precedent in the previous isolation of 3-chloro-4-(dichloro-methyl)-5-hydroxy-2(5*H*)-furanone, MX, **2a** in Fig. 1, which accounted for most of the observed Ames mutagenicity in the Kraft paper bleaching process and in the chlorination of drinking water in contact with humic material. MX was first identified by Holmbolm [4,5], and has recently been shown to be carcinogenic in rats [6]. MX is of considerable interest because of its demonstrated presence in drinking water supplies [7] and the still unresolved question of its possible importance in human health [8]. MX, with a molar mutagenicity in the range 2600–10 000 revertants per nanomole in TA-100, is the most potent mutagenic chlorine disinfection product ever reported. The molar mutagenicity of the direct-acting mutagen (**1c)** is 1450 rev/nmol in tester strain TA-100 without microsomal activation [1].

For strong electrophiles such as MX and the chloroimides, it has been difficult to isolate specific reaction products with nucleophilic reagents [9–11]. Early observations of DNA strand breaks caused by MX [12] have been supplemented by more recent studies showing that such reactions may be complex at the molecular level [13,14]. The bacterial mutagenicity of the MX family of compounds can be eliminated or reduced by reaction with nucleophilic reagents [9–11]. The mutagenicity produced by **1c** in poultry chiller water can be rapidly eliminated by L-cysteine, *N*-acetyl-L-cysteine and other common nucleophilic reagents [15] added to poultry chiller water.

MX and the mutagenic tetrachloroimide from our simulated food processing mixture are similar in chemical structure in that both are chlorinated five-membered ring heterocycles (**2a** vs. **1c**). Thus it was of interest to explore the possible similarity of structure-activity effects on Ames mutagenicity for the chlorofuranones [16–21] and the chloroimides. In this work, we attempt to link the toxicology of these two different classes of electrophilic compounds with the demonstration that calculated energies of the LUMOs and the calculated radical ion heats of formation ($\Delta H_f^{rad} - \Delta H_f$) yield similar correlations with observed Ames mutagenicity. E_{LUMO} and $\Delta H_f^{rad} - \Delta H_f$ were chosen because they are likely indicators of electrophilic reactivity. We include synthetic methods, mutagenicity data and computational results for chloroimides having both endo- and exocyclic double bonds; all the known MX analogs have endocyclic double bonds only. Open-ring analogs of MX make minor contributions to the total Ames mutagenicity of chlorinated drinking water [22]. There was no evidence in our studies of poultry chlorination for additional imides, or for the occurrence of open-ring analogs [1].

2. Materials and methods

2.1. Chemical synthesis

The synthesis of four chlorinated 2,5-pyrrolidinediones having exocyclic double bonds was reported

previously [1]. For the current study, three additional chloroimides and a single mixed bromo-chloroimide having an endocyclic double bond (Fig. 1, **1e**–**h**) were synthesized. Three of the four newly-synthesized compounds are direct-acting mutagens in TA-100.

Compounds to be chlorinated were added to a solution of chlorine in CCl_4 and exposed to direct sunlight for one to eight days, usually producing a complex mixture of compounds. Typically, 10–15% excess chlorine was present initially; compounds of low solubility were first dissolved in trifluoroacetic acid. Purifications of the reaction mixtures were carried out by silica gel chromatography using a specially prepared phenyl silica packing. The column packing was prepared by heating 60 g silica gel 60 H, dried at 175°C, in 220 ml benzyl alcohol in a bomb for 3 h at 200°C; product was thoroughly rinsed with acetone and ether and oven-dried to give 67.2 g packing. This product was further treated with 15 ml hexamethyldisilazane in boiling benzene. After cleaning and drying, 67.4 g of packing was obtained. Use of this packing facilitated separation of chloroimide products from less polar artifacts present in the reaction mixtures.

Analytical HPLC analyses utilized a 4.6 mm × 25 cm phenyl column (Microsorb-MV, Rainin Instrument Co.) and normal phase chromatography: hexane to 2% isopropanol in 8 min, then to 4% isopropanol in 8 min, then held at 4% isopropanol at 13 min, all at 1 ml/min. GC-MS analyses were carried out on a VG 7070/HS mass spectrometer (Micromass, Manchester, UK) and HP 5830 gas chromatograph (Hewlett-Packard, Palo Alto, CA) or on a Finnigan GCQ ion trap mass spectrometer (Thermoseparations, San Jose, CA) using 0.25 mm i.d., 0.25 μm, 30 m DB-5 or DB-5MS (5% phenyl, 95% methyl silicone) capillary columns (J & W Scientific, Folsom, CA) with splitless injection. Electron ionization (EI) spectra were recorded at 70 eV ionizing voltage. Typically, GC runs used linear temperature programs of 3°C/min from 60 to 280°C/min, and splitless injection at 220°C. The ion source temperature was 180°C and transfer lines to the mass spectrometer were maintained at 230°C. Retention index (RI) values were assigned by linear interpolation between the retention times of *n*-alkane standards. Proton (400 MHz) and ^{13}C NMR (100 MHz) spectra were determined in $CDCl_3$ solutions on a Bruker ARX400 spectrometer. Chemical shift values (δ) are in ppm relative to tetramethylsilane as internal standard ($\delta = 0.0$ ppm).

Preparation of 3-chloro-4-dichloromethyl-1H-pyrrole-2,5-dione (**1*e***). Citraconimide was first prepared as previously described [1,23]. The mixture obtained from a four-day chlorination of citraconimide was distilled under vacuum with bath temperature to 220°C. The residue was dissolved in toluene, treated with activated carbon, filtered through silica gel, then re-distilled with bath temperature to 160°C. The major component, RI 1551 by gas chromatography, was concentrated by silica gel chromatography and finally separated from an impurity 3,3-dichloro-4-dichloromethylene-2,5-pyrrolidinedione, by chromatography on a silica gel column with eluting solvent 2% ether in hexane. Crystals formed in the neat material after a week. The solid was recrystallized from CCl_4 to give **1e**, m.p.: 70.0–70.7°C (MS: *m/z* 215 (24), 213 (26) (M^+), 180 (26), 178 (39), 170 (11), 160 (13), 109 (59), 107 (100). ^1H NMR: δ6.61 (s, 2H), 7.79 (br s, 1H); ^{13}C NMR: δ164.33, 162.77, 137.34, 135.90, 58.02; UV: λ_{max}, 232 nm).

Preparation of 3-chloro-4-chloromethyl-1H-pyrrole-2,5-dione (**1*f***). Chloromethyl maleic anhydride, prepared by the procedure of Schreiber et al. [24], was chlorinated, yielding 3-chloromethyl-3,4-dichlorosuccinic anhydride, which was twice distilled under vacuum to produce 3-chloro-4-chloromethylmaleic anhydride (RI 1206; MS: *m/z* 182 (24), 180 (35) (M^+), 147 (23), 145 (68), 138 (24), 136 (38), 101 (54), 73 (100); ^1H NMR: δ4.40 (s); ^{13}C NMR: δ30.90, 138.26, 139.37, 158.70, 160.67). Stirring the anhydride in a mixture of urea and salt at 130°C converted it to the imide [22], which was purified by silica gel chromatography and recrystallized several times from CCl_4 to give **1f**, m.p.: 98.2–98.8°C (MS: *m/z* 181 (52), 179 (83) (M^+), 161 (36), 146 (32), 144 (98), 136 (41), 73 (100). ^1H NMR: δ4.36 (s, 2H), 7.82 (s, 1H); ^{13}C NMR: δ166.28, 163.55, 138.30, 136.49, 31.16; UV: λ_{max}, 230 nm).

Preparation of 3-chloro-4-methyl-1H-pyrrole-2,5-dione (**1*g***). Compound **1g** was prepared from citraconic anhydride (Aldrich Chemicals, 99% pure by GC analysis) by the procedure of Earl et al. [23] (MS: *m/z* 147 (32), 145 (100) (M^+), 102 (19), 74 (17), 64 (47), 39 (39); UV: λ_{max}, 230 nm).

Preparation of 3-bromomethyl-4-choromaleimide (**1*h***). Compound **1g** (1.2 g), was combined with

trifluoroacetic acid (2 ml), and bromine (1 ml) and then exposed to sunlight for seven days. After removal of the acid and bromine by evaporation, the resulting residue was chromatographed on silica gel with eluting solvents CCl_4, benzene and ether. The diethyl ether eluate contained **1h** (RI = 1547) and 3,4-dibromo-3-chloro-4-methylprrolidindione (RI = 1664). **1h** was crystallized from CCl_4 to an estimated purity (GC) of 97%. ^{1}H NMR (400 MHz) δ 4.20 (s, CH_2, 2H), 7.93 (broad s, NH, 1H); ^{13}C NMR (100 MHz) δ 166.27 (C=O), 163.66 (C=O), 137.08 (C–Cl), 136.99 (C–CH_2Br), 15.36 (CH_2Br).

Preparation of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (*MX*, **2*a***). We followed the method of Padmapriya [24]. Final purification of product was on silica gel H pre-washed with hydrochloric acid, then rinsed and dried.

Caution. The compounds reported in this study, with the exception of **1g**, are mutagenic in the Ames mutagenicity assay using *Salmonella typhimurium* strain TA-100 without microsomal activation. Therefore caution should be exercised in their use and disposal.

2.2. Mutagenisis assay

Haloimide samples were dissolved in 1,4-dioxane and tested for mutagenicity in the Ames/*Salmonella* assay using the standard plate incorporation method [25]. For MX, measurements were carried out in both DMSO and in peroxide-free 1,4-dioxane, which gave the same result within statistical error (data not shown). All measurements were conducted in *S. typhimurium* tester strain TA-100 without activation by rat liver homogenate fraction S9. Mutagenicity was calculated from the linear portion of the dose-response curves using three to five concentrations in the linear range of the assay, which was determined by preliminary range-finding experiments. Positive control plates contained 0.4 μl per plate of methyl methane sulfonate. Mean molar mutagenicity values (M_m), expressed as revertants per nanomole, were calculated from the slopes of the dose-response curves as determined by linear regression. Duplicate plating was used to determine the dose-response curves for all compounds. Compound **1g** was non-mutagenic, exhibiting less than twice the number of background revertants [25], as shown in Table 1.

2.3. Computational methods

Molecular modeling calculations utilized an INSIGHT II integrated software package (Biosym-MSI) with a Silicon Graphics Indigo workstation. The initial structures prepared using the Sketcher module were submitted to preliminary geometry optimization using molecular mechanics methods with the INSIGHT II DISCOVER interface in the BUILDER module. Following these initial approximations of structure, semi-empirical molecular orbital and ΔH_f calculations were performed with MOPAC 6.0 using the MNDO-PM3 parameterization set. All molecules were optimized using the default BFGS minimization type. Peptide corrections had no effect on the calculations. The MOPAC keyword PRECISE for termination of the calculations was set 100-fold below the default value as in previous work [17]. The gradient norm was 5 and the shift level was 15. Pulay's convergence method and 200 self-consistent field iterations were utilized in the calculations. The gradient type was NLLSQ. For all cases, the Herbert's or Peter's test was satisfied in BFGS.

To avoid trapping a molecule with rotatable groups in a local minimum, calculations were carried out for each 10° increment of bond rotation when appropriate using the Torsion command in INSIGHT II. Each structure so obtained was submitted to MOPAC and the confomer having the lowest ΔH_f was taken as the most stable structure. For calculations on neutral molecules, the lowest electronic state was used and the charge was zero. For calculations on radical anions, the doublet electronic state was used and the charge was −1.

3. Results and discussion

3.1. Mutagenicity

In addition to performing the standard tests for evaluating the activity of the TA-100 tester strain used in our work, we directly compared the response of this strain to MX independently synthesized in our laboratory. Our measured value is 7825 rev/nmol for the bacterial mutagenicity of MX in TA-100, without S9 activation, as shown in Table 1. This value falls within the range reported in the literature of about 2800 to

Table 1
Ames mutagenicity measurements in *S. typhimurium* strain TA-100[a]

Compound	Dose (μg/plate) (nmol/plate)[b]	Rev/plate	Mm (slope(rev/nmol))[c]	r^2
MX	0.0200 (0.092)	988; 876	7825	0.966
	0.0100 (0.046)	749; 654		
	0.0075 (0.034)	495; 654		
	0.0050 (0.023)	398; 402		
	0.0025 (0.011)	336; 349		
Solvent control[d]	(0)	232; 248; 204; 220	490	0.981
1e	0.500 (2.43)	1402; 1238		
	0.250 (1.16)	655; 589		
	0.125 (0.58)	379; 366		
	0.050 (0.23)	232; 248; 204; 220		
Solvent control	(0)	199; 215		
1f	0.400 (2.22)	1249; 1224	459	0.975
	0.300 (1.67)	801; 893		
	0.200 (1.11)	640; 576		
	0.100 (0.56)	348; 379		
Solvent control	0	195; 213		
1g	200 (1380)	Kill	<0.3	
	150 (1035)	Kill		
	100 (690)	223; 198		
	50	179; 179		
Solvent control	0	195; 213		
1h	0.500 (2.23)	1373; 1449	545	0.999
	0.250 (1.11)	753; 785		
	0.100 (0.45)	412; 422		
Solvent control	(0)	184; 219		

[a] All measurements in 1,4-dioxane. The positive control, methyl methane sulfonate, gave >1200 rev/plate in all assays at 0.4 μg/plate.
[b] M_m, molar mutagenicity, calculated by multiplying the dosage (μg/plate) by 1000 and dividing by the molecular weight of the compounds in g/mol.
[c] Slope in rev/nmol, by linear regression.
[d] Background counts above 200 are typical for use of 1,4-dioxane as solvent (B.N. Ames laboratory, personal communication).

above 10 000 rev/nmol [16,18]. Thus in their attempts to correlate mutagenicity values with computed properties, LaLonde et al. [17] used 4000 rev/nmol as the mutagenicity of MX, and Tuppurainen et al. [18], used a value of 6300 rev/nmol for MX. Because our measured value for MX fell in the range of literature values, no adjustments were made for comparisons of chloroimide mutagenicities with data obtained elsewhere for MX and its various analogs. Furthermore, there was no solvent dependency for the bacterial mutagenicity of MX in TA-100, but for the chloroimides, mutagenicities measured in DMSO or ethanol solutions were significantly lower than in 1,4-dioxane solution; 800 rev/nmol for **1c** in ethanol vs. about 1500 rev/nmol in 1,4-dioxane [1]; thus all TA-100 mutagenicity data reported in this work is based on measurements carried out in 1,4-dioxane. The 1,4-dioxane produces higher background counts than DMSO, but as indicated in the table, the values are well within an acceptable range [25].

The structures of the haloimide compounds are shown in Fig. 1. For the computational study we synthesized four novel five-carbon haloimides having structures containing endocyclic double bonds (**1e–h**). The imide containing a single chlorine (**1g)** was non-mutagenic in TA-100 by the criteria of Prival and Dunkle [26], but the imides with two and three chlorines (**1e** and **1f**), and the single mixed bromo, dichloro compound (**1h)** were all strongly mutagenic. They exhibited responses in TA-100 of

Table 2
Computed molecular properties and Ames mutagenicity values for chloroimides and chlorofuranones

Compound	M_m[a] (rev/nmol)	(ln M_m)	Reference	E_{LUMO} (eV)			$\Delta H_f^{rad} - \Delta H_f$		
				This work[b]	[17]	[18]	This work[b]	[17]	[18]
1a	0.24	−1.427	[1]	−1.00			−38.284		
1b	7.7	2.041	[1]	−1.21			−44.246		
1c	1450	7.279	[1]	−1.36			−47.736		
1d	28	3.332	[1]	−1.59			−50.882		
1e	490	6.194	(b)	−1.85			−58.513		
1f	459	6.129	(b)	−1.69			−53.790		
1g	<0.3[c]	<−1.20	(b)	−1.41			−45.70		
1h	545	6.301	(b)	−1.89			−59.604		
MX, 2a	7825	8.965	(b)	−1.46	−1.53	−1.51	−50.55	−52.7	−54.19
MX, 2a	3840	8.253	[17]						
MX, 2a	6298	8.748	[18]						
2b	177	5.176	[20]	−1.32	−1.32	−1.29	−47.16	−47.2	−48.15
2c	579	6.361	[20]	−1.30	−1.31	−1.32	−47.83	−47.6	−49.46
2d	4.920	1.593	[20]	−1.05	−1.08	−1.09	−41.44	−41.5	−43.49
2e	3.870	1.353	[20]	−1.11	−1.13	−1.08	−42.22	−43.4	−43.23
2f	0.306	−1.184	[20]	−0.85	−0.90	−0.85	−37.54	−37.5	−37.13
2g	0.473	−0.749	[16]	−0.90	−0.91	−0.91	−34.38	−34.4	−39.04
2h	0.404	−0.906	[17]	−0.93	−0.93		−38.82	−35.8	
2I	7.24	1.979	[21]	−1.15	−0.97	−1.24	−40.54	−41.9	−46.70
2j	0.171	−1.766	[21]	−0.93	−0.93	−1.02	−34.34	−34.4	−40.59

[a] Molecular mutagenicity in Ames tester strain TA-100 without metabolic activation.
[b] This work, see Table 1.
[c] Non-mutagenic, see Table 1.

about 500 rev/nmol, as shown in Table 1. Table 2 summarizes our previously measured values of mutagenicity for the three exocyclic chloroimides (**1a–c**), the four-carbon compound (**1d**), and the previously reported values for the halofuranones (**2a–j**). Fig. 1 shows the structures of the chlorofuranones which we have used for comparison with our mutagenicity and computational data for **1a–h**.

For the tri-chloroimide isomers **1e** (endocyclic double-bonded) vs. **1b** (exocyclic double-bonded), both with two chlorines on the exocyclic carbon, the increase in mutagenicity for the internal vs. external double bond is a factor of about 60. In like manner the dichloroimide with an endocyclic double bond **1f** (459 rev/nmol) has about 1900 times the mutagenicity of **1a** (0.24 rev/nmol), although in this case the exocyclic carbon has 1 and 2 bonded chlorines, respectively. The increased mutagenicity for the chloroimides which have an endocyclic double bond would be consistent with the localization of positive charge at C-4, the likely site for a 1,4-Michael addition reaction. A positive charge at C-4 would be inductively stabilized by the exocyclic mono- or dichloromethyl group. For the chloroimides with exocyclic double bonds, the Michael addition site would more likely be the exocyclic carbon, where directly-bonded chlorine atoms would be less stabilizing because of resonance. The relatively greater difference in mutagenicity between **1f** vs. **1a** is consistent with the location of two vs. one chlorine on the exocyclic carbon for **1a**. In **1f** both chlorines may inductively stabilize the positive charge at C-4, whereas for **1a**, with charge on C-6, there is no inductive stabilization by the two chlorines.

Interestingly, the tetrachloroimide (**1c**), which has the highest mutagenicity of all the chloroimides, is the only detectable product of chlorination in the simulated food processing mixture that we examined. It was not possible to synthesize a 4-chlorine imide with internal double bonds. However, the increase in mutagenicity between the di-chloro to tri-chloro compounds **1e** and **1f** is very small, suggesting that addition of a fourth chlorine should have little effect. Therefore **1c** appears to be the

most potent mutagen for the chloroimide series of compounds. This conclusion is consistent with the mutagenicities of the chlorohydroxyfuranones, where the increase in mutagenicity between the mono- and dichlorohydroxyfuranones (**2g**, **2c**) is large, a factor of 1200, but the relative increase from addition of a third chlorine to give MX (**2a**) is only a factor of 6.6. The bromine-containing imide (**1h**), had mutagenicity comparable to its structural analog (**1f**). This result is consistent with similar studies reported recently for mixed halogen-substituted 4-methyl-2(5H) furanones by LaLonde [27].

3.2. Computational results

There is good evidence that the halogenated imides may behave chemically as electrophiles from previous studies of the chemistry of citraconimide [23], and **1c** [15]. Success by others in relating the potency of Ames mutagens to computed molecular properties has been based on the idea that the energy of the lowest unoccupied molecular orbital (E_{LUMO}), and the electron affinity ($\Delta H_f^{rad} - \Delta H_f$) serve a measure of the reactivity toward nucleophilic reagents. We have therefore investigated the calculated E_{LUMO} and radical anion heats of formation ($\Delta H_f^{rad} - \Delta H_f$) as indicators of mutagenicity for this new series of compounds.

Semi-empirical computations using MNDO-PM3 gave satisfactory calculations of both the LUMO energy and ($\Delta H_f^{rad} - \Delta H_f$) in all cases, yielding MOPAC-derived bond distances and angles which were in excellent agreement with those obtained by X-ray crystallography for **1b** and **1c** [1] (data not shown). The computed structures are essentially planar for both compounds, in agreement with the X-ray crystallography results.

Table 2 summarizes the computed values of LUMO, ($\Delta H_f^{rad} - \Delta H_f$) and measured mutagenic response in TA-100 of all compounds reported in this study. Comparisons with literature values of E_{LUMO} and ($\Delta H_f^{rad} - \Delta H_f$) are included. For the thirteen structures taken from previous work, we re-computed the LUMO energies and radical anion heats of formation using the Biosym INSIGHT II software package. For the LUMO energy values, the deviations from published data of LaLonde and Tuppurainen are on the order of 0.1 eV or less except for compound **2i**, where the difference was −0.18 and 0.09 eV, respectively. The computed differential negative ion radical heats of formation agreed well with LaLonde's reported values, the maximum deviation being 1.4 Kcal/mol for **2i**; however, much larger deviations, up to 6.2 Kcal, were observed from Tuppurainen's calculations of ($\Delta H_f^{rad} - \Delta H_f$). We did not re-measure the mutagenicities for any of the furanone compounds except MX.

Fig. 2a shows the E_{LUMO} values plotted against the mutagenicity for ten chlorofuranones and the six chloroimides, including the compound in our study containing bromine (**1h**). Notably, the E_{LUMO} values for both the chlorofuranones and the chloroimides with exocyclic double bonds are well-correlated. The least squares fit to the combined set of data for the 13 chlorofuranones and chloroimides with external double bonds gives the following equation:

$$\ln M_m = -17.10 E_{LUMO} - 17.01 \quad (r^2 = 0.921) \quad (1)$$

which is plotted in Fig. 2a. If we exclude the values derived from the chlroimides and use only the re-calculated values of E_{LUMO} for the chlorofuranones, the equation for the least squares line is

$$\ln M_m = -16.1 E_{LUMO} - 16.5 \quad (r^2 = 0.953) \quad (2)$$

This second equation differs slightly from that originally given by LaLonde, $\ln M_m = -14.97 E_{LUMO} - 14.37$ ($r^2 = 0.925$) [17] because we have used re-calculated values of E_{LUMO} using our software (see Table 2).

Using Eq. (1), the predicted TA-100 response for the chloroimides **1a–c** is 1.1, 39.7 and 516 rev/nmol, respectively. The calculations give a good semi-quantitative prediction of the measured mutagenic potencies of 0.24, 7.7 and 1450 rev/nmol obtained in our laboratory for these compounds (Table 2).

The calculated negative anion radical heat of formation data gave comparable results. The least squares fit to data consisting of the recalculated, ($\Delta H_f^{rad} - \Delta H_f$) values for the ten chlorofuranones and the chloroimides with exocyclic double bonds gives the following equation, again using the published data for mutagenicities of the chlorofuranones shown in Table 2.

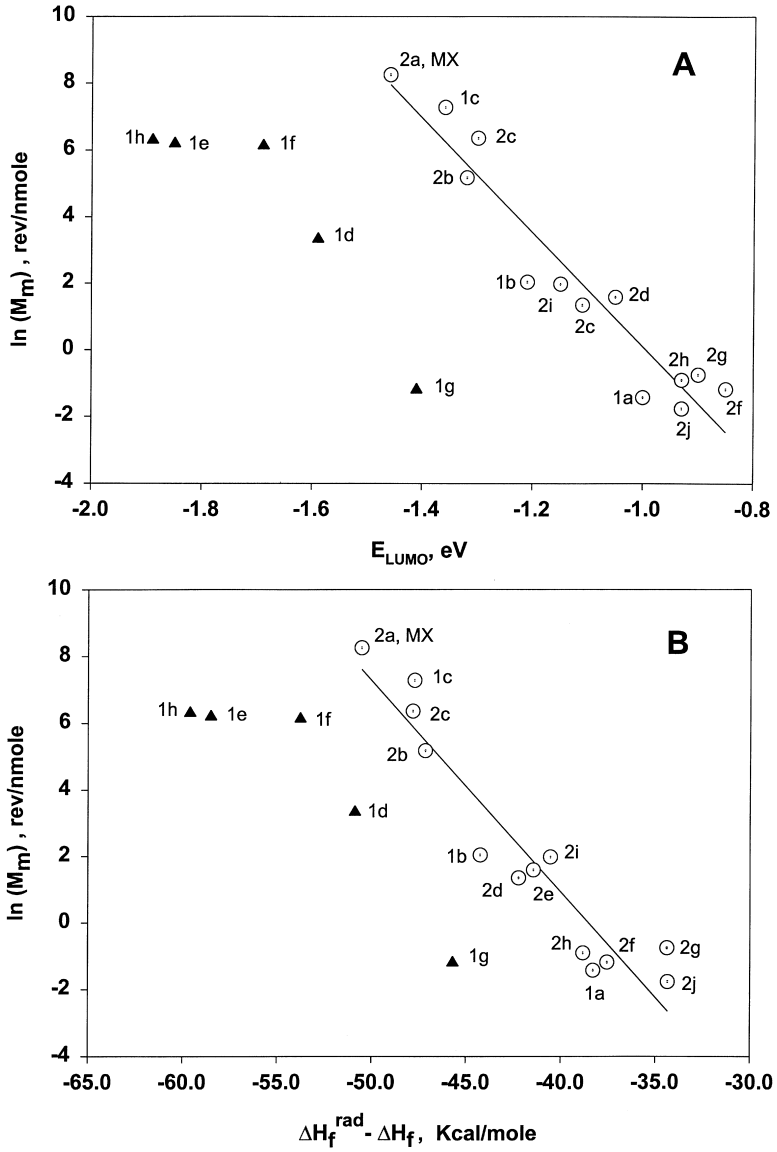


Fig. 2. (a) Plot of energy of lowest unoccupied molecular orbital, LUMO, computed from MNDO-PM3, for chloroimides and chlorofuranones against natural log of mutagenicity in Ames tester strain TA-100 without microsomal activation. (b) Plot of radical ion stability vs. natural log of mutagenicity in TA-100. Least squares lines include all compounds except the chloroimides with internal double bonds (**1d–h**).

$$\ln M_{\mathrm{m}} = -0.633(\Delta H_{\mathrm{f}}^{\mathrm{rad}} - \Delta H_{\mathrm{f}}) - 24.39 \quad (r^2 = 0.903) \tag{3}$$

which is plotted in Fig. 2b. Eq. (3) predicts the mutagenicity of **1a–c** to be 0.85, 37.3 and 339 rev/nmol, respectively, again in reasonable agreement with the measured values for the chloroimides with exocyclic double bonds. For the chlorofuranones only, but using our recalculated values for $(\Delta H_{\mathrm{f}}^{\mathrm{rad}} - \Delta H_{\mathrm{f}})$, we obtain the following:

$$\ln M_{\mathrm{m}} = -0.602(\Delta H_{\mathrm{f}}^{\mathrm{rad}} - \Delta H_{\mathrm{f}}) - 22.96 \quad (r^2 = 0.930) \tag{4}$$

also in good agreement with the published equation of LaLonde, et al. [17], $\ln M_m = -0.538(\Delta H_f^{rad} - \Delta H_f) - 20.38 \quad (r^2 = 0.949)$.

The correlation of E_{LUMO} and $(\Delta H_f^{rad} - \Delta H_f)$ with mutagenicity from Eqs. (1) and (3) for the chloroimides with exocyclic double bonds and the chlorofuranone derivatives would significantly over-predict mutagenicity if applied directly to the endocyclic imides **1e–h**. Dichloromaleimide (**1d**) with four carbons, also lies below the line established by the exocyclic imides and the chlorofurnanones. The published chorofuranone data include one four-carbon compound, mucochloric acid (**2i**) which fits the same line as the five-carbon chlorofuranones for both E_{LUMO} and $(\Delta H_f^{rad} - \Delta H_f)$ [17]. The fact that this four-carbon chlorofuranone fits the same equation as the five-carbon analogs suggests that double bond position may be the important structural parameter in establishing the correlation. In any case, the endocyclic chloroimides appear to follow a different LUMO-mutagencity relationship than their counterparts with exocyclic double bonds.

4. Conclusions

We have presented the mutagenicity results for seven chlorinated 2,5-pyrrolidine diones, analogs of the compound 3,3-dichloro-4-(dichloromethylene)-2,5-pyrrolidinedione previously isolated from the chlorination of simulated poultry chiller water. Six of the seven synthetic chloroimides were direct-acting mutagens in Ames tester strain TA-100. Semi-empirical molecular calculations of E_{LUMO} and $(\Delta H_f^{rad} - \Delta H_f)$, for a sub-set of the chloroimides having exocyclic double bonds gave the same correlation with measured TA-100 mutagenicity as demonstrated by others for the mutagenic chlorofuranones, of which the widely studied mutagen MX is a member, suggesting that these different classes of Ames bacterial mutagens may share a common chemistry and toxicology. Such agreement is unusual considering the simplicity of the calculations used to predict the mutagenicity. The computational results provide a convenient means to evaluate the effect of substituents on mutagenicity for the chloroimides.

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References

[1] W.F. Haddon, R.G. Binder, R.Y. Wong, L.A. Harden, R.E. Wilson, M. Benson, Potent bacterial mutagens produced by chlorination of simulated poultry chiller water, J. Agric. Food Chem. 44 (1996) 256–263.
[2] M.S. Masri, Chlorinating poultry chiller water: the generation of mutagens and water reuse, Food Chem. Toxicol. 24 (1986) 923–930.
[3] J.E. Schade, L.-S. Tsai, L. Tong, R. Wilson, J.T. MacGregor, Extraction of mutagens from chlorinated poultry chiller water, J. Food Sci. 55 (1990) 635–639.
[4] B.R. Holmbolm, R.H. Voss, R.D. Mortimer, A. Wong, Isolation and identification of an Ames-mutagenic compound in Kraft chlorination effluents, Tappi 64 (1981) 172–174.
[5] B. Holmbolm, L. Kronberg, P. Backlund, B.-A. Langvik, J. Hemming, Formation and properties of 3-chloro-4(dichloromethyl)-5-hydroxy-2(5H)-furanone, a potent mutagen in chlorinated waters, in: R.L. Jolley (Ed.), Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol. 6, Lewis, Chelsea, MI, 1990, pp. 125–135.
[6] H. Komulainen, V.-M. Kosma, S.-L. Vaittinen, T. Vartiainen, E. Kaliste-Korhonen, S. Lötjönen, R.K. Tuominen, J. Tuomisto, Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone in the rat, J. Natl. Cancer Inst. 89 (1997) 848–856.
[7] J.R. Meier, W.F. Blazak, R.B. Knohl, Mutagenic and clastogenic properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: a potent bacterial mutagen in drinking water, Environ. Mol. Mutagen. 10 (1987) 411–424.
[8] R.L. Melnick, G.A. Boorman, V. Dellarco, Water chlorination, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and potential cancer risk, J. Natl. Cancer Inst. 89 (1997) 832–833.
[9] Y. Ishiguro, R.T. LaLonde, C.W. Dence, Mutagenicity of chlorine-substituted furanones and their inactivation by reaction with nucleophiles, Environ. Toxicol. Chem. 6 (1987) 935–946.
[10] R.T. LaLonde, S. Xie, Glutathione and *N*-acetylcysteine inactivations of mutagenic 2(5H)-furanones from the chlorination of humics in water, Chem. Res. Toxicol. 6 (1993) 445–451.
[11] M. Watanabe, H. Kobayashi, T. Ohta, Rapid inactivation of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) a potent mutagen in chlorinated drinking water, by sulfhydryl compounds, Muat. Res. 312 (1994) 131–138.
[12] L. Chang, F.B. Daniel, A.B. DeAngelo, DNA strand breaks induced in cultured human and rodent cells by

chlorohydroxyfuranones-mutagens isolated from drinking water, Teratog. Carcinog. Mutagen. 11 (1987) 103–114.
[13] S. Knasüller, E. Zöhrer, E. Kronberg, M. Kundr, Franzén, R.R. Hermann-Schulte, Mutational spectra of *Salmonella typhimurium* revertants induced by chlorohydroxyfuranones by-products of chlorine disinfection of drinking water, Chem. Res. Toxicol. 9 (1996) 374–381.
[14] R. LaLonde, F. Ramdayal, Mucochloric acid action on ΦX174 DNA: a comparison to other chlorine-substituted 2(5H)-furanones, Chem. Res. Toxicol. 10 (1997) 205–210.
[15] M. Friedman, R.E. Wilson, K.L. Stevens, Inactivation of a tetrachloroimide mutagen from simulated processing water, J. Agric. Food Chem. 43 (1995) 2424–2427.
[16] R.T. LaLonde, G.P. Cook, H. Perakyla, L. Bu, Structure-activity relationships of bacterial mutagens related to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: an emphasis on the effect of stepwise removal of chlorine from the dichloromethyl group, Chem. Res. Toxicol. 4 (1991) 540–545.
[17] R.T. LaLonde, H. Leo, H. Perakyla, C.W. Dence, R.P. Farrell, Associations of the bacterial mutagenicity of halogenated 2(5H)-furanones with their computed MNDO-PM3 computed properties and mode of reactivity with sodium borohydride, Chem. Res. Toxicol. 5 (1992) 392–400.
[18] K. Tuppurainen, S. Lötjönen, R. Laatikainen, T. Vartiainen, Structural and electronic properties of MX compounds related to TA100 mutageneicity. A semi-empirical molecular orbital QSAR study, Mutat. Res. 266 (1992) 181–188.
[19] A. Poso, K. Tuppurainen, J. Gynther, Modelling of molecular mutagenicity with comparative molecular field analysis (CoMFA). Structural and electronic properties of MX compounds related to TA100 mutagenicity, J. Mol. Struct. (Theochem) 304 (1994) 255–260.
[20] R.T. LaLonde, G.P. Cook, H. Perakyla, C.W. Dence, Effect on mutagenicity of the stepwise removal of hydroxyl group and chlorine atom from the 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H) furanone: ^{13}C NMR chemical shifts as determinants of mutagenicity, Chem. Res. Toxicol. 4 (1991) 35–40.
[21] R.T. LaLonde, G.P. Cook, H. Perakyla, C.W. Dence, Contribution of 5-hydroxyl group to the mutagenicity of mucochloric acid, Environ. Toxicol. Chem. 9 (1990) 687–691.
[22] L. Kronberg, T. Varianinen, Ames mutagenicity and concentration of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and of its geometric isomer E-2-chloro-3-(dichloromethyl)-4-oxo-butenoic acid in chlorine-treated tap waters, Mutat. Res. 206 (1988) 177–182.
[23] R.A. Earl, F.W. Clough, L.B. Townsend, Chemical investigations of citraconimide, J. Heterocycl. Chem. 15 (1978) 1479–1483.
[24] A.A. Padmapriya, G. Just, N.G. Lewis, Synthesis of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, Can. J. Chem. 63 (1985) 828–832.
[25] B.N. Ames, D.M. Maron, Revised methods for the Salmonella mutagenicity test, Mutat. Res. 113 (1983) 173–215.
[26] M.J. Prival, V.C. Dunkle, Re-evaluation of the mutagenicity and carcinogenicity chemicals previously identified as false positives in the *Salmonella typhimurium* mutagenicity assay, Environ. Mol. Mutagen. 13 (1989) 1–24.
[27] R.T. LaLonde, L. Bu, A. Henwood, J. Fiumano, L. Zhang, Bromine-, chlorine-, and mixed halogen-substituted 4-methyl-2(5H) furanones: synthesis and mutagenic effects of halogen and hydroxyl group replacements, Chem. Res. Toxicol. 10 (1997) 1427–1436.