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Ab initio MO Studies of the Mutagenic Properties of Allylic Chloropropenes[#]

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Abstract

Motivation. It has become clear that there are two metabolic pathways involved in the mutagenic activation of chloropropenes by S9 mix. It is also clear that the dominant pathway depends upon the substituted positions of chlorine atoms. We investigated the relationship between the mutagenic activity of chloropropenes and their electronic structures as obtained by molecular orbital calculations.

Method. The calculations of charge densities were performed for some correlation levels, such as MP4SDQ/6–31G** and MP3/6–31++G**. Moreover, the charge densities were calculated by the CHELPG method for MP2/6–31G** level. Furthermore, a fully optimized calculation of protonated species was also performed.

Results. The charge densities calculated by Mulliken's approximation could not explain the experimental results regarding the mutagenic activities of the epoxidative metabolic pathway. On the other hand, the CHELPG charge densities showed good correspondence to the experimental results for the mutagenic activities. With compounds other than 2,3–dichloropropene, the calculation of protonated chloropropenes showed that the HCl molecule became detached and allylic cation was produced.

Conclusions. It is thought that the metabolic pathways of chloropropenes depend partly on the electronic structure of chloropropenes and partly on the electronic structure of subsequent intermediate compounds.

Keywords. *Ab initio* MO; allylic chloropropenes; mutagenic properties; charge densities; protonated chloropropenes; allylic cations.

1 INTRODUCTION

The electronic structures of carcinogenic substances and mutagenic substances have been studied by using the molecular orbital (MO) method [1–25]. Pullman's work regarding the electronic structure of polycyclic aromatic hydrocarbons was an early success for the MO theory of studying chemical carcinogenicity. Pullman's theory is well known as the theory of so–called *K*–region and *L*–region [1,2]. This theory was examined at the time when only the π–electron was able to be dealt

[#] Dedicated to Professor Nenad Trinajstić on the occasion of the 65th birthday.

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with. The central feature of the methodology is investigating the correlation between structure and activity, and the method includes some of values acquired by MO calculation as the independent variable. The same technique was used in recent research on quite large compounds [3–6]. In recent years, several papers have appeared that analyze the carcinogenic activity of nitroso compounds, amino compounds, and other chemical compounds by presenting the results of theoretical calculation [7–10].

During the last ten years the development of the electronic computer and the revision and refinement of programs for *ab initio* molecular orbital calculations, such as Gaussian series, make it possible for us to treat rather large biological molecules easily. In particular, in order to investigate carcinogenic phenomena, MO has been applied to molecules including nucleic acid bases and their mutagenic derivatives [11,12]. In addition to a calculation involving the nucleic acid bases themselves, many calculations were performed in order to study the interaction between members of a base pair or between a pair of base–analogous substances [13–25].

Some important observations were obtained through conducting calculations regarding nucleic acid bases. For example, it was shown that the existence of a nonplanar base pair is possible [16–18], and the possibility of making a stable structure being neither a Hoogsteen nor Watson–Crick structure was shown in ref. 19. Furthermore, the effect of a solvent was considered on the basis of calculations with a continuous solvent model [20–23]. Thus consideration has been added from various viewpoints. Hobza and Sponer referred in their study regarding nucleic acid base pairs to the importance of arguing not only by enthalpy but also by entropy, and proposed that the introduction of molecular dynamics to research in this field is necessary [24]. However, they indicated at the same time that it is important that a precise calculation of enthalpy be performed; that is, it is important to raise the accuracy of molecular orbital calculation. In recent years, higher order–electronic correlations have been considered in the calculations regarding nucleic acid base pairs [25].

Halogenated organic compounds have attracted interest for years as mutagenic substances and carcinogenic substances [26–31]. Among them, the many vinylchlorides and allylic chloropropenes have been studied with respect to their mutagenic or carcinogenic properties [27,28]. As for allylchloride and 1,3–dichloropropene, their carcinogenicity was proved by research on mice [29]. On the other hand, there also exist reports in which the electronic structures of vinylchlorides, chloropropanes, and chloropropenes are studied by molecular orbital calculation [32–36].

Neudecker and Henschler examined the indirect mutagenic activity of allylic chloropropenes by measuring the metabolic activity under the existence of rat–liver homogenate fraction (S9) [30]. In their research, they used the enzyme inhibitors to trace the activated metabolic intermediates in the metabolic processes. They found two metabolic pathways. The two pathways are shown in Figure 1. That shown in Figure 1 (a) is called the epoxidative pathway and that in Figure 1 (b) is called the

hydrolytic–oxidative pathway. It was also thought that the essential metabolic intermediates in the pathways shown in Figure 1 (a) and (b) are epoxides and acroleins, respectively.

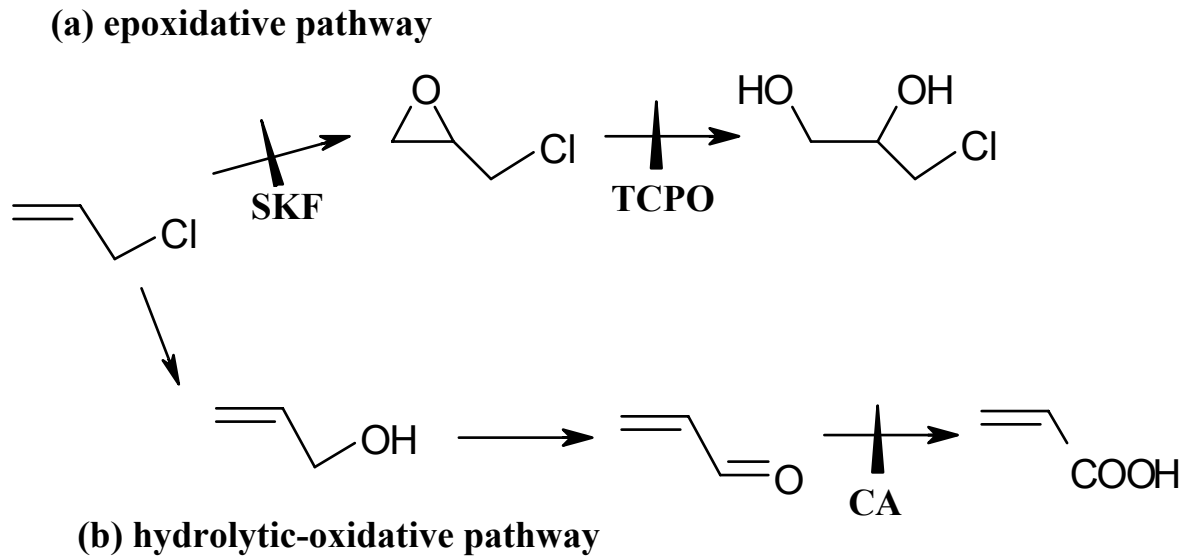


Figure 1. Metabolic pathways for allylic chloropropenes from Ref. [30]. **SKF**:KF525, **TCPO**:1,1,1–trichloropropene–2,3–oxide, **CA**:cyanamide.

In their research, six chloropropenes, allylchloride (**1**), 1,3–dichloropropene (**2**), 2,3–dichloro–1–propene (**3**), 1,2,3–trichloropropene (**4**), 1,2,3,3–tetrachloropropene (**5**) and hexachloropropene, were dealt with. The six chloropropenes are classified into three categories based on the metabolic modes. The first category includes **1** and **2**; its metabolic activity occurs through pathway (*b*). The second category includes **3** and **5** and its metabolic activity occurs through pathway (*a*). The third category includes **4** and its metabolic activity occurs through both pathways. In particular, when the inner carbon atom of the C=C–C sequence is substituted by a chlorine atom, the carbon atom becomes electron–deficient because of the –I effect of the substituent. Therefore epoxidation becomes easy, and metabolic pathway (*a*) occurs.

In this study, we examined whether calculated results that are in agreement with the conclusion of Neudecker and Henschler are obtained by researching the electronic structure of five allylic chloropropenes (**1**–**5**) from which the metabolism process differs clearly with the substituted position of chlorine. For these compounds, the molecular orbital calculation without electronic correlation has been performed by Hagen and Stolevik [32]. They reported only the stable structures of the compounds. Since their paper does not refer to electronic structure, the calculated charge densities of gas phase chloropropenes were investigated here. Because previous research has pointed out the existence of allylic cations, especially with respect to the hydrolytic–oxidative pathway [37–39], the structural change induced by proton addition was also examined here.

2 METHODS OF CALCULATION

Structure optimization was performed at the MP2/6–31G** level, and it was performed further for some local stable structures at the MP3/6–31G**, MP4SDQ/6–31G**, MP2/6–31++G**, and MP3/6–31++G** levels. The stable structure of protonated chloropropenes was calculated with the MP2 level of electronic correlation by using the 6–31G** and 6–31++G** basis sets. All calculations were performed by the use of the Gaussian98 program [40] being installed in the SGI–Origin–3400 computer of Toyama Medical and Pharmaceutical University.

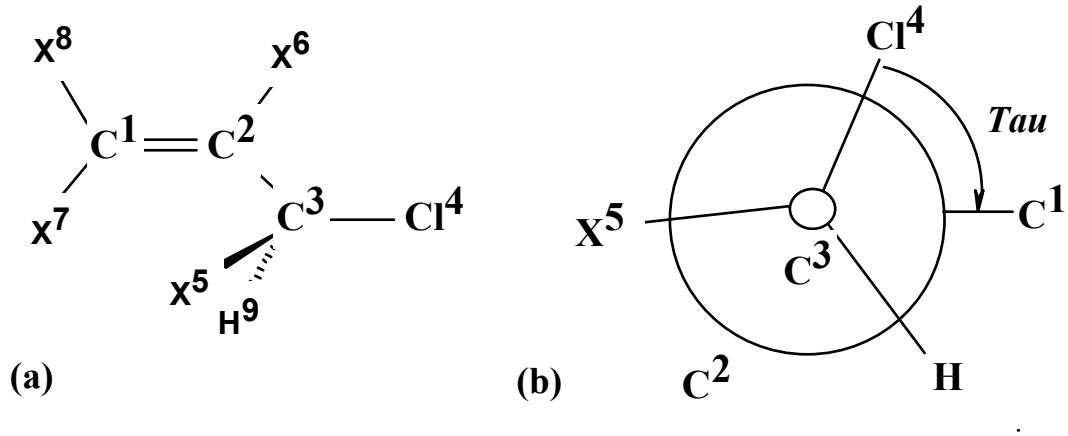


Figure 2. The numbering of the atoms in allylic chloropropenes and the definition of rotation angle of Cl^4: (*a*) numbering, (*b*) definition of rotation angle, ***Tau***. X^i means a hydrogen or chlorine atom. In following cases X^i is a chlorine atom; X^5 in **5***c* and **5***t*; X^6 in **3**, **4***c*, **4***t*, **5***c*, and **5***t*; X^7 in **2***c*, **4***c*, and **5***c*; X^8 in **2***t*, **4***t*, and **5***t*.

The end C atom in the double bond of C=C–C in **2**, **4**, and **5** was substituted by a chlorine atom. The two directions of the substitution are *cis*– and *trans*–directions, which are defined against the end C atom in the single bond of the C=C–C sequence. In reference 30, of course, the experiment was performed for *cis*– and *trans*–isomers independently. However, their report does not suggest that different metabolic pathways are needed for the *cis*– and *trans*–isomers. In the text after this, the subscripts *c* and *t* will be used to distinguish the *cis*– and *trans*–isomers. For example, we abbreviate the *cis*– and *trans*–isomers of **2** as **2***c* and **2***t*.

3 RESULTS AND DISCUSSION

3.1 Stable structure

There exist some rotational isomers of each allylic chloropropene around the C^2–C^3 bond axis, and the superscripts in C^2–C^3 are shown in Figure 2(a). In order to obtain the potential curve with changes of the rotation angle, the definition of which is shown in Figure 2(b), *ab initio* calculation for each compound was performed at the MP2/6–31G** level. In order to calculate the potential curves, the rotation angle was set up at every 15° in the range of 0° to 180° for **1**–**4** and in the range of 0° to 360° for **5**. The potential curves are shown in Figure 3.

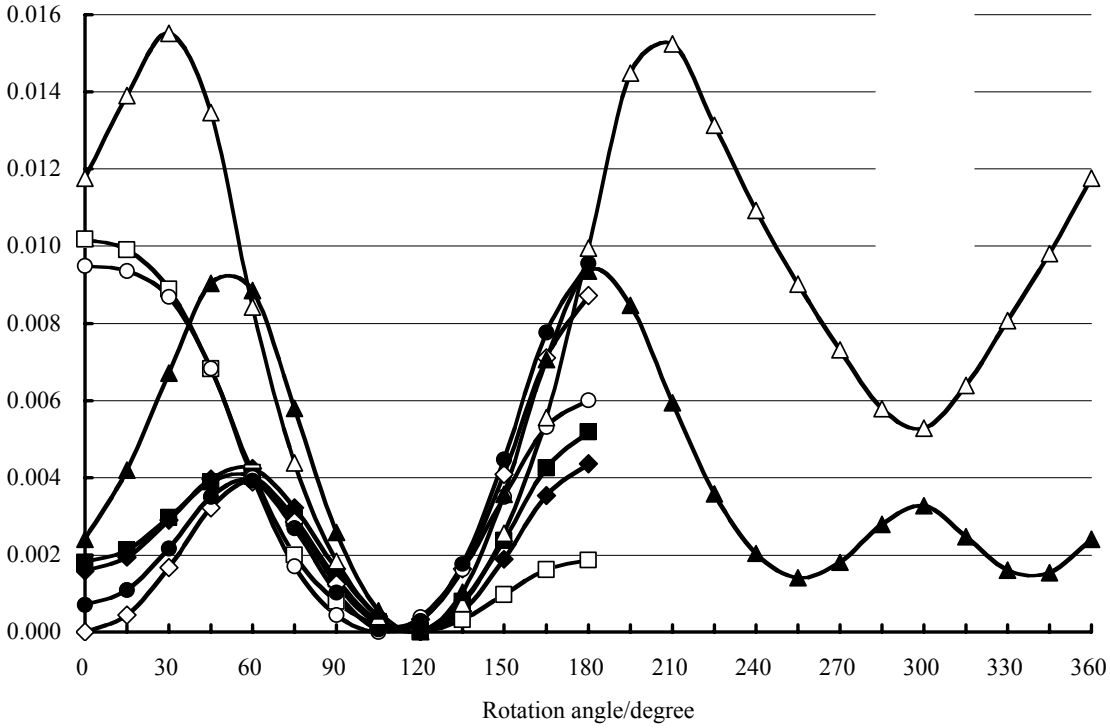


Figure 3. Potential profiles for chloropropenes calculated using MP2/6–31G**, as a function of rotation angle of Cl^4. The definition of the angle is given in Figure 2(b). **1**: allylchloride, **2***c*: 1,3–dichloropropene (*cis*), **2***t*: 1,3–dichloropropene (*trans*), **3**; 2,3–dichloropropene, **4***c*: 1,2,3–trichloropropene (*cis*), **4***t*: 1,2,3–trichloropropene (*trans*), **5***c*: 1,2,3,3–tetrachloropropene (*cis*), **5***t*: 1,2,3,3–tetrachloropropene (*trans*).

Figure 3 clarifies that the partial stable structures for **1**–**4** have rotation angles near 0° and near 120°, and these correspond to the *syn* conformer and *gauche* conformer, respectively. For **5***c*, partial stable structures exist at a rotational angle near 120° and near 300°, and these correspond to the *syn* conformer and *anti* conformer, respectively. Although there are three stable structures for **5***t* at a rotational angle near 120°, near 250°, and near 340°, the last two are mirror images of each other.

Table 1. Relative energies of isomers in each allylic chloropropene. (kcal/mol)

Compound [a]		6–31G**			6–31++G**	
		MP2	MP3	MP4SDQ	MP2	MP3
1	*syn*	0.990	0.937	0.909	1.289	1.249
	gauche	0	0	0	0	0
2*c*	*gauche*	0	0	0	0	0
2*t*	*syn*	1.133	0.977	0.970	1.547	1.378
	gauche	0	0	0	0	0
3	*syn*	0	0	0	0.412	0.302
	gauche	0.087	0.186	0.182	0	0
4*c*	*gauche*	0	0	0	0	0
4*t*	*syn*	0.440	0.211	0.248	0.942	0.686
	gauche	0	0	0	0	0
5*c*	*gauche*	0	0	0	0	0
	syn	3.293	3.179	3.222	3.331	3.201
5*t*	*syn*	0.906	0.660	0.733	1.276	1.039
	gauche	0	0	0	0	0

[a] The terms *syn* and *gauche* are defined for the Cl^4 atom in Figure 2.

Substantially, there are two structures of the angle near 120° and near 250° which correspond to the *syn* and *gauche* conformers, respectively. Hagen and Stolevik also performed the calculation for these compounds using HF/6–31G* [32]. The rotation angles for the stable structures in their results correspond well to those in Figure 3.

For the local stable structures shown in Figure 3, the effects of higher–order electronic correlation and of large basis sets were examined by performing the fully optimized calculations at the MP3/6–31G**, MP4SDQ/6–31G**, MP2/6–31++G**, and MP3/6–31++G** levels. The results of those energy values are summarized in Table 1. Except in **3**, it is shown that the conformation of the most stable structure for each compound never changes through all the levels of calculation used in this study. The most stable structure is the *gauche* conformer for **1**, **2**, and **4**, and it is the *syn* conformer for **5***c* and **5***t*.

Table 2. Bond lengths of some main bonds in allylic chloropropenes. (Å)

Comp.	Bond	6–31G**			6–31++G**		Exp.
		MP2	MP3	MP4SDQ	MP2	MP3	
	$C^1=C^2$	1.3372	1.3333	1.3364	1.3408	1.3366	1.344 [a]
1	$C^2–C^3$	1.4901	1.4947	1.4953	1.4903	1.4948	1.506 [a]
	$C^3–Cl^4$	1.7956	1.7988	1.8008	1.7971	1.8006	1.802 [a]
	$C^1=C^2$	1.3376	1.3323	1.3355	1.3405	1.3348	1.341 [b]
2*c*	$C^2–C^3$	1.4871	1.4926	1.4930	1.4870	1.4924	1.508 [b]
	$C^3–Cl^4$	1.7941	1.7965	1.7986	1.7969	1.7993	1.805 [b]
	$C^1=C^2$	1.3361	1.3309	1.3341	1.3388	1.3331	1.338 [c]
2*t*	$C^2–C^3$	1.4883	1.4936	1.4940	1.4883	1.4937	1.508 [c]
	$C^3–Cl^4$	1.7949	1.7973	1.7993	1.7970	1.7991	1.801 [c]
	$C^1=C^2$	1.3368	1.3316	1.3350	1.3398	1.3341	1.334 [d]
3	$C^2–C^3$	1.4923	1.4967	1.4973	1.4925	1.4969	1.504 [d]
	$C^3–Cl^4$	1.7877	1.7913	1.7931	1.7893	1.7928	1.776 [d]
	$C^1=C^2$	1.3420	1.3346	1.3387	1.3439	1.3361	
4*c*	$C^2–C^3$	1.4884	1.4941	1.4944	1.4881	1.4937	
	$C^3–Cl^4$	1.7781	1.7911	1.7931	1.7905	1.7934	
	$C^1=C^2$	1.3422	1.3349	1.3389	1.3445	1.3368	1.365 [e]
4*t*	$C^2–C^3$	1.4911	1.4965	1.4969	1.4909	1.4963	1.467 [e]
	$C^3–Cl^4$	1.7883	1.7910	1.7931	1.7902	1.7931	1.800 [e]
	$C^1=C^2$	1.3428	1.3352	1.3396	1.3448	1.3365	1.340 [f]
5*c*	$C^2–C^3$	1.4926	1.4992	1.4994	1.4924	1.4992	1.489 [f]
	$C^3–Cl^4$	1.7757	1.7786	1.7811	1.7773	1.7801	1.774 [f]
	$C^1=C^2$	1.3430	1.3355	1.3396	1.3453	1.3372	1.333 [f]
5*t*	$C^2–C^3$	1.4944	1.5009	1.5011	1.4945	1.5009	1.492 [f]
	$C^3–Cl^4$	1.7769	1.7796	1.7821	1.7783	1.7810	1.777 [f]

[a] Ref. [43], [b] Ref. [44], [c] Ref. [45], [d] Ref. [46], [e] Ref. [47], [f] Ref. [48]

For **3**, the different bases sets result in a different conformer as the most stable structure; that is, the *syn* conformer is the most stable structure in the results from the 6–31G** basis set and the *gauche* is the most stable structure from the 6–31++G** bases set. However, the energy difference between the two stable structures of **3** is very small, and it is smaller than the energy difference between the maximum stable structure and the 2nd stable structure in almost all of the other compounds. Due to this finding, only the *gauche* conformer was examined for **3** in the following

arguments. The calculation results of the bond distance for some main bonds are summarized in Table 2, and the calculation results of the valence bond angle and dihedral angle, including the Cl^4 atom, are summarized in Table 3.

It turns out as shown in Table 2 that the difference in each bond distance as determined by the calculation methods is small, and that the conformity of the calculated results to the experimental ones is also good. For all compounds, on the other hand, the C=C bond lengths obtained by the calculation of HF/6–31G* in reference 32 are shorter than the experimental ones by about 0.02 Å. It cannot be determined whether the shortening propensity depends on the lack of electronic correlation or the size of the basis sets until the other levels of calculation, such as MP2/6–31G* or HF/6–31++G**, are performed. It can be said at least that the calculations used in this study provide satisfactory results for the bond lengths.

Table 3. Valence bond angles, $<Cl^4C^3C^2$, and dihedral angles, $\tau Cl^4C^3C^2C^1$, in allylic chloropropenes (degree)

Comp.		6–31G**			6–31++G**		Exp.
		MP2	MP3	MP4SDQ	MP2	MP3	
1	$<Cl^4C^3C^2$	111.4	111.4	111.4	111.1	111.2	110.3 [a]
	$\tau Cl^4C^3C^2C^1$	119.1	119.4	119.9	117.8	118.3	122.7 [a]
2*c*	$<Cl^4C^3C^2$	110.8	110.8	110.8	110.8	110.7	111.9 [b]
	$\tau Cl^4C^3C^2C^1$	117.3	119.7	120.4	111.9	114.1	132.7 [b]
2*t*	$<Cl^4C^3C^2$	111.4	111.3	111.3	111.2	111.2	110 [c]
	$\tau Cl^4C^3C^2C^1$	115.9	116.9	117.3	114.9	115.8	119 [c]
3	$<Cl^4C^3C^2$	112.4	112.5	112.5	112.2	112.2	113.1 [d]
	$\tau Cl^4C^3C^2C^1$	111.3	111.0	111.3	112.3	111.6	108.9 [d]
4*c*	$<Cl^4C^3C^2$	111.8	111.9	111.9	111.7	111.8	
	$\tau Cl^4C^3C^2C^1$	105.7	105.8	106.0	105.5	105.3	
4*t*	$<Cl^4C^3C^2$	112.4	112.4	112.4	112.1	112.2	110.9 [e]
	$\tau Cl^4C^3C^2C^1$	109.9	109.7	110.0	110.8	110.3	110 [e]
5*c*	$<Cl^4C^3C^2$	111.1	111.2	111.2	111.2	111.2	111.8 [f]
	$\tau Cl^4C^3C^2C^1$	117.7	117.6	117.7	117.7	117.7	
5*t*	$<Cl^4C^3C^2$	111.5	111.6	111.6	111.5	111.6	114.2 [f]
	$\tau Cl^4C^3C^2C^1$	117.6	117.6	117.7	117.8	117.7	

[a] Ref. [43]; [b] Ref. [44]; [c] Ref. [45]; [d] Ref. [46]; [e] Ref. [47]; [f] Ref. [48]

Only the results regarding the presently observed valence bond angle$<Cl^4C^3C^2$ and the dihedral angle $\tau Cl^4C^3C^2C^1$ related to the chlorine atom are shown in Table 3. Although there was no difference between the values of the angles determined by the calculation methods, the difference between the experimental value and the calculated value ranges from small to rather large. Especially in **2***c*, the difference is large, and seems to be too large as compared with the difference in other compounds. The reason for this result is unknown. Moreover, although the difference between the experimental values of the dihedral angles in *cis* and *trans* conformers is large in a pair like **2***c* and **2***t*, in the results from all levels of calculation there is no large difference between the results of the *cis* and *trans* conformers.

Table 4. Charge densities of the atoms in chloropropenes calculated using several methods. (au)

Comp.	Atom	Mulliken's analysis					CHELPG
		6–31G**			6–31++G**		
		MP2	MP3	MP4SDQ	MP2	MP3	MP2/6–31G**
1	C^1	–0.2427	–0.2377	–0.2344	–0.2853	–0.2667	–0.3347
	C^2	–0.0476	–0.0544	–0.0530	–0.0392	–0.0392	–0.0594
	C^3	–0.3271	–0.3214	–0.3133	–0.3119	–0.2897	–0.1034
	Cl^4	–0.0761	–0.0736	–0.0738	–0.0653	–0.0681	–0.2135
	H^5	0.1582	0.1570	0.1538	0.1569	0.1496	0.0612
	H^6	0.1309	0.1309	0.1288	0.1389	0.1323	0.0997
	H^7	0.1155	0.1136	0.1118	0.1128	0.1055	0.1520
	H^8	0.1239	0.1222	0.1204	0.1247	0.1173	0.1419
	H^9	0.1651	0.1632	0.1598	0.1684	0.1597	0.0493
2*c*	C^1	–0.2121	–0.2211	–0.2180	–0.3004	–0.2906	–0.1065
	C^2	–0.0509	–0.0509	–0.0501	0.0362	0.0044	–0.1156
	C^3	–0.3374	–0.3310	–0.3232	–0.3937	–0.3733	0.2042
	Cl^4	–0.0643	–0.0617	–0.0620	–0.0598	–0.0613	–0.2174
	H^5	0.1715	0.1698	0.1665	0.1718	0.1629	0.0294
	H^6	0.1492	0.1492	0.1475	0.1658	0.1579	0.0986
	Cl^7	–0.0042	–0.0028	–0.0026	0.0799	0.0724	–0.0770
	H^8	0.1678	0.1690	0.1657	0.1434	0.1579	0.1484
	H^9	0.1805	0.1795	0.1762	0.1568	0.1500	0.0359
2*t*	C^1	–0.2105	–0.2188	–0.2157	–0.3600	–0.3540	–0.1195
	C^2	–0.0418	–0.0426	–0.0419	0.0886	0.1015	–0.0615
	C^3	–0.3326	–0.3268	–0.3187	–0.3648	–0.3437	0.0251
	Cl^4	–0.0640	–0.0608	–0.0610	–0.0619	–0.0632	–0.1888
	H^5	0.1726	0.1707	0.1672	0.1755	0.1665	0.0778
	H^6	0.1536	0.1543	0.1523	0.1549	0.1476	0.1200
	H^7	0.1614	0.1624	0.1590	0.1319	0.1259	0.1686
	Cl^8	–0.0008	0.0007	0.0012	0.0751	0.0661	–0.0945
	H^9	0.1618	0.1608	0.1575	0.1607	0.1533	0.0730
3	C^1	–0.2368	–0.2240	–0.2229	–0.2089	–0.1793	–0.3233
	C^2	–0.0378	–0.0589	–0.0555	0.0160	–0.0052	0.1336
	C^3	–0.3208	–0.3113	–0.3051	–0.4523	–0.4218	–0.0267
	Cl^4	–0.0455	–0.0435	–0.0436	–0.0394	–0.0431	–0.1660
	H^5	0.1803	0.1784	0.1747	0.1730	0.1642	0.0927
	Cl^6	0.0110	0.0149	0.0146	0.0884	0.0856	–0.1122
	H^7	0.1343	0.1322	0.1307	0.1388	0.1311	0.1548
	H^8	0.1459	0.1445	0.1429	0.1255	0.1171	0.1499
	H^9	0.1693	0.1677	0.1641	0.1590	0.1514	0.0970
4*c*	C^1	–0.2036	–0.2041	–0.2033	–0.1871	–0.1627	–0.0731
	C^2	–0.0422	–0.0570	–0.0544	0.0608	0.0448	0.0727
	C^3	–0.3287	–0.3185	–0.3127	–0.5317	–0.5016	0.0131
	Cl^4	–0.0370	–0.0344	–0.0348	–0.0326	–0.0357	–0.1628
	H^5	0.1881	0.1868	0.1832	0.1608	0.1540	0.0859
	Cl^6	0.0309	0.0340	0.0345	0.1119	0.1077	–0.1005
	Cl^7	0.0235	0.0245	0.0257	0.0960	0.0871	–0.0642
	H^8	0.1847	0.1865	0.1831	0.1460	0.1397	0.1374
	H^9	0.1842	0.1824	0.1787	0.1760	0.1666	0.0916
4*t*	C^1	–0.2150	–0.2152	–0.2146	–0.4487	–0.4463	–0.1200
	C^2	–0.0407	–0.0559	–0.0532	0.2256	0.2298	0.1225
	C^3	–0.3224	–0.3129	–0.3068	–0.4476	–0.4183	–0.0904
	Cl^4	–0.0398	–0.0369	–0.0371	–0.0439	–0.0467	–0.1503
	H^5	0.1849	0.1832	0.1794	0.1761	0.1673	0.1107
	Cl^6	0.0517	0.0554	0.0555	0.1029	0.1002	–0.0762
	H^7	0.1735	0.1745	0.1712	0.1398	0.1333	0.1572
	Cl^8	0.0368	0.0383	0.0396	0.1276	0.1203	–0.0634
	H^9	0.1710	0.1696	0.1661	0.1683	0.1606	0.1099

Table 4. (Continued)

Comp.	Atom	Mulliken's analysis					CHELPG
		6–31G**			6–31++G**		
		MP2	MP3	MP4SDQ	MP2	MP3	MP2/6–31G**
5*c*	C^1	–0.2043	–0.2034	–0.2029	–0.2316	–0.2132	–0.0522
	C^2	–0.0238	–0.0377	–0.0362	–0.0074	–0.0128	0.0754
	C^3	–0.3318	–0.3332	–0.3253	–0.3173	–0.3040	–0.0142
	Cl^4	0.0237	0.0175	0.0262	–0.0242	–0.0244	–0.0874
	Cl^5	0.0234	0.0275	0.0262	–0.0242	–0.0244	–0.0875
	Cl^6	0.0628	0.0655	0.0659	0.1504	0.1452	–0.0670
	Cl^7	0.0341	0.0347	0.0361	0.1219	0.1119	–0.0563
	H^8	0.1909	0.1925	0.1890	0.1498	0.1434	0.1344
	H^9	0.2250	0.2266	0.2210	0.1825	0.1782	0.1548
5*t*	C^1	–0.2163	–0.2152	–0.2148	–0.4389	–0.4397	–0.1149
	C^2	–0.0217	–0.0357	–0.0343	0.1326	–0.1458	0.1577
	C^3	–0.3249	–0.3271	–0.3187	–0.2898	–0.2806	–0.1722
	Cl^4	0.0222	0.0264	0.0251	–0.0186	–0.0179	–0.0635
	Cl^5	0.0222	0.0264	0.0251	–0.0186	–0.0179	–0.0635
	Cl^6	0.0828	0.0860	0.0859	0.1412	0.1386	–0.0467
	H^7	0.1776	0.1784	0.1751	0.1437	0.1368	0.1569
	Cl^8	0.0494	0.0505	0.0519	0.1523	0.1447	–0.0532
	H^9	0.2087	0.2103	0.2048	0.1962	0.1901	0.1995

3.2 Charge density

The charge densities were calculated by Mulliken's population analysis at all levels of the calculations and also by the CHELPG method [41,42] at the MP2/6–31G** level. The values of the charge densities of all atoms are listed in Table 4. All the hydrogen atoms in the molecules treated in this work have the positive charge densities. The charge densities of Cl^4 atoms of **1**, **2***c*, **2***t*, **3**, **4***c*, and **4***t* in all methods of the calculations are negative. However, the Mulliken's charge densities of Cl^4 and Cl^5 atoms of **5***c* and **5***t* in the results by the calculations with 6–31G** basis set are positive while the corresponding values with 6–31++G** basis set and with PCHELG method are negative. Mulliken's charge densities of chlorine atoms binding to C^1 or C^2 atoms are positive, with few exceptions of **2***c* and **2***t*. All of the CHELPG charge densities of the chlorine atoms are negative.

In any case, no explicit evidence by which the differences between epoxydative pathway and the hydrolytic–oxidative pathway can be explained was found in these charge densities of hydrogen and chlorine atoms. It is shown that the Mulliken's charge densities of C^1 and C^3 in all levels of the calculations are negative with a large absolute value, and the charges of C^2 have a small absolute value. The common feature in **3**, **4**, and **5**, which are metabolized through the epoxidative pathway, is that the inner carbon atom of the C=C–C sequence is substituted by a chlorine atom. From this, Neudeker and Henshler predicted that the net charge of an inner carbon must be positive according to the –I effect of the chlorine atom. In conflict with this prediction, their calculated net charges were found to be negative in the calculations performed with the 6–31G** basis set. Only the net charges of **2***c*, **2***t*, **4***c*, **4***t*, and **5***t* were positive in the calculation with the 6–31++G** basis set, although **2***c* and **2***t* have no chlorine atom which binds to the inner carbon atom. The net charges of

the inner carbon in **3** and **5***c* are negative, although the inner carbon atom is bound with a chlorine atom. This finding also disagrees with their prediction. On the other hand, the result of the charge density found by the CHELPG method conforms to their prediction. The change of the charge density of C^2 from **1** to **5***t* is shown in Figure 4. This figure shows that the change of the density is small in the calculations with the 6–31G** basis set; that is, it turns out that the influence of the number and position of the chlorine substitution on the charge density of C^2 is small.

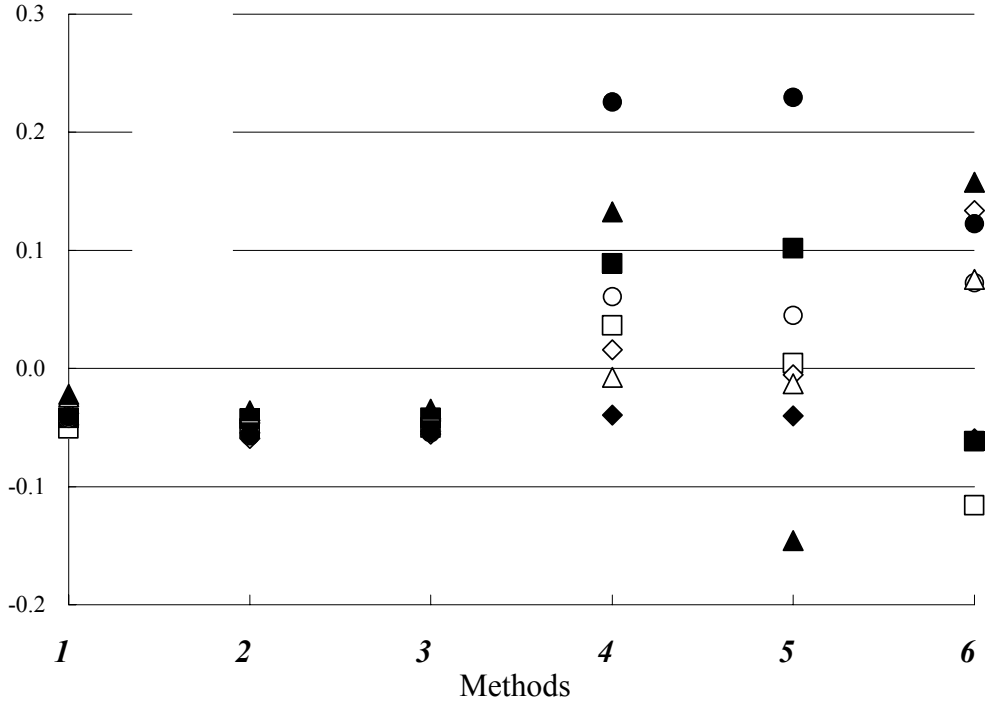
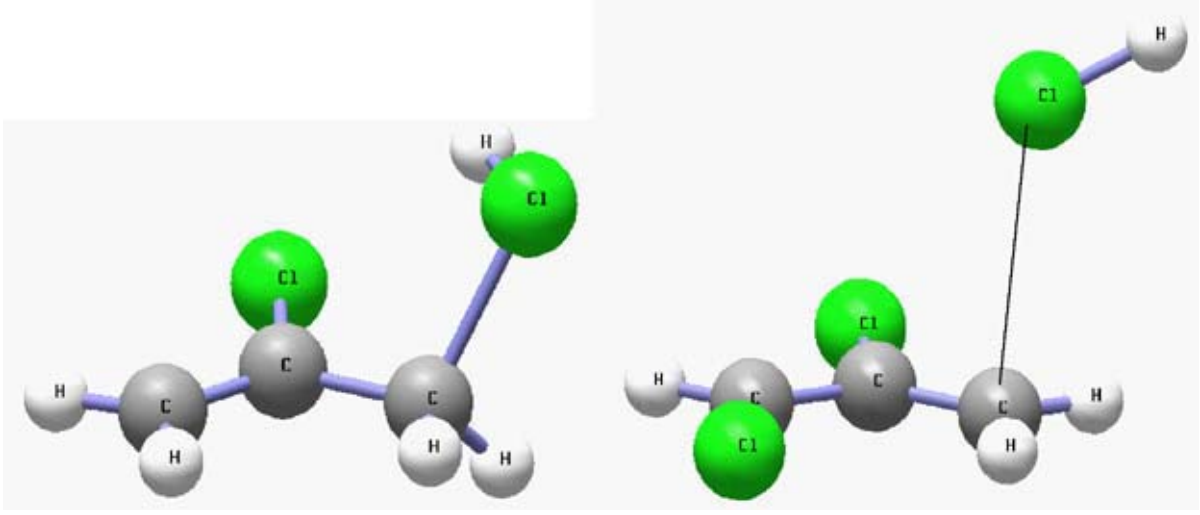


Figure 4. Change of the charge density of C^2 from **1** to **5***t* in the methods *1~6*. Methods *1*, *2*, *3*, *4*, and *5* are the Mulliken's population analysis with MP2/6–31G**, MP3/6–31G**, MP4SDQ/6–31G**, MP2/6–31++G**, and MP3/6–31++G**, respectively. Method *6* is CHELPG method in MP2/6–31G**.

Although not expressed in a figure, the results of the Mulliken's charge densities with 6–31G** basis set in Table 4 also show that the position and number of substituents have almost no influence on the charge density of C^1, C^3, and Cl^4. The Mulliken's charge density in the 6–31++G** basis set and the CHELPG charge density in the MP2/6–31G** calculation change greatly with the number of chlorine substitutions and with the position. Particularly in **3**, **4**, and **5** which have the chlorine atom binding to the C^2 atom, the CHELPG charge densities of the C^2 atom become positive, while in other compounds they become negative. The fact that the allylic chloropropenes being metabolized via the epoxidative pathway indicate positive charges for the C^2 atom in the CHELPG method supports the supposition that the electrostatic interaction is greatly concerned with the first step of the mutagenic process, because of the methodology in the CHELPG method. Due to this situation, we are very interested in the evaluation of electrostatic potentials and it would be a future work.

3.3 Addition of a proton

In accordance with the suggestion from the experimental mutagenic activity of allylic cations, the protonated allylic chloropropenes were optimized by the MP2/6–31G** and MP2/6–31++G** methods, although the hydrolysis mechanism changes with the pH of the solution. The protonated allylic chloropropenes were also examined for the possibility of decomposition into the allylic cations and HCl. It is thought that a proton makes a bond to the atoms, the charge densities of which are negative. It is thought that according to the Mulliken's charge densities it is easy to add a proton to the C^1 atom or C^3 atom, and it is difficult to add a proton to the Cl^4 atom. On the other hand, the CHELPG charge distribution shows the tendency for the addition of a proton to the C^1 atom and to the Cl^4 atom to be fairly easy. Here, a full optimization calculation of the protonated allylic chloropropenes was performed with three types of initial conformation on the MP2/6–31G** and MP2/6–31++G** levels. The initial value of the distance between H^+ and the Cl^4 atom was set to a unique value of 1.3Å in the three types of conformation. The initial value of the angle, $<H^+Cl^4C^3$, was initially set to 110.0° in the three types of conformation. The initial value of the dihedral angle, $\tau H^+Cl^4C^3C^2$, was set to 0°, 120°, or –120°, respectively, in each type of conformation. Since the optimized structures of both levels resembled each other, only the results obtained by the calculation with the 6–31++G** basis set were considered here. All of the results were classified into two kinds. That is, in the first case, the original structure of an allylic chloropropene is maintained and changes slightly when H^+ approaches the Cl^4 atom. In another case, the Cl^4 atom separates from C^3 and then HCl and an allylic cation are created. When the former is called the H^+ addition type and the latter is called the HCl separation type, only **3** becomes the H^+ addition type and all the others become the HCl separation type. Examples of these two types of stable structure are shown in Figure 5.



(*a*) (*b*)

Figure 5. Two types of stable structure of protonated allylic chloropropenes: (*a*) protonated 2,3–dichloropropene which is an example of the H^+ addition type, and (*b*) protonated *cis*–1,2,3–trichloropropene which is an example of the HCl separation type.

Figure 5(a) is an example of **3**; the calculated distance of C^3 and Cl^4 is 1.9376 Å, and the calculated distance of the added proton and Cl^4 is 1.2959 Å. On the other hand, Figure 5(b) is an example of **4***c*; the distance of C^3 and Cl^4 is 3.0113 Å, and the H–Cl distance is 1.2748 Å. It can be said that the HCl part is similar to an isolated HCl molecule because the bond distance of an isolated HCl becomes 1.2701 Å by the MP2/6–31++G** level of calculation.

The distances between C^3 and Cl^4 are 2.8588, 3.0778, 3.7010, 3.6848, 3.2323, and 3.3302 Å, respectively, in **1**, **2***c*, **2***t*, **4***t*, **5***c*, and **5***t*. In **3**, the total charge of HCl was 0.6134au, which is close to the +1au of a proton. On the other hand, the total charges of HCl in other cases were in the range of 0.0121–0.0745au, which means that the HCl part seems to be almost isolated.

Although a compound which reached different stable structures depending on the initial conformation of a proton was observed, and a compound which grew into the same stable structure altogether was also observed, no compound that becomes the H^+ addition type from a certain initial conformation of proton and the HCl separation type from another initial conformation was seen.

4 CONCLUDING REMARKS

Neudecker and Henshler found that allylic chloropropenes which are metabolized via the epoxidative pathway have the inner C atom of the C=C–C sequence substituted by a Cl atom. This fact means that the inner C atom is electron deficient because of the –I effect of the substituent. Although the expectation that the inner C atom bears a positive charge was not realized in Mulliken's charge densities, it was realized in the charge densities calculated by the CHELPG method. From this finding, it was thought that an electrostatic interaction is important for the first step of the epoxidative metabolic pathway.

By proton addition to the allylic chloropropenes the optimized structures became a decomposed feature which comprised the HCl and allylic cation being separated from each other. The only exception was **3**. If it is assumed that the existence of an allylic cation is participating in mutagenic activity, the calculated result for **3**, which could not create an allylic cation, is in agreement with the experimental result that **3** is not metabolized by the hydrolytic–oxidative pathway.

On the other hand, the calculated results for **5***c* and **5***t* cannot explain the fact of not being metabolized via the hydrolytic–oxidative pathway. The point described above suggests that it may be necessary to examine the electronic properties of intermediates in the metabolic process. It may also be necessary to examine the hydrolysis of allylic chloropropenes in neutral aqueous solution. These examinations will be the subject of a future investigation.

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Biographies

Hiroyuki Shinoda is research associate of Physical Chemistry at the Faculty of Pharmaceutical Science, Toyama Medical and Pharmaceutical University (Japan). After obtaining the Ph.D. degree in physical chemistry from Waseda University, Dr. Shinoda investigated IR and Raman intensities by theoretical calculations. His research interest is in differences of chemical properties of biological substances between in gas–phase and in aqueous–phase.

Michio Sayama is assistant professor of the department of material systems Engineering and life science, Faculty of engineering, Toyama University (Japan). After obtaining the Ph.D. degree in Toyama Medical and Pharmaceutical University, Dr. Sayama undertook postdoctoral research at Maryland University (USA). Recently, Dr. Sayama engages himself in research of metabolic activation of carcinogen. His research area spreads from analytical science and quantum chemistry to biochemistry and molecular biology. Sayama's interest is the fusion of vital phenomenon and quantum chemistry.

Yoshihiro Mori is research associate at the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University (Japan). He obtained the Ph.D. degree in physical chemistry from University of Tokyo. Dr. Mori has investigated the reactivity of molecules solubilized in a small water or organic cluster with several experimental methods such as mass spectroscopy combined with a supersonic molecular beam technology and laser–induced transient absorption spectroscopy. Recently, Dr. Mori has a great interest in electric percolation phenomena occurred via fusion of small water droplets in a reversed micellar solution.

Keiichi Kawano is professor of Structural Biology at the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University (Japan) from 1998. After obtaining the Ph.D. degree in protein physical chemistry from Osaka University, Dr. Kawano was postdoctoral fellow of Nagoya University Medical School (Japan) and Forsyth Dental Center (Boston), research associate and lecturer of Kyushu University, and associate professor of Hokkaido University. In 2002, Dr. Kawano was visiting professor Toronto University. Recently, Dr. Kawano concentrated on the search for protein conformation analysis, supported by "protein 3000 project". Dr. Kawano's research interest is in protein–protein and protein–drug interaction analysis mainly by using NMR.