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STRUCTURE–ACTIVITY RELATIONSHIP IN HALOGEN AND ALKYL SUBSTITUTED ALLYL AND ALLYLIC COMPOUNDS: CORRELATION OF ALKYLATING AND MUTAGENIC PROPERTIES

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Abstract—In a series of allylic chloroolefins and their non-allylic isomers the significance of the allylic structure and the influence of methyl and chlorine substituents on the direct mutagenic activity in *Salmonella typhimurium* (TA 100) was tested. The direct mutagenic potentials correlate well with the alkylating activities as measured in the nitrobenzyl-pyridine (NBP) test. In contrast to allyl chloride, the vinylic chloroolefins 2-chloro-1-propene and 1-chloro-1-propene did not show any direct mutagenic and alkylating properties. Monomethylated allylic chlorides are six to thirty times more mutagenic: 3-chloro-2-methyl-1-propene <3-chloro-1-butene <1-chloro-2-butene. The non-allylic isomers 2-chloro-2-butene and 4-chloro-1-butene, however, are not directly mutagenic. In spite of a higher alkylating potency, bimethylated allylic chlorides did not show an increase in mutagenicity if compared with monomethylated derivatives: 3-chloro-2-methyl-1-butene <1-chloro-2-methyl-2-butene. 1-Chloro-1-cyclohexene lacks mutagenic and alkylating activity, whereas 3-chloro-1-cyclohexene is comparable to allyl chloride in both respects. Dichloropropenes are also more directly mutagenic than allyl chloride: 2,3-dichloro-1-propene <<*trans*-<*cis*-1,3-dichloropropene. Benzyl chloride exerted the highest alkylating activity of all substances tested in this survey, and is about fifty times more mutagenic than allyl chloride. Addition of rat liver S-9 mix was followed by a distinct decrease in the mutagenicity of directly mutagenic substances, the only exception being 2,3-dichloro-1-propene, which demonstrated an increase by a factor of 35. Under the same conditions, vinylic chloroolefins are activated and become mutagenic to various degrees. Only 1-chloro-1-cyclohexene and the homoallylic compound 4-chloro-1-butene are negative both in the presence and absence of S-9 mix.

Haloolefins have increasingly gained attention as a widely used class of potentially mutagenic and carcinogenic substances, some of which, e.g. vinyl chloride [1], have recently been identified as human carcinogens. Usually an enzymatic epoxidation by mono-oxygenases is considered to be the critical step in the formation of the ultimate carcinogens [2]. In a preceding paper, however, we showed that a special group of haloolefins, the allyl and allylic compounds, may act as directly alkylating and mutagenic substances which do not require metabolic activation for mutagenic activity [3]. This direct mutagenicity is the result of a high S_N1 and S_N2' reactivity of allyl and allylic molecules, which is decisively influenced by the chemical properties of the leaving group in the allylic position [4, 5]. Though the nature of the leaving group is a very important aspect, it is not the only determining factor for the alkylating, mutagenic and possible carcinogenic potency in allylic compounds. These compounds have found extensive use and distribution in our environment, both as synthetic and naturally occuring products, e.g. as plastic monomers, pesticides, flavourings, perfumes and pharmaceuticals.

In this paper we discuss the influence of further substituents, especially methyl and chlorine substitutions in different positions in an allylic molecule, on its alkylating and mutagenic activity.

MATERIALS AND METHODS

Sources and criteria for purity of chemicals tested in this study are listed in Table 1.

The test procedures for determination of mutagenic activity in a modified Ames *Salmonella typhimurium* assay system and for determination of alkylating properties with 4-(*p*-nitrobenzyl)-pyridine (NBP-test) have been described previously [3]. The concentration of the S-9 mix has been kept identical (4 mg S-9 protein/ml mix, i.e. 1 mg S-9 mix protein/ml incubation volume). The problem of volatility, which is encountered with all compounds of the present series, has been tackled by using a modified liquid test system similar to that described by Rannug *et al.* [6].

RESULTS

All compounds tested for mutagenic and alkylating activity in our program are listed in Table 1. Ames test results are shown in Fig. 1.

(1) *Allyl chloride* (**1**), *2-chloro-1-propene* (**2**) *and* *1-chloro-1-propene* (**3**). Considering the mutagenicity and alkylating potency of allyl chloride (**1**), which has been described earlier [4, 5, 7] and discussed in detail from molecular theoretical aspects [3], we started our comparative study with its two

Table 1. Sources and criteria for purity of tested compounds. For chemical structures see Table 2

Compound	Source*	Method of purification and purity†	Confirmation of structure by NMR‡
1 Allyl chloride	A	Distilled (b.p. 45°), prep. g.l.c., 100%	6.28–5.05 (m, 3 H), 4.00 (d, 7 Hz, 2 H)
2 2-Chloro-1-propene	B	Distilled (b.p. 24°), prep. g.l.c., 100%	5.08 (s, 2 H), 2.10 (s, 3 H)
3 1-Chloro-1-propene	B	Without purification	6.07–5.52 (m, 2 H), 1.70 (m, 3 H)
4 3-Chloro-2-methyl-1-propene	A	Distilled (b.p. 71°), prep. g.l.c., 100%	4.93 (m, 2 H), 3.97 (s, 2 H), 1.80 (s, 3 H)
5 3-Chloro-1-butene	D	Distilled (b.p 65°), prep. g.l.c., > 99.8%	6.30–4.98 (m, 3 H), 4.51 (qi, 7 Hz, 1 H), 1.61 (d, 7 Hz, 3 H)
6 1-Chloro-2-butene	A	Distilled (b.p. 80°), prep. g.l.c., 100%	6.03–5.26 (m, 2 H), 3.93 (d, 6 Hz, 2 H), 1.63 (d, 6 Hz, 3 H)
7 2-Chloro-2-butene§	E	Distilled (b.p. 71°), prep. g.l.c.	5.56 (q, 7 Hz, 1 H), 1.98 (s, 3 H), 1.60 (d, 7 Hz, 3 H)
8 4-Chloro-1-butene	E	Without purification, 100%	6.15–5.48 (m,1 H), 5.18 (m, 1 H), 4.93 (m, 1 H), 3.51 (t, 7 Hz 2 H), 2.46 (q, 7 Hz 2 H)
9 3-Chloro-2-methyl-1-butene	F	Prep. g.l.c., > 96%	4.93 (m, 2 H), 4.57 (q, 7 Hz, 1 H), 1.85 (s, 3 H), 1.62 (d, 7 Hz, 3 H)
10 1-Chloro-2-methyl-2-butene	F	Distilled (b.p. 108°), prep. g.l.c., > 96%	5.50 (q, 7 Hz, 1 H), 3.91 (s, 2 H), 1.55 (d, 7 Hz, 6 H)
11 2,3-Dichloro-1-propene	B	Distilled (b.p. 35°/100 mm), prep. g.l.c., 100%	4.10 (s, 2 H), 3.73 (m, 2 H)
12 *cis*-1,3-Dichloropropene	C	Distilled (b.p. 43°/100 mm), prep. g.l.c., 100%	6.27–5.75 (m, 2 H), 4.17 (d, 6 Hz, 2 H)
trans-1,3-Dichloropropene	C	Distilled (b.p. 51°/100 mm), prep. g.l.c., 100%	6.45–5.77 (m, 2 H), 4.00 (d, 6 Hz, 2 H)
13 3-Chloro-1-cyclohexene	E	Distilled (b.p. 52°/25 mm), prep. g.l.c., 85%	5.87 (m, 2 H), 4.65 (m, 1 H), 2.10 (m, 6 H)
14 1-Chloro-1-cyclohexene	E	Without purification, 96.5%	5.73 (m, 1 H), 2.15 (m, 4 H), 1.65 (m, 4 H)
15 Benzyl chloride	A	Distilled (b.p. 50°/11 mm), 100%	7.33 (s, 5 H), 4.53 (s, 2 H)

* A, Merck, Darmstadt, F.R.G.; B, Riedel-de Haën, Seelze, F.R.G.; C, Deutsche Shell GmbH, Frankfurt, F.R.G.; D, EGA-Chemie, Steinheim, F.R.G.; E, ICN Pharmaceuticals, New York, U.S.A.; F, synthesized in this laboratory by the method described by L.F. Hatch *et al.* [15]

† Purity was detected by gas–liquid chromatography. Procedures for preparative scale and analytical gas liquid chromatography are given in detail in a preceding paper [3].

‡ NMR spectra are measured with a 60-MHz Varian EM-360 A spectrometer in $CDCl_3$ solutions with tetramethylsilane as internal standard. Data are reported as follows: chemical shift in ppm, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qi = quintet, m = multiplet), coupling constants in Hz, number of protons.

§ Contains 32% 2-chloro-1-butene as shown by NMR.

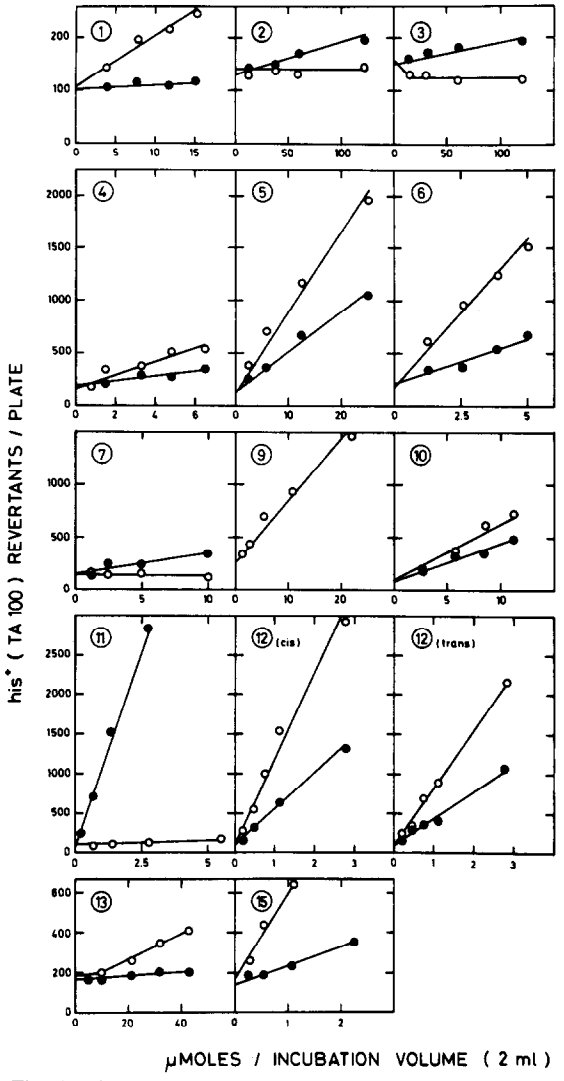


Fig. 1. The mutagenicities of those compounds listed in Table 1 which are positive in the Ames-test without (○) and/or with (●) S-9 mix. The numbers of the substances correspond to the numbers in Table 1 and Table 2.

isomers, 2-chloro-1-propene (**2**) and 1-chloro-1-propene (**3**). Both compounds exhibited no direct mutagenic or alkylating activity, since both lack an allylic structure. In contrast to allyl chloride, these isomers are vinyl-like in structure and, therefore, lack the high electrophilicity of allyl chloride, which correlates with the S_N-1, S_N-2 and S_N-2′ reactivity [8]. For this reason a direct mutagenic and alkylating potency is not to be expected. Addition of S-9 mix to the incubation mixture, however, has quite a different effect on allyl chloride and its isomers. While the weak direct mutagenicity of allyl chloride decreases in the presence of rat liver homogenate [3], 2-chloro-1-propene and 1-chloro-1-propene, which are inactive *per se*, become slightly mutagenic under these conditions. The metabolic activation of these non-allylic halooefins to mutagenic derivatives contrasts remarkably to the situation with allyl chloride.

(2) 3-*Chloro*-2-*methyl*-1-*propene* (**4**), 3-*chloro*-1-*butene* (**5**) *and* 1-*chloro*-2-*butene* (**6**). These three compounds can be regarded as methylated allyl chlorides, in each case the methyl group being located on a different one of the respective three C atoms of allyl chloride. All of these compounds have an allylic structure and should therefore be expected to be directly mutagenic and alkylating due to the high electrophilicity characteristic for allylic molecules. This theoretical expectation is fully confirmed by our experimental findings. All three compounds show a distinct direct mutagenic as well as alkylating activity. As a consequence of the methyl group substitution on any one of the three C atoms of allyl chloride, there is a 6- to 30-fold increase in mutagenicity. The molecular theoretical interpretation will be discussed below. In the presence of rat liver homogenate there is in each case a more or less distinct decrease in mutagenicity. This is consistent with what has been observed so far with all other directly mutagenic allyl compounds tested in our laboratory. To date, there has been only one exception from this general rule [2, 3-dichloro-1-propene (**11**)], which will be dealt with below.

(3) 2-*Chloro*-2-*butene* (**7**) *and* 4-*chloro*-1-*butene* (**8**). Both compounds are isomeric to those described in section (2), the principal difference being that they lack the allylic structure. In the case of 2-chloro-2-butene, the Cl-leaving group is directly bound to an olefinic C atom. With the homoallylic compound 4-chloro-1-butene, there is an interjection of a methylene group between the double bond and the C atom with the leaving group. In both cases the allylic structure and its characteristic reactivity is lacking. Consequently, as expected from theoretical considerations, both compounds are negative in regard to direct mutagenicity and alkylating potential. In the presence of S-9 mix, however, these two non-allylic haloolefins behave quite differently: 4-chloro-1-butene does not gain any detectable mutagenic activity even with high concentrations of S-9, whereas 2-chloro-2-butene is clearly activated by the S-9 mix, thus becoming a rather strong mutagen.

(4) 3-*Chloro*-2-*methyl*-1-*butene* (**9**) *and* 1-*chloro*-2-*methyl*-2-*butene* (**10**). These compounds can be regarded as bimethylated derivatives of allyl chloride. Both contain the allylic structure and are therefore alkylating and directly mutagenic. Although their mutagenicity and alkylating potencies are distinctly higher than those of allyl chloride, a comparison with the monomethylated derivatives 3-chloro-2-methyl-1-propene (**4**), 3-chloro-1-butene (**5**) and 1-chloro-2-butene (**6**) already discussed above clearly shows that the introduction of a second methyl group into the allylic molecule does not necessarily lead to a further increase, but rather to a certain decrease in direct mutagenicity. It is conceivable that a steric hindrance is responsible for this phenomenon.

(5) 2, 3-*Dichloro*-1-*propene* (**11**), cis-1, 3-*dichloropropene* (**12**) *and* trans-1, 3-*dichloropropene* (**12**). These three compounds may be regarded as allyl chloride-like molecules with a second chlorine substituent on one of the two olefinic C atoms. Compared with allyl chloride, this second chlorine atom induces a more or less distinct increase in mutagen-

icity depending on its position and on the steric constellation of the molecule. With 2, 3-dichloro-1-propene this increase is relatively small (about 3-fold), but in the case of the 1, 3-dichloropropenes a striking increase in alkylating as well as in direct mutagenic activity is observed. The mutagenicity of *cis*- and *trans*-1, 3-dichloropropene has already been described by DeLorenzo [9] and ourselves [10]. DeLorenzo used another testing system and did not check the purity of his samples, which might explain some quantitative differences between his and our results.

Compared with allyl chloride, *trans*-1, 3-dichloropropene is more mutagenic by a factor of about 70; *cis*-1, 3-dichloropropene demonstrated an even higher factor of about 100. Addition of S-9 mix, however, reveals a fundamental difference in the response of these isomers to enzymatic action. While the 1, 3-dichloropropenes lose mutagenic potential in the presence of S-9 mix, as expected, the isomeric, 2, 3-dichloropropene gains considerable mutagenic activity under these conditions. This is the first and only case of metabolic activation of a genuine allylic compound, as tested in the present study. This will be discussed further below.

(6) *3-Chloro-1-cyclohexene* (**13**) *and 1-chloro-1-cyclohexene* (**14**). Of these isomeric compounds only 3-chloro-1-cyclohexene (**13**) contains an allylic moiety; in 1-chloro-1-cyclohexene (**14**) the chlorine substituent is directly bound to the olefinic C atom. Thus the situation with these two compounds is very similar to that of allyl chloride (**1**) and its non-allylic isomers 2-chloro-1-propene (**2**) and 1-chloro-1-propene (**3**), as discussed above. In regard to both direct mutagenicity and alkylating potency, the allylic compound 3-chloro-1-cyclohexene (**13**) is very similar to allyl chloride (**1**). 1-Chloro-1-cyclohexene (**14**) is lacking any such activity.

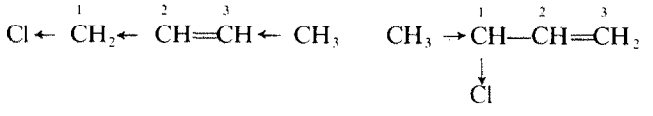
(7) *Benzyl chloride* (**15**). Benzyl chloride may be regarded in this context as an 'allylic' compound because of the easy formation of a benzyl cation in connexion with high S_N-1 reactivity. However, it does not undergo any S_N-2′ mechanism. The aromatic ring substitutes for the C = C double bond in the genuine allylic structure. Its mutagenicity exceeds by far that of allyl chloride, a fact which can easily be explained by the formation of a benzyl cation, which is highly stabilized by mesomerism, the positive charge being distributed over the methyl group and the *ortho* and *para* positions of the aromatic ring. The much lower value of revertants found by McCann *et al.* [11] may be explained by these authors' use of another testing procedure (probably high volatility in the open system, lower number of cells per plate, etc.).

DISCUSSION

The results of this comparative study of the mutagenic and alkylating activities in methyl group- and chlorine-substituted allylic and non-allylic haloolefins can easily be explained by relatively simple basic chemical considerations. As both types of substitutions may have different consequences for the chemical behaviour of a molecule, their influence on the reactivity of allylic compounds will be discussed separately.

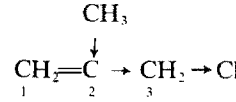
Effects of alkyl substituents. Displacement of a carbon-bound hydrogen atom by a methyl group increases mutagenicity and alkylating potency if the substitution takes place in an allylic structure. This is clearly demonstrated by comparing the data of allyl chloride (**1**) with its three methylated derivatives 3-chloro-2-methyl-1-propene(**4**), 3-chloro-1-butene (**5**) and 1-chloro-2-butene (**6**). These increases result partly from a positive inductive effect (+I effect) and partly from a positive mesomeric effect (+M effect), in this case hyperconjugation, of the alkyl substituents. Such influences are expected to be most effective if the substitution involves a carbon atom in the 1- or 3-position of the allylic structure. In this case the +I effect of the alkyl group has two important consequences:

(1) The polarity of the C–Cl bond is increased by the electron shifting effect of the alkyl group, thus favouring splitting off the chloride ion [8]. This effect may differ considerably in intensity (Scheme 1 vs Scheme 2), which is clearly reflected in the mutagenic activity (Table 2). By this, a general increase in S_N-1, S_N-2 and S_N-2′ reactivities is achieved.



1-chloro-2-butene (**6**) 3-chloro-1-butene (**5**)

Scheme 1. High activation of the C–Cl bond by +I of the methyl group in the 1- or 3-position of the allylic structure.



3-chloro-2-methyl-1-propene (**4**)

Scheme 2. Minor activation of the C–Cl bond by a methyl group in the 2-position.

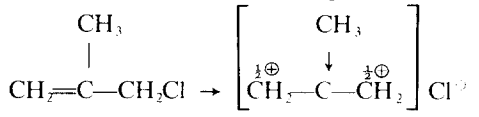
(2) The allylic cation is stabilized by the +I effect of the alkyl group [12]. This includes an increase in S_N-1 reactivity. In the case of 3-chloro-2-methyl-1-propene(**4**), however, this stabilizing effect is rela-

Table 2. Structures, mutagenicity *in vitro* (Ames test *S. typhimurium* TA 100), and alkylating properties (NBP-test) of various chlorine- and alkyl-substituted allylic and non-allylic olefinic hydrocarbons. Chemical names of compounds under identical numbers as in Table 1

No.	Substance	Mutagenicity (revertants/μmole) −S-9mix	Mutagenicity (revertants/μmole) +S-9mix	NBP-test (ΔE_{560nm})
1	$CH_2{=}CH{-}CH_2Cl$	9	1	0.285
2	$CH_2{=}C(Cl){-}CH_3$	0	<1	0
3	$CH(Cl){=}CH{-}CH_3$	0	<1	0
4	$CH_2{=}C(CH_3){-}CH_2Cl$	65	25	0.570
5	$CH_2{=}CH{-}CH(CH_3)Cl$	78	37	*
6	$CH(CH_3){=}CH{-}CH_2Cl$	270	85	1.035
7	$CH(CH_3){=}C(Cl){-}CH_3$	0	20	0
8	$CH_2{=}CH{-}CH_2{-}CH_2Cl$	0	0	0
9	$CH_2{=}C(CH_3){-}CH(CH_3)Cl$	58	n.d. †	*
10	$CH(CH_3){=}C(CH_3){-}CH_2Cl$	52	36	2.057
11	$CH_2{=}C(Cl){-}CH_2Cl$	26	925	0.248
12	$CH(Cl){=}CH{-}CH_2Cl$ *cis*	1075	450	2.240
	trans	700	340	1.933
13		6	1	0.312
14		0	0	0
15		430	95	2.925

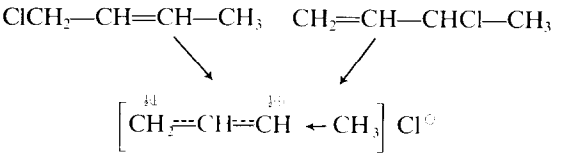
* NBP-test not applicable in this case; see Scheme 5 in the text.
† Not determined, no more compound available.

tively small, because the methyl group is not located on one of the 1- or 3-carbon atoms (Scheme 3) bearing a part of the positive charge:



Scheme 3.

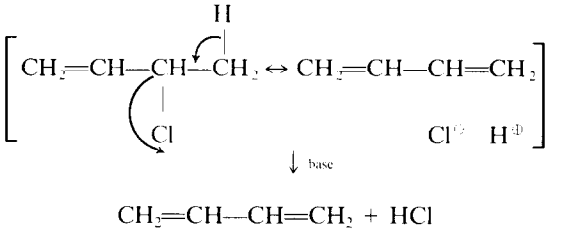
1-Chloro-2-butene (**6**) and 3-chloro-1-butene (**5**) form the same cation, the mechanism of stabilization being as follows:



Scheme 4.

The effect of Scheme 1, leading to a general increase in reactivity, prevails in 3-chloro-1-butene (**5**), but is also valid in 1-chloro-2-butene (**6**), because of the electron shift exerted by the methyl group towards the double bond. The cation stabilizing effect is equally present both in 1-chloro-2-butene (**6**) and 3-chloro-1-butene (**5**). With 3-chloro-2-methyl-1-propene (**4**), however, it is not evident, because in this case the methyl group is not located on one of the 1- or 3-carbon atoms. For this reason, the increase in alkylating and mutagenic potency is relatively small in 3-chloro-2-methyl-1-propene (**4**) when compared with allyl chloride (**1**).

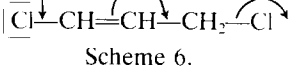
Comparing the mutagenicity of 3-chloro-1-butene (**5**) and 1-chloro-2-butene (**6**), the mutagenic activity of the former is smaller than expected from the theoretical considerations discussed above. It is conceivable that its lower mutagenicity is the result of a side reaction favoured by the hyperconjugation effect of the methyl group located on the chlorine substituted carbon atom:



Scheme 5.

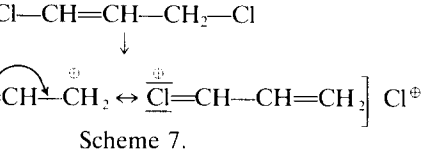
This side reaction is particularly favoured in the presence of HCl-trapping amino groups [13]. For this reason, the NBP-test does not function with compounds of this structure [i.e. (**5**) and (**9**)] (see Table 2). NBP itself and the strong amino base present in this test can act as HCl-trapping agents.

Influence of chlorine substituents. In contrast to methyl groups which exert a +I effect, chlorine substituents cause a −I effect. Yet the substitution of a hydrogen by chlorine in one of the outer carbon atoms of the allylic structure also results in increased mutagenic and alkylating activity. Compared with allyl chloride (**1**), we found a rise in mutagenicity of about 70-fold with *trans*-1, 3-dichloropropene (**12**) and of about 100-fold with the *cis*-isomer (**12**). This can be explained by the chlorine-induced positive mesomeric (+M) effect, which exceeds the inductive electron withdrawal and by which the C–Cl bond in allylic position is further destabilized [8]:



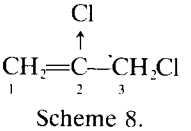
Scheme 6.

The electron release exerts an increased stabilization of the allyl cation:



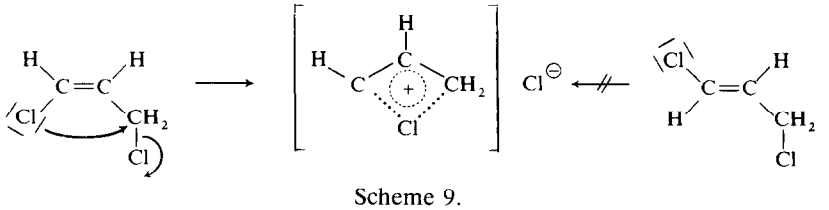
Scheme 7.

This effect is not possible when the chlorine substituent is located on the central (C_2) atom of the allylic structure:



Scheme 8.

Due to the −I effect of the chlorine substituent in the C_2 positon, this compound exerts a lower alkylating activity in the NBP test than allyl chloride. Its direct mutagenicity, however, is higher than that of allyl chloride and, in remarkable contrast both to all other allylic compounds tested in this series as well as to findings by DeLorenzo *et al.* [9], addition of S-9 mix distinctly increases the mutagenic potency of 2, 3-dichloro-1-propene (**11**). The reason for this striking discrepancy in response to S-9 mix is now under study in our laboratory and will be dealt with in a separate paper. The +M effect, however, cannot provide an explanation for the differences in alkylating and mutagenic potency in the *cis* and *trans* isomers of 1, 3-dichloropropene (**12**). The higher thermodynamic stability and lesser degree of steric hindrance of the *trans* isomer also cannot be the only reasons for this difference in mutagenicity and alkylating activity. We suppose that a neighboring group effect from the second chlorine substituent is a major cause for this discrepancy:



Scheme 9.

This effect can lead to a further destabilization of the allylic C–Cl bond. In the case of the *cis* configuration, the resulting cation can be stabilized by the formation of a chloronium-type ion, which for steric reasons is impossible in the case of *trans*- 1, 3-dichloropropene.

Chloronium ions of this kind are postulated as a transition state in addition reactions of chlorine with double bonds. Meanwhile the existence of chloronium ions has been made likely by spectroscopic methods [14].

The significance of metabolic epoxidation. Corresponding to earlier findings [3], this series of substances, too, shows direct mutagenic and alkylating activity exclusively in compounds of genuine allylic structure. Substances similar to allylic compounds, but without a leaving group in the allylic position, did not show any alkylating and/or direct mutagenic potency. Mutagenicity in these substances, if any, is observed only in the presence of S-9 mix. With allylic compounds, however, addition of S-9 mix generally leads to a decrease in mutagenicity.

These findings and the good correlation of direct mutagenicity and chemical reactivity in allylic compounds are another strong argument for our thesis that a metabolic expoxidation is not a prerequisite for mutagenic activity and that other molecular mechanisms must be taken into account in an interpretation of mutagenicity data in this special group of haloolefins.

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