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The Stereochemistry of Tritium Labeling of Hexachlorocyclohexane Isomers. I. Gas Exposure Method*

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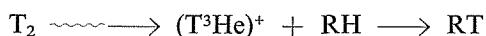
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Four isomers of Hexachlorocyclohexane were labeled with tritium by gas exposure method. From each tritiated product, the parent compound and its isomers which are expected to produce by exchange with inversion were isolated in radiochemically pure state by isotope dilution method. Further, intramolecular distributions of the activities in α,γ and δ -isomer tritiated were determined by chlorination. The results suggest that stereochemically less stable isomer such as γ -isomer is subject to inversion labeling and that a close relation exists between the intramolecular distribution of the activity in a parent compound and the yields of tritiated isomers obtained by inversion labeling.

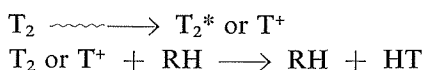
Labeling of organic compounds by exposure to tritium gas was first reported by Wilzbach¹⁾ and is consequently called "Wilzbach" labeling. Because of the great interest in the fundamental reaction mechanism as well as the widespread practical application of the method for labeling complex compounds of biological and medical importance, extensive studies on the subject have been reported.²⁾

Although the detailed mechanism of tritium labeling by gas exposure is so far not fully worked out, gas phase investigations have shown that the labeling occurs by following mechanisms.

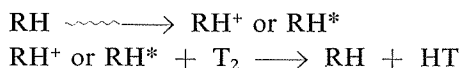
- 1) Reaction of $(T^3He)^+$ ions from the decay of molecular tritium with the substrate.



- 2) Reaction of excited or ionized tritium molecules with the substrate.



- 3) Reaction of excited or ionized compound molecules with tritium.



The above mechanisms have been proposed from the results of experiments with homogeneous systems and are therefore not necessarily applicable to the labeling of liquid and solid substrates.

Stereochemical changes which occur during the exchange of hydrogen atom for

* For a preliminary communication on this work, see M. Hamada and E. Kawano, *Tetrahedron Lett.*, **1969**, 4679

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tritium by gas exposure in the condensed phase have been received particular attention. Gordon, Intrieri and Brown³⁾ obtained labeled 1,2,3,5-tetra-O-acetyl-D-ribofuranose with apparently complete retention of configuration and Riese and Wilzbach⁴⁾ showed that 30% racemization occurred during the tritiation of (–)-mandelic acid, and Crawford and Garnett⁵⁾ reported the predominant retention of configuration in labeling of (+)- and (–)-2-octyl phthalate. Recently, Angyal, Garnett and Hoskinson⁶⁾ have demonstrated that Wilzbach labeling of crystalline (–)-inositol proceeds with a high retention of configuration, whereas its liquid hexa-O-methyl derivative undergoes the predominant inversion.

Isomers of hexachlorocyclohexane were chosen for this study because of the following reasons.

- 1) Inversion of any carbon atom in a parent compound produces a stereo-isomer and most of such isomers can be separated by liquid chromatography.
- 2) Intramolecular distribution of tritium in each isomer can be determined by chlorination of the compound.
- 3) It is possible to reveal the effect of the reaction in case of sterically less stable isomer (γ -isomer).

In the reaction, certain amount of each of α , β , γ and δ -isomers of 1,2,3,4,5,6-hexachlorocyclohexane were allowed to contact with 1 to 2 curies of tritium gas for 15 to 20 days. After exposure to tritium gas, the excess tritium was removed by vacuum transfer. The column partition chromatography which was developed by Ramsey and Patterson⁷⁾ was very convenient for the separation of isomers of hexachlorocyclohexane from above tritiated product. Certain amount of an hexachlorocyclohexane which was intended to separate, were added as carrier to a tritiated product and the mixture was subjected to the separation by the column chromatography. Chemical purity of each fraction collected by the column chromatography was checked by electron capture gas chromatography. A number of fractions which were found to be chemically pure,

Table I. Specific Activities of Hexachlorocyclohexanes

Compound exposed to tritium	Product	Specific activity, $\times 10^6$ dpm/mg parent
γ -Isomer	Crude material	19.79
	γ -Isomer (parent)	11.68
	α -Isomer	3.505
α -Isomer	Crude material	6.006
	α -Isomer (parent)	4.353
	γ -Isomer	0.0136
	δ -Isomer	0.0154
δ -Isomer	Crude material	14.76
	δ -Isomer (parent)	9.621
	α -Isomer	0.511
	β -Isomer	1.694
	γ -Isomer	0.0377
β -Isomer	Crude material	7.803
	β -Isomer (parent)	1.542
	δ -Isomer	0.893

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were combined and the same column chromatography was repeated until specific activity of the compound becomes to be constant.

Table I records the specific activities of tritium found in parent molecule and the other labeled isomers, given as dpm/mg of irradiated parent molecule, which is obtained by multiplying the measured specific activity by the isotopic dilution factor. Since reaction conditions of gas exposure to each isomer are considerably different, it is impossible to compare directly the results of each experiment. However, except in the case of β -isomer, the specific activity found in parent compound reached approximately 60% of total activity obtained.

From the data on the distribution of specific activities found in parent and its isomers in each tritiation, it is obvious that the ratio of radioactivity of an isomer obtained with inversion at one carbon atom to that with retention (parent) widely varies with the steric configuration of a compound tritiated. Correlations among the isomers of hexachlorocyclohexane which are expected to form by substitution with inversion at one carbon atom of cyclohexane ring are shown in Fig. 1.

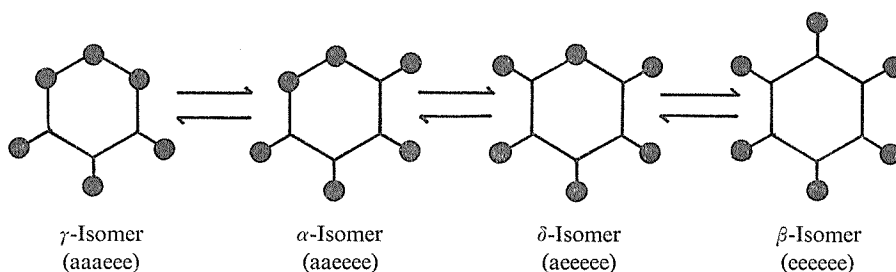


Fig. 1. Possible Inversion Scheme of Hexachlorocyclohexanes

A black circle connected with a short line or on the cyclohexane ring represents the chlorine atom in an equatorial bond or in an axial bond, respectively.

In the tritiation of γ -isomer, the activity of α -isomer which is obtained from the tritiated product of γ -isomer is approximately 30% of the activity of the parent compound. However, the activity of γ -isomer separated from the tritiated product of α -isomer is only about 0.3% of the activity of the parent. This demonstrates that the labeling with inversion at one carbon atom of γ -isomer is much easier than the labeling with that of α -isomer. The differences among activity yields obtained by inversion labeling may be largely depend on the steric hindrance of two carbon-chlorine bonds which exist axially at *meta* positions of cyclohexane ring of γ -isomer.

Both of the activities of δ - from α -isomer and α - from δ -isomer are small. In general, sterically unstable isomer is subject to inversion labeling to stable one and stable isomer is much less susceptible to inversion labeling, especially to the labeling to unstable one. A labeled isomer which is formed by exchange with inversion at two carbon atoms of the ring such as γ - from δ -isomer is very few as being expected.

In order to determine the intramolecular distribution of tritium in labeled compounds, α -, γ - and δ -isomers prepared by the above reaction were chlorinated under appropriate conditions.⁸⁾ Schemes of the chlorination are shown in Fig. 2. From the

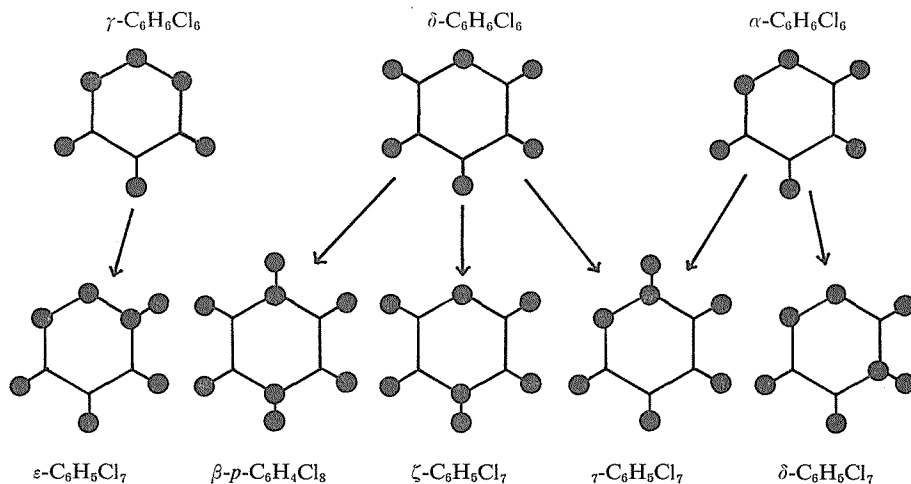


Fig. 2. Scheme of Chlorination of Hexachlorocyclohexanes

chlorinated products, isomers of hepta- and octa-chlorocyclohexane whose configurations had been established were separated in radiochemically pure state by the column chromatography described above. The intramolecular distribution of activities in original hexachlorocyclohexane was calculated from the activities of original and higher chlorinated compounds.

In the case of γ -hexachlorocyclohexane, the distribution of tritium is shown as the values at two different carbon positions because the ring conversion of the isomer represents same conformation. In cases of α - and δ -hexachlorocyclohexane, the distributions are represented as the values at three and four different carbon positions, respectively.

Table II. Specific Activities of Each Position and Intramolecular Distribution in Hexachlorocyclohexane

Isomer*		Position	Specific activity, $\times 10^6$ dpm/m mol.	Distribution
γ -C ₆ H ₆ Cl ₆		1	1.76	1.00
		2	0.20	0.11
δ -C ₆ H ₆ Cl ₆		1	0.77	0.06
		2	3.38	0.27
		3	12.69	1.00
		4	12.18	0.96
α -C ₆ H ₆ Cl ₆		1	14.63	1.00
		2	3.01	0.21
		3	12.88	0.88

* Figures of each position of cyclohexane were ring conveniently assigned to represent the positions having same chemical nature

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Table II illustrates the intramolecular distribution of tritium in 3 isomers of hexachlorocyclohexane. The reaction of He^3H^+ ions formed from the β -decay of molecular tritium with organic compounds is known to predominate under conditions ensuring that the tritiated product formed through radiolytic process are reduced to an insignificant fraction by decreasing the concentration of tritium gas.^{9,10} Assuming that each carbon of the cyclohexane ring of hexachlorocyclohexane is equally protonated by the ions in the initial stage of tritiation process under conditions employed here, such protonated intermediates may decompose to produce the mixture of the observed labeled compounds with retention, inversion or dechlorination.

Since two chlorine atoms at *meta* positions of cyclohexane ring in γ -isomer are sterically hindered, carbon positions bonded to these chlorine atoms are considered to be subject to inversion during tritiation. As indicated in Table II, the activities of γ -hexachlorocyclohexane are distributed much more in C-1 position than in C-2 where the steric hindrance exists. The activity of α -isomer which is the inversion product at C-2 position of γ -isomer is attained to 30% of that of parent as shown in Table I. Therefore, considering that protonated intermediate at C-2 position of γ -isomer decomposes either to parent tritiated at C-2 with retention or to α -isomer with inversion at C-2 of γ -isomer and that most of the intermediates at C-1 of γ -isomer change to parent tritiated at C-1 with retention, such a difference between the activities at C-1 and C-2 positions in the tritiated γ -isomer seems reasonable.

The result obtained in the case of δ -isomer demonstrates that both activities at C-1 and C-2 position are less than those at C-3 and C-4 position. As indicated in Table I, both activities of β - and α -isomer obtained by inversion labeling at C-1 and C-2 of δ -isomer are comparatively high (18% and 5%, respectively). Although isomers by inversion at C-3 and C-4 of δ -isomer have not been isolated, the intramolecular distribution may be explained by the estimation that more inversion would take place at C-1 and C-2 than at C-3 and C-4 positions of cyclohexane ring. In the case of α -isomer, the activity at C-2 position is less than activities at C-1 and C-3 positions. However, from Table I, activity of γ - or δ -isomer derived from inversion labeling at C-2 or C-1 position of α -isomer, respectively, is very low compared with that of parent. Therefore, further investigation is necessary for the explanation on the lower activity at C-2 position of α -isomer.

EXPERIMENTAL

Materials. Isomers of hexachlorocyclohexane were used after the recrystallization of commercial reagent (Tokyo Kasei Co.) and the check of the purity by gas chromatography. Tritium gas was purchased from Radiochemical Center (U. K.). Silicic acid (Mallinckrodt, U.S.A.), nitromethane and *n*-hexane were used as received.

Tritiation procedure. One to 2 curies of tritium gas is introduced by vacuum transfer into a reaction vessel containing certain amount of an isomer of hexachlorocyclohexane to be tritiated. The vessel containing tritium gas and the organic compound is stored at room temperature for 14 to 20 days. Reaction conditions are summarized in Table III.

After irradiation time, the excess tritium gas was transferred to a storage vessel.

Table III. Tritiation of Hexachlorocyclohexanes

Compound	Weight, mg	Tritium gas, Ci	Storage time, days
γ -Isomer	500	2.0	14
α -Isomer*	500	1.9	18
δ -Isomer*	500	1.8	20
β -Isomer	250	1.0	18

* α - And δ -isomers were subjected to exposure with tritium gas recovered from the reaction of γ - and α -isomer, respectively.

Aliquots of each tritiated product were then diluted with appropriate amount of original or one of the other isomers of hexachlorocyclohexane which is intended to separate. Each of these products is subjected to purification by the liquid chromatography.

Liquid chromatography. The column of liquid partition chromatography was prepared as follows. To 20 g of silicic acid was added 10 ml of nitromethane. After mixing thoroughly and then making a slurry by adding the solution of *n*-hexane saturated with nitromethane, the slurry was poured into chromatographic tube (60 cm long \times 2 cm inside diameter), and pressure was applied on to it. When the gel become so firm that it retains its shape on tipping, the pressure was released.

The sample was dissolved in minimum amount of *n*-hexane saturated with nitromethane. The solution was transferred to the column and pressure was applied. At the instant all the solution had sunk into the gel, the pressure was released. Then the tube was filled with *n*-hexane saturated with nitromethane and fractionation was begun. Percolated solution was fractionated in certain volume a fraction by a fraction collector.

The chemical purity of each fraction was checked by an electron capture gas-chromatography (OV-17 3%, 5 m on chromosorb W, AW, DMSC; 180°C; He, 30 ml/min). Relative retention time of the column partition and the gas chromatography of hexachlorocyclohexanes and the higher chlorinated cyclohexanes are illustrated in Table IV. Several fractions in which a compound are in chemically pure state were collected together and fractionation with the column chromatography was repeated. When specific activities measured separately in three times of repetition of the chroma-

Table IV. Relative Retention Time of Hexachlorocyclohexanes and the Higher Chlorinated Cyclohexanes on the Column Partition and the Gas Chromatography.

Compound	Column chromatography, Silicic acid	Gas chromatography, OV-17
γ -C ₆ H ₆ Cl ₇	1.0	1.28
ϵ -C ₆ H ₆ Cl ₇	1.1	1.89
δ -C ₆ H ₆ Cl ₇	1.4	1.79
ζ -C ₆ H ₆ Cl ₇	2.4	—
α -C ₆ H ₆ Cl ₆	2.5	1.00
γ -C ₆ H ₆ Cl ₆	3.8	1.36
δ -C ₆ H ₆ Cl ₆	10.6	1.99
β -C ₆ H ₆ Cl ₆	—	1.67

tography of a chemically pure compound became constant, the compound was regarded as radiochemically pure.

Chlorination of hexachlorocyclohexanes. Chlorination of γ -, δ - and α -isomers of hexachlorocyclohexane was performed as follows. Certain amount of a radiochemically pure isomer was dissolved in carbon tetrachloride and calculated amount of chlorine gas also dissolved in carbon tetrachloride. Both solutions were placed in a reaction vessel and the mixture was irradiated internally with 12 W low pressure mercury lamp under cooling with ice. Irradiation time to each isomer was determined by preliminary experiment with authentic non-active compound.

After irradiation, solvent was removed and chlorinated compounds in the product were separated in radiochemically pure state by the column partition chromatography as mentioned above. Specific activities of starting and the chlorinated compounds are shown in Table V. From these values, specific activities at each carbon position and therefore, intramolecular distributions of activity in 3 hexachlorocyclohexane isomers were calculated (Table II).

Table V. Specific Activities of Hexa-, Hepta- and Octachlorocyclohexanes

Starting compound	Chlorinated compound	Specific activity, $\times 10^6$ dpm/m mol.	Steric conformation
γ -C ₆ H ₆ Cl ₆	—	4.301	a a a e e e
—	ϵ -C ₆ H ₆ Cl ₇	4.104	ae a a e e e
δ -C ₆ H ₆ Cl ₆	—	41.66	a e e e e e
—	γ -C ₆ H ₆ Cl ₇	38.28	ae a e e e e
—	ζ -C ₆ H ₆ Cl ₇	32.90	a e e ae e e
—	β - <i>p</i> -C ₆ H ₄ Cl ₈	32.13	ae e e ae e e
α -C ₆ H ₆ Cl ₆	—	61.06	a a e e e e
—	γ -C ₆ H ₆ Cl ₇	46.43	ae a e e e e
—	δ -C ₆ H ₆ Cl ₇	48.18	a a e ae e e

Radioassay. Radioactivities of each compound were determined by liquid scintillation spectrometry using external standard method (Packard Tricarb-3820).

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