a little larger value with the new deproteinizing agent, which might form $[AlF_6]^{3-}$ with the reagent and hence slight turbidity in the alkaline medium.

The result of parallel tests on acetone and acetoacetic acid in blood with 2,4-dinitrophenylhydrazine method[8] is shown in Table III. All data well agreed in both methods. The precision of the present method was examined by carrying out 40 separate analysis on two blood solutions. The standard deviation was 0.25 mg./dl. (4.2%) for a mean acetone value of 5.9 mg./dl., and 0.39 mg./dl. (2.4%) for a mean value of 16.5 mg./dl.

This method of determination is simple to treat with a large number of samples, gives reliable results, and may be suitable for a clinical laboratory.

The authors are indebted to Messrs. H. Matsui and M. Hikita, and Miss Y. Soeda for the spectral measurements.

Summary

The sum of acetone and acetoacetic acid in blood was successfully determined with trinitrobenzene as the color developing agent. The method is simple to carry out with a large number of samples, and gives reproducible results.

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168. Masao Nishikawa : Infrared Spectra of Thiourea and its Inclusion Compounds. III.*1 Conformational Studies of Halocyclohexanes Trapped in Thiourea.

(*Research Laboratories, Takeda Chemical Industries, Ltd.**2)

In the preceding paper of this series,*1 description was made on the establishment of a new method of studying infrared dichroism of various molecules trapped in thiourea inclusion compounds. In that method, single-crystals of thiourea adducts, about 2 mm. long and 50 μ in diameter, were arranged side by side on a thin sodium chloride plate and their infrared spectra were measured with polarized radiation by the use of an infrared microscope attachment. It was suggested*1 that the physical state of guest molecules in thiourea channels could be investigated by this technique, and several simple examples were shown there. In the present study, the conformations of halocyclohexane molecules trapped in thiourea channels have been studied, and an outline of the results was reported as a communication.[1] It is well known that halocyclohexanes exist in equilibrium between two chair conformations either with an equatorial or an axial C-X bond. The abundance ratio or equilibrium constant of these two conformations can be best determined by nuclear magnetic resonance, infrared and other physicochemical methods and numerous investigations concerning this

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*1 Part II. This Bulletin, **10**, 1205 (1962).

*2 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (西川正夫).

[1] M. Nishikawa: Chem. & Ind. (London), 1963. 256

conformational analysis have been published.[2~10] Although the detailed data of these works were not necessarily in good agreement with one another, it has become clear that in all halocyclohexanes the equatorial conformation is predominant over the axial one in the vapour, liquid and solid states, as well as in solutions in various organic solvents. According to the most reliable experiments carried out with nuclear magnetic resonance and infrared spectroscopy,[9,10] the abundance ratio [equatorial]/[axial] for bromocyclohexane in carbon disulfide solution is 2.8 at 25°, and those for chloro- and iodo-cyclohexanes would be of nearly the same value. It would be of interest to know whether this ratio is kept even if these molecules are enclosed in rigid thiourea channels.

Experimental

IR dichroism was measured with a Hitachi IR spectrophotometer Model EPI-2 equipped with an IR microscope attachment Model IM-2P and an AgCl polarizer. A NaCl prism was used. Oriented crystal samples were prepared by either of the following methods. (1) Single-crystals of thiourea inclusion compounds were arranged on a NaCl "deck glass" using a polarized microscope. (2) Crystals were grown in a definite direction under thermal gradient. The detailed procedures were described in the previous paper.*1 Of these two methods, the former is preferable since with the latter method there is a possibility that molecules which do not form adducts might contaminate samples.

The absorption frequencies were measured with a Perkin Elmer Model 221 IR spectrophotometer installed with a prism-grating interchange assembly. Adduct crystals were immersed in liq. paraffin of high viscosity and smashed in a mortar. Then the IR spectra were measured as mull. With this procedure, adducts did not appear to have decomposed since no absorption bands attributable to rhombic thiourea[11] were detected and the parts of the spectra attributable to enclosed molecules were essentially the same as those determined by the single-crystal method.

The abundance ratio [axial]/[equatorial] in thiourea adducts was also determined as Nujol mull at 25°. The area intensities were obtained by the graphical integration of the absorbances over an appropriate frequency interval which covers the absorption band in question. Intensity measurements in CS_2 solution were made under the same conditions as those of Jensen and Gale[9]; $1M$ in CS_2 at 25°.

Halocyclohexanes and *trans*-1,2-dihalocyclohexanes were all prepared by well-known authentic procedures, and their inclusion compounds were prepared as usual.*1

Results and Discussion

Infrared spectra of chlorocyclohexane-, bromocyclohexane-, and iodocyclohexane-thiourea adducts determined by the method described above are shown in Figs. 1~3, respectively. In those figures, the adduct spectra are compared with those of halocyclohexanes in the liquid state and in carbon tetrachloride solution. In many cases, spectra of guest molecules enclosed in thiourea channels are substantially the same as those measured in the liquid state or solution.*1 In the present case, however, there were found remarkable differences between them, although the X-ray powder diffraction patterns of these adducts were essentially the same as that of thiourea adducts of chemically inert compounds (*e.g.*, decalin). This observation indicates that thiourea channels did not suffer either physical or chemical changes on the inclusion of halocyclohexane

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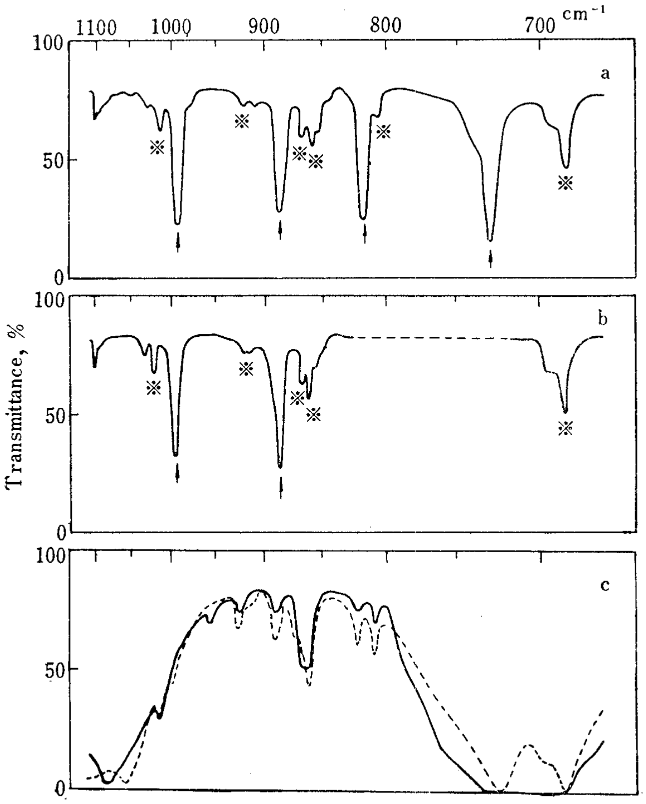
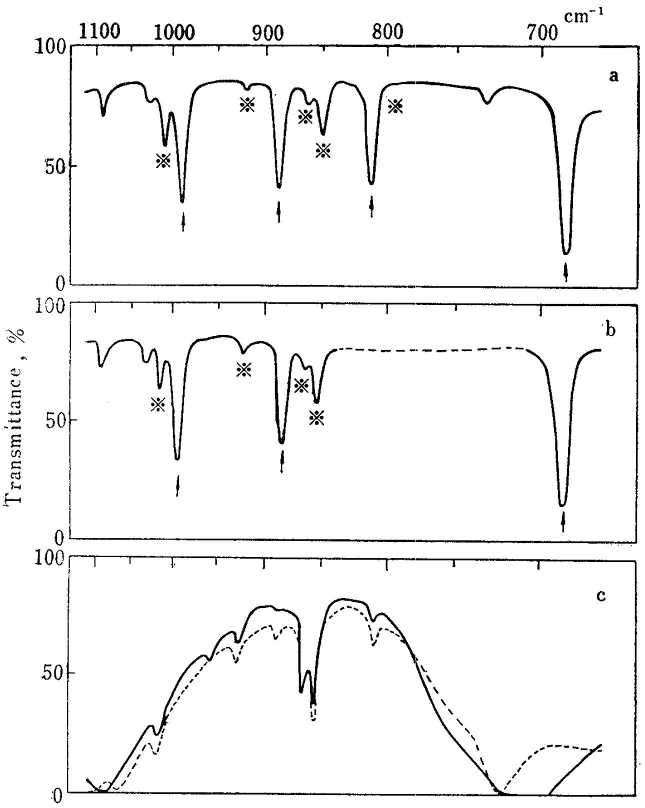
2) M. Larnandie: Compt. rend., **235**, 154 (1952); *Idem*: J. Phys. radium, **15**, 650 (1954).
3) P. Klaeboe, J.J. Lothe, K. Lunde: Acta Chem. Scand., **10**, 1465 (1956).
4) P.A. Pickerino, C.C. Price: J. Am. Chem. Soc., **80**, 4931 (1958).
5) K. Kozima, K. Sakashita: Bull. Chem. Soc. Japan, **31**, 796 (1958).
6) E.L. Eliel: Chem. & Ind. (London), 568 (1959).
7) C.G. LeFevre, R.J.W. LeFevre, R. Roper, R.K. Pierens: Proc. Chem. Soc., **1960**, 117.
8) G. Chiurdogle, L. Kleiner, W. Masschelein, J. Reisse: Bull. soc. chim. Belg., **69**, 143 (1960).
9) F.R. Jensen, L.H. Gale: J. Org. Chem., **25**, 2075 (1960).
10) A.J. Berlin, F.R. Jensen: Chem. & Ind. (London), 998 (1960).
11) M. Nishikawa, H. Mima, T. Kanzawa: This Bulletin, **10**, 1199 (1962).



a : Liquid state
b : CCl_4 soln.
c : Thiourea adduct
—— With the polarized light, electric vector perpendicular to the C-axis
- - - Parallel to it

Fig. 1. Infrared Spectra of Chlorocyclohexane



a : Liquid state
b : CCl_4 soln.
c : Thiourea adduct
—— With the polarized light, electric vector perpendicular to the C-axis
- - - Parallel to it

Fig. 2. Infrared Spectra of Bromocyclohexane

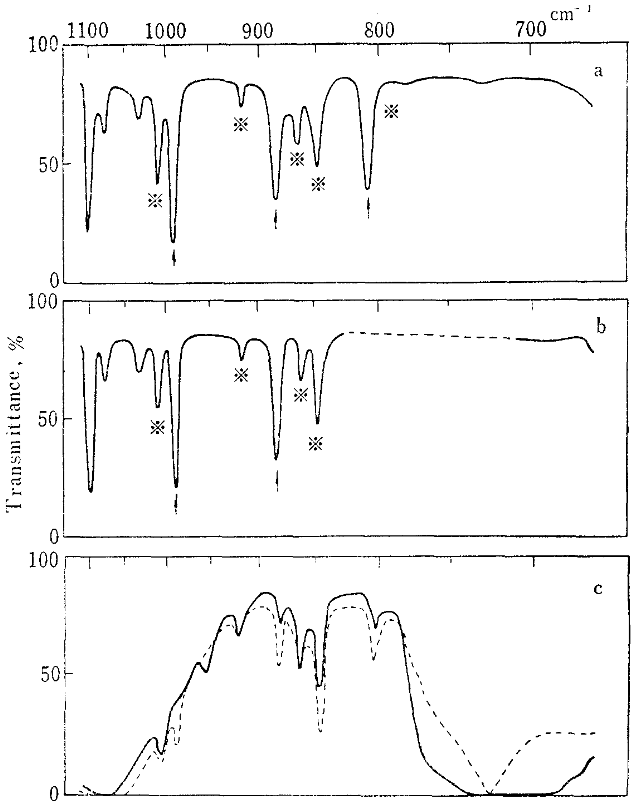


Fig. 3. Infrared Spectra of Iodocyclohexane

a : Liquid state
b : CCl_4 soln.
c : Thiourea adduct
—— With the polarized light, electric vector perpendicular to the C-axis
- - - Parallel to it

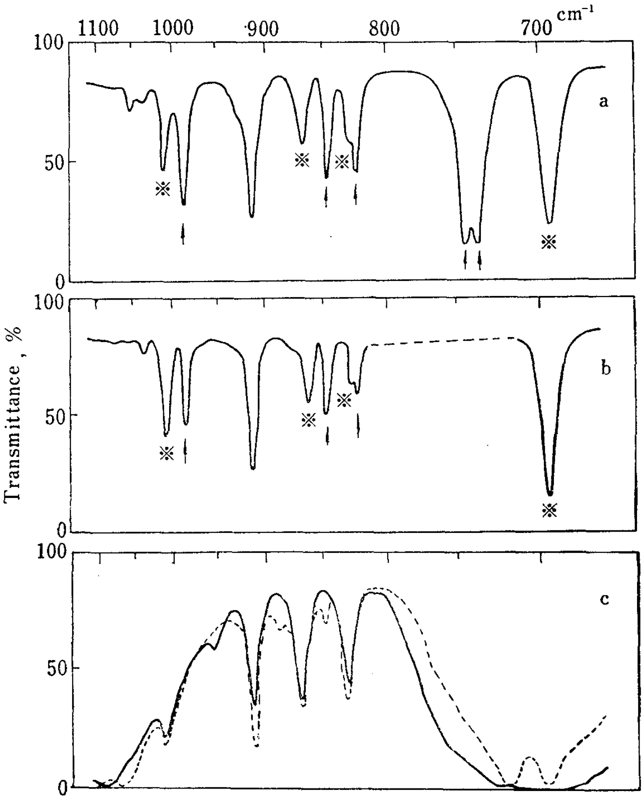


Fig. 4. Infrared Spectra of *trans*-1,2-Dichlorocyclohexane

a : Liquid state
b : CCl_4 soln.
c : Thiourea adduct
—— With the polarized light, electric vector perpendicular to the C-axis
- - - Parallel to it

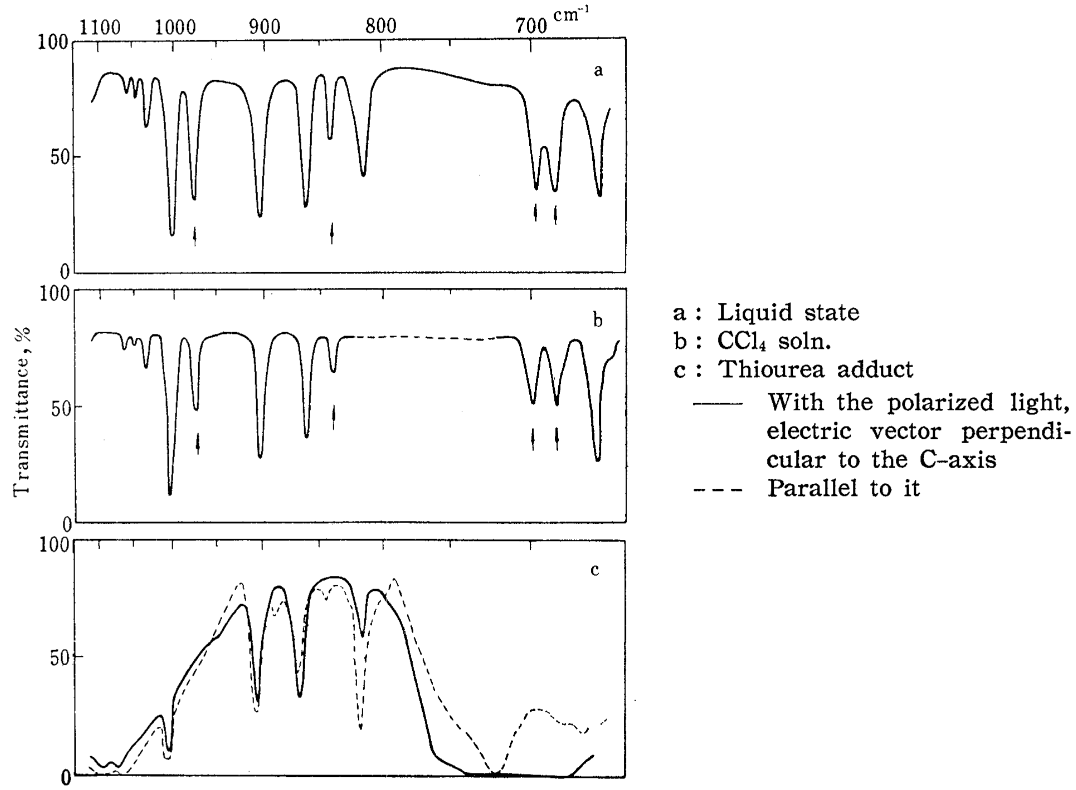


Fig. 5. Infrared Spectra of *trans*-1,2-Dibromocyclohexane

molecules. In fact the absorption spectra of the ether extract of the thiourea adducts were found to be due entirely to halocyclohexanes. Therefore the possibility of selective adduct formation with certain impurities which might have existed in a small amount in the starting materials would be discarded.

When thiourea adducts are formed, new bands which are absent in the spectra of the liquid state or of the solutions do not appear but the absorption bands pointed by arrows in the figures decrease in intensity as much as those bands marked with stars increase, as listed in Table I. These spectral changes from liquid halocyclohexanes to their thiourea adducts actually suggest some conformational changes in the guest molecules.

Formerly, Klaeboe, *et al.*[3] and Kozima and Sakashita,[5] independently, have investigated the spectrum of chlorocyclohexane in the solid anisotropic modification at —150°

Table I. Intensity Changes of Halocyclohexanes on the Formation of Thiourea Adducts

Frequencies of absorption bands (cm^{-1})			Intensity change
Chlorocyclohexane	Bromocyclohexane	Iodocyclohexane	
1016	1009	1006	increase
994	987	988	decrease
922	918	917	increase
889	885	872	decrease
867	861	862	increase
859	850	848	〃
818	810	807	decrease
807	804	804	increase
(728)[a]	685	—	decrease
683	(658)[a]	—	increase

a) The bands in brackets are obscured by strong absorptions due to thiourea.

and noted the disappearance of a set of absorption bands of medium intensities in the liquid state. This set, which has been assigned by these investigators to the conformational isomer with the axial C–Cl bond,*[3] well coincides with that of the bands which increased in intensity on the formation of thiourea adducts. On the contrary, the other bands which have been assigned to the equatorial isomer coincide with those which decreased in intensity. Therefore, it is reasonable to assume that chlorocyclohexane molecules exist predominantly in the axial conformation in thiourea channels. Usually the equilibrium constant between the two conformational isomers is determined by the intensity measurement of the C–Cl stretching bands associated with both isomers. In the present case, however, the spectral region where the C–Cl stretching band of the equatorial isomer is expected to appear is blanketed by the strong thiourea absorption. Also in the case of bromocyclohexane, the C–Br stretching band of the axial isomer is hidden under the thiourea absorption band and such technique of conformational analysis was not applicable for either case. But it must be noticed in Figs. 1 and 2 that the C–Cl stretching band of the axial isomer of chlorocyclohexane is clearly observed at 683 cm^{-1} in thiourea adduct whereas there is no sign of absorption at the position where the C–Br stretching vibration of the equatorial isomer of bromocyclohexane should absorb (685 cm^{-1}), although these two stretching have nearly the same vibrational frequency. This observation, combined with comparative consideration with Klaeboe's assignment,[3)] would indicate the predominance of the axial conformation of bromocyclohexane as well as chlorocyclohexane in thiourea channels.

As for iodocyclohexane, the vibrational assignment for the two conformational isomers does not appear to be made as yet except for the C–I stretching vibrations, with which the predominance of the equatorial isomer in the liquid state has been established.[2)] As the C–I stretching frequencies do not lie in the sodium chloride region, these bands were not available for conformational analysis in this work. However, the strong similarity of spectral changes from the liquid state to thiourea adducts among these three halocyclohexanes (Table I) would suggest the same phenomenon, that is, the inversion of the predominance of the C–X bond conformation. The results would lead to the following classification for vibrational bands of iodocyclohexane in the region between 1020 and 750 cm^{-1}; the bands at 1006, 917, 862, 848, and 804 cm^{-1} arising from the axial isomer and those at 988, 872, and 807 cm^{-1} from the equatorial one. As will be discussed in the next paragraph, the intensity of the band at 917 cm^{-1} may be partly due to the equatorial isomer.

The assignments made by Klaeboe, *et al.* and by Kozima and Sakashita should be revised in part judging from the data of the present experiments. First, the bands at 920 cm^{-1} of chlorocyclohexane and at 919 cm^{-1} of bromocyclohexane have been attributed to their equatorial isomer because they remain in the spectra of the solid anisotropic modification. Corresponding bands were found, however, also in the spectra of their thiourea adducts at nearly the same position; chlorocyclohexane, 922 cm^{-1} and bromocyclohexane, 918 cm^{-1}. Since these bands were not found in the spectra of other thiourea adducts, they would not be originated from thiourea, the host molecule. They are not due to a certain impurity either, for the adducts of chloro-, bromo-, and also iodo-cyclohexane exhibited these bands at similar positions with similar intensities. It seems to be appropriate, therefore, to assume that at these positions the bands originated from the axial and equatorial isomers overlap as unresolved bands. Secondly, in assigning spectra of bromocyclohexane Klaeboe, *et al.*, have correlated the band at 846 cm^{-1} in the solid anisotropic modification with that at 850 cm^{-1} in the liquid state, which consequently was attributed to the equatorial isomer. This is not strictly correct because the band corresponding with the one at 850 cm^{-1} in the liquid state was found to increase in intensity on adduct formation. Thus, this band would arise from the axial isomer. The absorption band of the equatorial isomer in the liquid state may be located at the unresolved inflection on the low frequency side of the 850 cm^{-1} band.

Klaeboe, *et al.* have presumed that an inflection of the band at 811 cm^{-1} in the case of bromocyclohexane is possibly due to the breathing vibration of the axial isomer. This was confirmed by the appearance of the strong band at 804 cm^{-1} when inclusion compounds were formed.

From the intensity changes as listed in Table I, it may be possible to make correspondence between bands due to the axial isomer and those due to the equatorial isomer. Taking chlorocyclohexane as an example, the band at 1016 cm^{-1} of the equatorial isomer would correspond to the band at 994 cm^{-1} of the axial one. The band at 922 cm^{-1} would be due to the two isomers as discussed before. The band corresponding to the equatorial one at 889 cm^{-1} would be splitted into two moderately strong bands at 867 and 859 cm^{-1} for the axial conformation. The splitting increases with the mass of the halogen atoms. The breathing vibrations lie at 818 cm^{-1} and 807 cm^{-1} for the equatorial and axial isomer, respectively.

The isomer ratio [axial]/[equatorial] in thiourea adducts could be calculated more quantitatively. As mentioned before, C–X stretching vibrations are of little use for this purpose and the following approximate method had to be employed. Using the ratio [equatorial]/[axial] of 2.80 for bromocyclohexane (recalculated from $\Delta F=610$ cal./mole) obtained by Jensen and Gale[9)] in the carbon disulfide solution at 25° as a standard, the spectrum of bromocyclohexane was recorded under the same condition, and the integrated intensity (A_{ecs_2}) of the band of the equatorial isomer at 885 cm^{-1} and that (A_{acs_2}) of the sum of the intensities of the bands of the axial isomer at 861 and 850 cm^{-1} were obtained by weighing. The ratio of the molecular extinction coefficients of these bands could be calculated from the ratio of the integrated intensities and from the value 2.80, by the following formula :

$$\frac{K_{C-Br(e)}}{K_{C-Br(a)}}=\frac{A_{ecs_2}}{A_{acs_2}}\times\frac{1}{2.80}=0.44$$

where the ratio A_{ecs_2}/A_{acs_2} was determined from the average data of four experiments. Here one has to make several assumptions for further development of this method. The ratio of the molecular extinction coefficients calculated above was assumed to be constant even halocyclohexane molecules are enclosed in thiourea channels. The fact that the spectra of guest molecules in thiourea adducts are nearly identical with those of solutions in nonpolar solvent, would suggest the validity of this assumption. As thiourea adducts dissociate in solution, spectral measurement has had to be made as Nujol mull, in which case light scattering was assumed to have small effect on the integrated intensities of the bands in question and Beer–Lambert's Law should also hold within the accuracy of this approximation. Moreover, in the case of chlorocyclohexane and iodocyclohexane, there is no suitable value available for standards of the isomer ratios. So it must be necessary to place another assumption. In this work, the ratio $K_{C-Br(e)}/K_{C-Br(a)}$ was assumed to be invariable among all the halocyclohexanes dealt with. Though at first glance the validity of this assumption may seem to be rather doubtful, the isomer ratio K'_{cs_2} of these compounds in carbon disulfide solutions at 25° calculated under this assumption was found to be in good agreement with the ratio K_{area} or K_{shift} obtained by Berlin and Jensen[10)] at −81°, in view of the temperature difference of both experiments. They are tabulated in Table II. In the table, the data from the work conducted by Jensen, *et al.* are listed in the second to sixth columns. In the seventh column, the ratios of area intensities in carbon disulfide solutions are shown, from which K'_{cs_2}'s were determined. Each K'_{cs_2} is smaller than K_{shift} by about 0.5 and is

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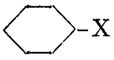
[*3] Only the equatorial one was considered to remain in the solid anisotropic modification.

Table II. Isomer Ratios of the Halocyclohexanes

	Jensen's work[9,10]					Present work				
						CS₂ soln.		Thiourea adducts		
-X	ΔF_{area}[a]	K_{area}[a]	ΔF_{shift}[b]	K_{shift}[b]	K_{cs_2}[c]	A_{ecs_2}/A_{acs_2}	K'_{cs_2}	A_{eA}/A_{aA}	K_A	ΔF_A
	(cal./mole)	(−81°)	(cal./mole)	(−81°)	(25°)	(25°)	(25°)	(25°)	(25°)	(cal./mole)
X = Cl	513	3.84	478	3.49	—	1.30	3.0	0.16	0.37	590
Br	480	3.51	439	3.15	2.8	1.23	2.8	0.11	0.26	790
I	431	3.09	407	2.90	—	1.05	2.4	0.25	0.56	350

a) Obtained from the areas under the (H-C-X) peaks of NMR[10]
b) Obtained from the chemical shifts of the above peaks[10]
c) Obtained from IR[9]

smaller than K_{area} by about 0.7. These results would suggest that the last assumption is valid within the error of this method. Based on these assumptions, therefore, the isomer ratios of halocyclohexanes in thiourea adducts were calculated by the use of the ratios of integrated intensities obtained by the technique described in the experimental part and the results which are shown in the next two columns of Table II reveals an interesting phenomenon. The axial conformation is the most favorable for bromocyclohexane ([axial]:[equatorial] = 4:1) and its preference decreases in order of chlorocyclohexane (3:1) and iodocyclohexane (2:1). The order is in sharp contrast with the case of the liquid state or solutions where the ratio [equatorial]/[axial] decreases in proportion with the mass of the halogen atoms attached to the cyclohexane ring at least among the molecules treated in the present work. These facts would show that the isomer ratio in thiourea adducts depends upon the steric factor of the host and guest molecules, that is, the shape and size of the container and the contents, and the axial form of bromocyclohexane would seem to fit most adequately with the space provided by thiourea channels.

There could be the following three possible cases when the coexistence of these two conformational isomers is realized in thiourea adducts.

(1) Two isomers form a complex in a fixed ratio in thiourea channels. In this case, the isomer ratio must be an integer.

(2) The isomer ratio depends on the condition under which adduct crystals are grown and once halocyclohexane molecules are enclosed in channels, their conformation is not able to change either from axial isomer to equatorial one or *vice versa*.

(3) Both isomers are in dynamic equilibrium in thiourea channels.

Since it has been known that two molecules of halocyclohexanes are enclosed in a unit cell of rhombohedral thiourea crystal,[12] it would not be energetically profitable to form a complex of the isomer ratio 1:4, 1:3, or 1:2 in the crystal. So the first case would be unlikely. To check the second case, adduct crystals of chlorocyclohexane and iodocyclohexane were prepared under several different conditions but the isomer ratio obtained was found to be constant every time. A part of this experiments is shown in Table III.

Table III. The Ratio of Integrated Intensities A_{eA}/A_{aA} of Iodocyclohexane under Different Conditions of Crystallization

Conditions of crystallization	A_{eA}/A_{aA}
Crystallized instantaneously by rapid cooling	0.25
Crystallized slowly taking three or four days	0.26
Crystallized at temperature above 60°	0.25
Crystallized at temperature between 25°∼0°	0.26
Crystallized at temperature between −20°∼−70°	0.23

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[12] W. Schlenk: Ann., **573**, 142 (1951).

The last case is, therefore, concluded to be most probable at present and if so, free energy differences ΔF_A in thiourea channels could be calculated from K_A and the results are also listed in the last column in Table II.

Turning now to *trans*-1,2-dichlorocyclohexane and *trans*-1,2-dibromocyclohexane, there are also several papers[13~15] which dealt with their conformational problem by infrared methods. According to them, the former compound takes only diequatorial form on crystallization at low temperature, whereas the latter freezes into the diaxial form, although both are in equilibrium between two conformations in the liquid state or in solutions. The factors which determine the conformation would be largely intramolecular steric or electrostatic ones. But as in thiourea channels these factors have been found to become less important and an intermolecular steric interaction has a greater effect, the diequatorial conformation was expected to be much more unfavorable in these two molecules than in the three halocyclohexane molecules treated above. The experimental results obtained are shown in Figs. 4 and 5. Both *trans*-1,2-dihalocyclohexanes were found to exist almost exclusively in the diaxial conformation in thiourea channels. Thus the above expectation was found to be the case.

There is another point to be noticed. In spectra of halocyclohexane-thiourea adducts, all the bands attributed to the axial isomers show little infrared dichroism but, on the other hand, those attributed to the equatorial ones exhibit fairly strong parallel dichroism along the C-axis of the crystal. This would show that the axial isomers could orientate at random or rotate freely in thiourea channels but the equatorial ones could not do so because of their "knob" projected out in their molecular plane. In the case of *trans*-1,2-dihalocyclohexanes, the chloro derivative would be relatively unrestricted in channels whereas the breathing vibration of the bromo derivative suggests that the two fold axis of the compound mostly takes the same direction as that of the C-axis of the adduct crystal. This phenomena might be also explained by less mobility of the latter derivative owing to the bulkiness of the bromine atoms.

The author expresses his deep gratitude to Dr. H. Mima for his kind guidance throughout the course of this work, and to Takeda Chemical Industries, Ltd. for permission to publish. He is also grateful to Mr. A. Takabatake and Miss F. Kasahara for experimental assistance.

Summary

The infrared spectra of halocyclohexanes and *trans*-1,2-dihalocyclohexanes trapped in thiourea channels were investigated by the use of the technique developed in the previous paper. It was revealed that these compounds took preferably the axial conformation of the C-X bond in thiourea channels. The abundance ratio of the two conformational isomers is found to depend upon the size and shape of these molecules and of the space provided by thiourea framework. From these results the bands attributable to the axial isomer were assigned definitely and the assignment made by previous workers was partly revised. The mobility or the orientation of these guest molecules in channels was also discussed and the dynamic equilibrium between the axial and equatorial conformations was proved in the case of halocyclohexanes.

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13) M. Larnandie: Compt. rend., **236**, 909 (1953).
14) K. Kozima, K. Sakashita, S. Maeda: J. Am. Chem. Soc., **76**, 1965 (1954).
15) P. Klaeboe, J. J. Lothe, K. Lunde: Acta Chem. Scand., **11**, 1677 (1957).