tals. Recrystallization from EtOH-H_2O gave slightly yellow plates (0.50 g.), m.p. 139～140°. *Anal.* Calcd. for $C_9H_9O_2N_3$: C, 56.54; H, 4.75; N, 21.98. Found : C, 56.61; H, 4.89; N, 21.83.

The IR spectrum of this compound was identical with that of XXIV above-obtained, and the mixed melting point showed no depression.

The authors are deeply grateful to Prof. Emeritus E. Ochiai of the Uneversity of Tokyo and Dr. K. Takeda, Director of this Laboratory, for their helpful advices and encouragements. Thanks are due to Mr. K. Aono for NMR spectral measurements, to the members of the Analysis Room of this Laboratory, and to Mr. S. Hashimoto for his technical assistance.

Summary

Reactions of 1-alkoxybenzimidazole (I) with nucleophilic reagents, acetic anhydride and some other reagents involving the nitrating mixture were examined. In these reactions, I behaves as an ordinary heteroaromatic N-oxide and its N-alkoxyquarternary salt, although the reactivity is not so large as the quarternary salt.

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[Chem. Pharm. Bull. 12 (3) 291 ～ 298]

UDC 547.496.3 : 548.2 : 543.422.4

41. **Masao Nishikawa and Akio Takabatake** : Infrared Spectra of Thiourea and its Inclusion Compounds. V.[*1] Use of Thiourea-d_4 as a Host Molecule.[*2]

(*Research Laboratories, Takeda Chemical Industries, Ltd.*[*3])

Considerable efforts have been devoted to investigations on the behavior of molecules trapped in clathrate compounds. Physicochemical methods, such as wide-line nuclear magnetic resonance,[1,2] magnetic susceptibility,[3] thermal analysis,[4] dielectric absorption,[5] electron spin resonance,[6] and infrared absorption,[7~9] are useful for such investigations. One of the authors (M. N.) also has attempted to study the state of guest molecules in thiourea channels by means of infrared spectroscopic analysis. Usually, absorption bands due to the host molecule (thiourea) are so strong that bands of guest molecules are masked by them almost completely and, consequently, all inclusion compounds exhibit nearly the same spectra when treated by ordinary Nujol mull or potassium bromide disk methods. By the technique described in previous papers of this series,[*1,10~12] however, some absorption bands of guest molecules can be observed in

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[*1] Part Ⅳ : This Bulletin, **11**, 1290 (1963).

[*2] Presented at Symposium on Molecular Structure, Sendai, Oct. 3, 1963.

[*3] Juso-nishino-cho, Higashiyodogawa-ku, Osaka (西川正夫, 高畑明夫).

1) D.F.R. Gilson, C.A. McDowell : Nature, **183**, 1183 (1959); *Idem* : Mol. Phys., **4**, 125 (1961).
2) H. Nakajima : Presented at International Symposium on Molecular Structure and Spectroscopy, Tokyo, September, 13, 1962.
3) H. Meyer, M.C.M. O'Brien, J.H. VanVleck : Proc. Roy. Soc., **A**, **243**, 414 (1957).
4) H.G. McAdie : Canad. J. Chem., **40**, 2195 (1962).
5) R.J. Meakins : Trans. Faraday Soc., **51**, 953 (1955).
6) O.H. Griffith, H.M. McConnell : Proc. Nat. Acad. Sci. U.S., **48**, 1877 (1962).
7) R.S. Drago, J.T. Kwon, R.A. Archer : J. Am. Chem. Soc., **80**, 2667 (1958).
8) R.A. Durie, R.J. Harrison : Spectrochim. Acta, **18**, 1505 (1962).
9) D.F. Ball, D.C. McKean : *Ibid.*, **18**, 933. (1963).
10) M. Nishikawa : Chem. & Ind. (Lonbon), 256 (1963).
11) *Idem* : This Bulletin, **11**, 977 (1963).
12) *Idem* : *Ibid.*, **10**, 1205 (1962).

the more or less transparent region of rhombohedral thiourea spectrum. From the absorption frequencies and/or dichroic behavior of these bands, the conformation, orientation as well as aggregational states of guest molecules in thiourea channels has been studied. But the transparent regions available for such study are limited and in some cases the states of guest molecules could not be elucidated satisfactorily. The difficulty has now been overcome by the complemental use of thiourea-d_4 as the host molecule; most of the strong bands of thiourea in the region between 1400 and 1000 cm^{-1} are displaced to lower frequency on deuteration and leave new transparent region.

Experimental

The instrument used for most of this work was a Hitachi Model EPI-2 IR spectrophotometer equipped with a NaCl prism and a microscope attachment, Model IM-2P. The dichroism was determined by the technique described in previous papers of this series.*[1,11,12] Since, in the present work, deuterated thiourea was used as the host molecule, the proton-deuteron exchange reaction with atmospheric water vapor was carefully retarded. Each spectroscopic measurement was repeated several times and the reproducibility was checked.

Thiourea-d_4 was prepared by three recrystallizations of reagent grade thiourea from 99.75% D_2O (Showa Denko K. K.) and then was dried and stored in a sealed tube. The best solvent for the crystallization of thiourea-d_4 adducts may be CH_3OD but in the present work a mixture of CH_3CN and D_2O was used for convenience. CH_3CN, with no active hydrogen, is miscible with D_2O freely and dissolves thiourea in a moderate amount. Moreover, CH_3CN does not form thiourea-adduct at all and its adsorption on adduct crystals is easily detected by the characteristic absorption bands at 2210 and 1040 cm^{-1}.

Guaranteed grade CH_3CN was distilled over P_2O_5 using Widmer fractionation column and 1/20 part of D_2O was added to the distillate. Into 5 ml. of this mixture, 0.7 g. of thiourea-d_4 was dissolved by heating and then about 0.8 g. of a guest compound was added. The amount of the guest compound added was different from case to case. After vigorous stirring, the mixture was cooled in an ice bath. Needle crystals separated were collected on a glass filter and Nujol or perfluorocarbon was applied immediately on them. By this method, thiourea-d_4 adducts were shielded from the atmosphere and neither decomposition nor protondeuteron exchange took place any further. Crystals were then spread on filter paper and excess Nujol or perfluorocarbon was removed. Single-crystals were oriented on a NaCl plate just as described before.[12]

Dioxane-thiourea adduct was too unstable to be prepared by this method. In this work, therefore, dioxane itself was used as solvent. It was dried over Na wire, distilled and D_2O was added (0.05 ml./ml.). In 5 ml. of the solvent 0.4 g. of thiourea-d_4 was dissolved and after addition of 0.5 ml. of *trans*-decalin, the solution was allowed to cool by means of a stream of H_2O. Fine needles grown from the bottom of the flask were treated in a similar manner as described before.

Results and Discussion

Since the introduction of isotopic atoms does not affect the electronic structure or the force fields, the replacement of four hydrogen atoms of thiourea by deuterium has little effect on its ability to form inclusion compounds. Thus the molecules which form adducts with thiourea can also be "guests" to thiourea-d_4 adducts.

On deuteration, however, all absorption bands associated with the NH group are shifted to the lower frequency.[13] The NH stretching bands at 3290 cm^{-1} are lowered by a factor of 1.37. The bending bands are shifted to 1185 and 1154 cm^{-1} and are decreased in intensity. The bands between 1200 and 1000 cm^{-1} (mainly due to the NH_2 rocking modes) and at 715 cm^{-1} (C–N stretching mode) are also displaced and these spectral regions become transparent as shown in Fig. 1.

The dichroism of bands of single crystal shown in Fig. 1 was the same as the bands of oriented thin crystal section of thiourea adduct grown under thermal gradient.[13] The assignments[13] of absorption bands of thiourea-d_4 were thus confirmed.

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[13] M. Nishikawa, H. Mima, T. Kanzawa: This Bulletin, **10**, 1199 (1962).

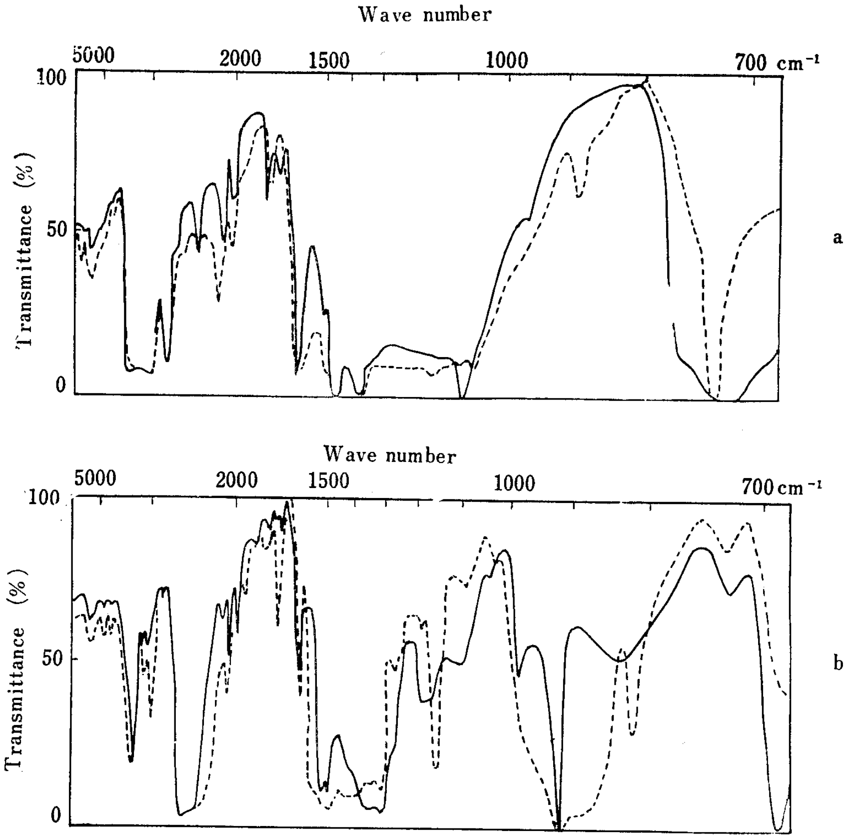

Fig. 1. Infrared Spectra of Orientated Rhombohedral Crystals of Thiourea (a) and Thiourea-d_4 (b)

—— and - - -, with the electric vector perpendicular and parallel, respectively, to the c-axis

From Fig. 1, it is expected that strong absorption bands of guest molecules in thiourea-d_4 channels would be observed in the regions between 2800 and 2600 cm^{-1}, 2200 and 1600 cm^{-1}, 1300 and 990 cm^{-1}, and 790 and 680 cm^{-1}. This means that by the complemental measurements of ordinary thiourea and thiourea-d_4 adducts, the whole spectra of guest molecules would be observed in the sodium chloride region except the regions between 3400 and 2800 cm^{-1} and between 1600 and 1300 cm^{-1}, where bands arising from CH_2 group of guest molecules would be located.

A typical example is shown in Fig. 2. *trans*-1,2-Dichlorocyclohexane molecule is in equilibrium between the diaxial and diequatorial conformations of the C-Cl bonds in the liquid state. In its thiourea adduct, however, the diaxial conformation exists almost exclusively owing to the intermolecular steric interaction between guest and host molecules.(11) Therefore, absorption bands due to the diaxial isomer would be intensified in the spectrum of the thiourea adduct. Accordingly, the bands at 1005, 864, and 827 cm^{-1} are assigned to this isomer. These assignments agree with those made by Klaeboe, *et al.*(14) The band at 909 cm^{-1} is considered to arise from the absorptions of both the diequatorial and diaxial isomers. In accord with this assignment, the bands of the diaxial isomer in the thiourea-d_4 adduct were assigned in the spectral regions between 1300 and 1050 cm^{-1} and between 730 and 680 cm^{-1}. The results are listed in Table I.

In a similar manner, the assignments of the bands due to the axial isomer of chlorocyclohexane and bromocyclohexane(11) were also made in the region between 1300 and 680 cm^{-1}. In both cases five or six bands attributable to the guest molecules were

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14) P. Klaeboe, J. J. Lothe, K. Lunde: Acta Chem. Scand., **11**, 1677 (1957).

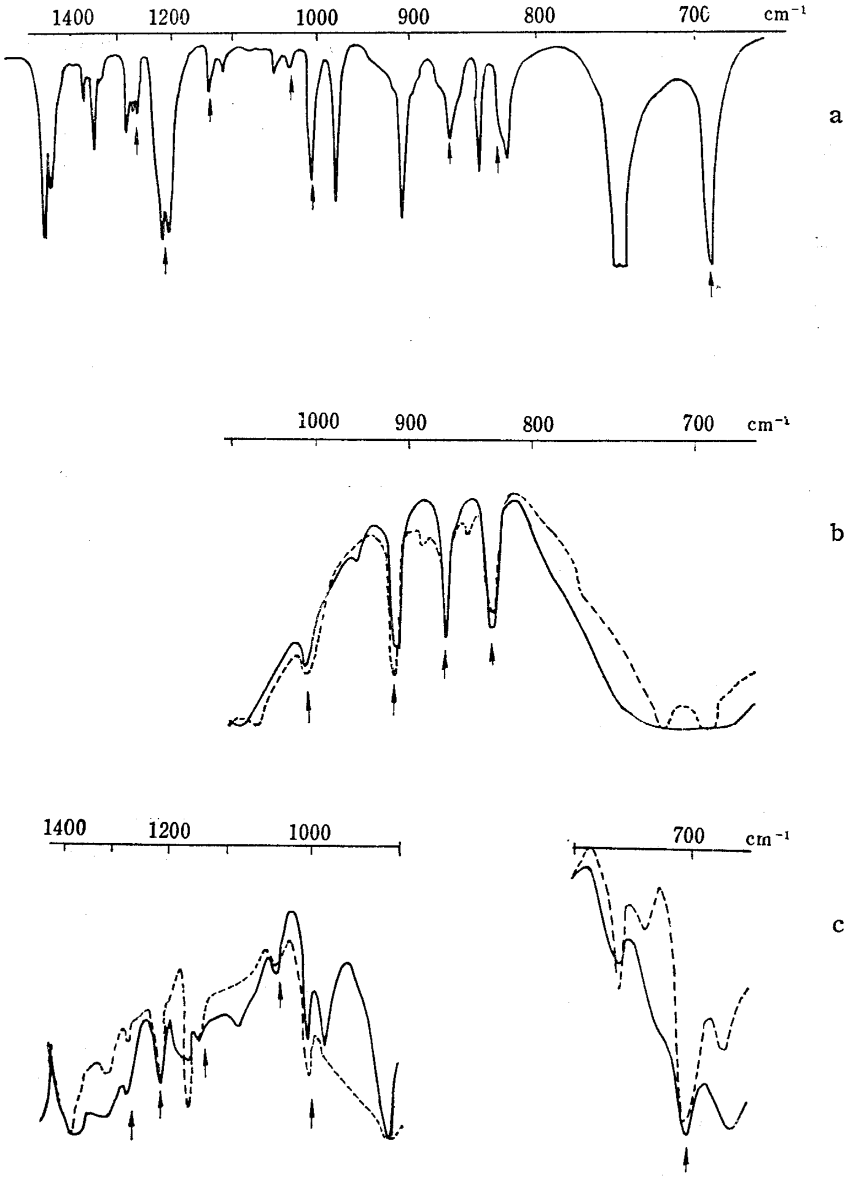

Fig. 2. Infrared Spectra of *trans*-1,2-Dichlorocyclohexane

a : liquid state; b : thiourea adduct; c : thiourea-d_4 adduct
—— and ---, with the electric vector perpendicular and parallel, respectively, to the c-axis

located as indicated by arrows in Fig. 3. In Table II, the intensity changes on thiourea-d_4 adduct formation are listed in comparison with the assignments made by Kozima and Sakashita[15] or by Klaeboe, *et al.*[16] It is noted that several bands are increased in intensity although they have been assigned to the equatorial isomer. These bands would actually be the hybrids of the bands of the axial and equatorial isomers just as for the 909 cm^{-1} band of *trans*-1,2-dichlorocyclohexane.

In a previous paper,[11] it was reported that in thiourea adducts all the bands assigned to the axial isomers of chloro-, bromo-, and iodo-cyclohexanes show little infrared dichroism, whereas those assigned to the equatorial isomers exhibit the dichroism parallel to the c-axis of the crystal. From this dichroic behavior, the axial isomers

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[15] K. Kozima, K. Sakashita : Bull. Chem. Soc. Japan, **31**, 796 (1958).
[16] P. Klaeboe, J. J. Lothe, K. Lunde : Acta Chem. Scand., **10**, 1465 (1956).

Table I. The Infrared Bands of *trans*-1,2-Dichlorocyclohexane and their Intensity Changes on the Formation of Thiourea of Thiourea-d_4 Adduct

Band cm^{-1}	Assignment[14]	Intensity change	Assignment (present work)
1276		decrease	diequatorial
1267		increase	diaxial
1255		decrease	diequatorial
1212	diaxial	increase	diaxial
1202	diequatorial	decrease	diequatorial
1138	diaxial	increase	diaxial
1121		decrease	diequatorial
1048		〃	〃
1031	diaxial	increase	diaxial
1005	〃	〃	〃
980	diequatorial	decrease	diequatorial
909		increase	
864	diaxial	〃	diaxial
844	diequatorial	decrease	diequatorial
827	diaxial	increase	diaxial
821	diequatorial	decrease	diequatorial
746	〃	〃	〃
738	〃	〃	〃
692	diaxial	increase	diaxial

were suggested to lie at random or rotate somewhat freely in thiourea channels but the equatorial isomers were suggested not to do so. The molecular model of the axial isomer is rather spherical as a whole but, for the equatorial isomer, the halogen atom is projected out of the cyclohexane ring so that the orientation of the equatorial isomer in thiourea channels will not be at random. Studies on their thiourea-d_4 adducts supported these presumptions. In fact, the C-Cl stretching band due to the equatorial

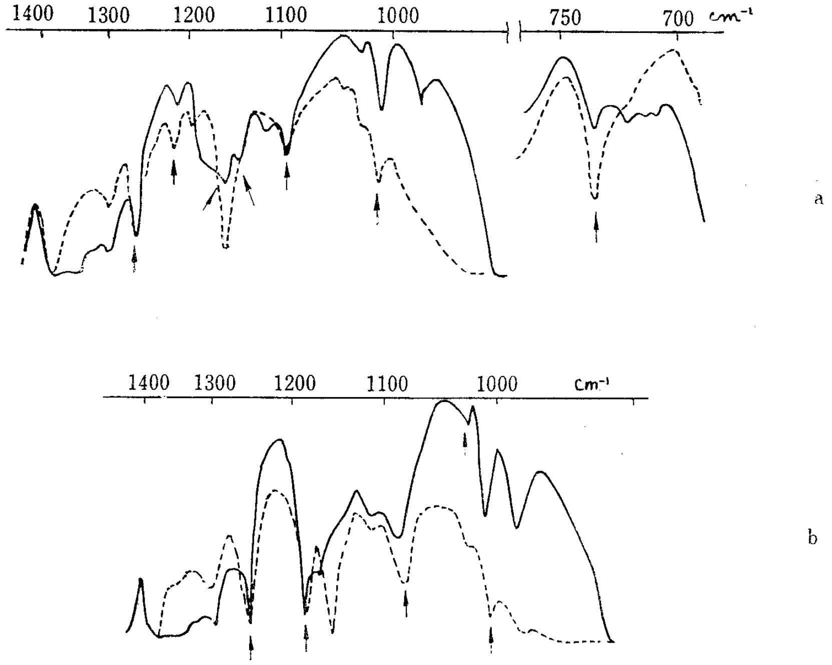

Fig. 3. Infrared Spectra of Halocyclohexane Thiourea-d_4 Adduct

a : chlorocyclohexane thiourea-d_4 adduct
b : bromocyclohexane thiourea-d_4 adduct
—— and - - -, with the electric vector perpendicular and parallel, respectively, to the c-axis

Table II. Infrared Bands of Halocyclohexanes and their Intensity Changes on the Formation of Thiourea-d_4 Adducts

Chlorocyclohexane			Bromocyclohexane		
Band cm^{-1}	Assignment[15)]	Intensity change	Band cm^{-1}	Assignment[16)]	Intensity change
993	equatorial	decrease	987	equatorial	decrease
1014	axial	increase	1009	axial	increase
1029	equatorial	〃	1026	equatorial	〃
1098	axial	〃	1086	equatorial ?	〃
1132	equatorial	decrease	1116	equatorial	decrease
1145	axial	increase	1172	axial	increase
1184	equatorial	decrease			
1215	〃	〃	1189	equatorial	decrease
1258	〃	increase	1252	〃	increase
1267	〃	decrease			

isomer of chlorocyclohexane in thiourea-d_4 channels showed strong dichroism parallel to the c-axis (Fig. 3), indicating that the equatorial C-Cl bond of this isomer was oriented parallel to the channel axis.

The orientations of cyclohexane and dioxane molecules in thiourea channels were also studied by the analyses of the infrared spectra of the thiourea-d_4 adducts. In the spectra of dioxanethiourea adduct, two bands at 889 (CH_2 rocking) and 874 cm^{-1} (ring

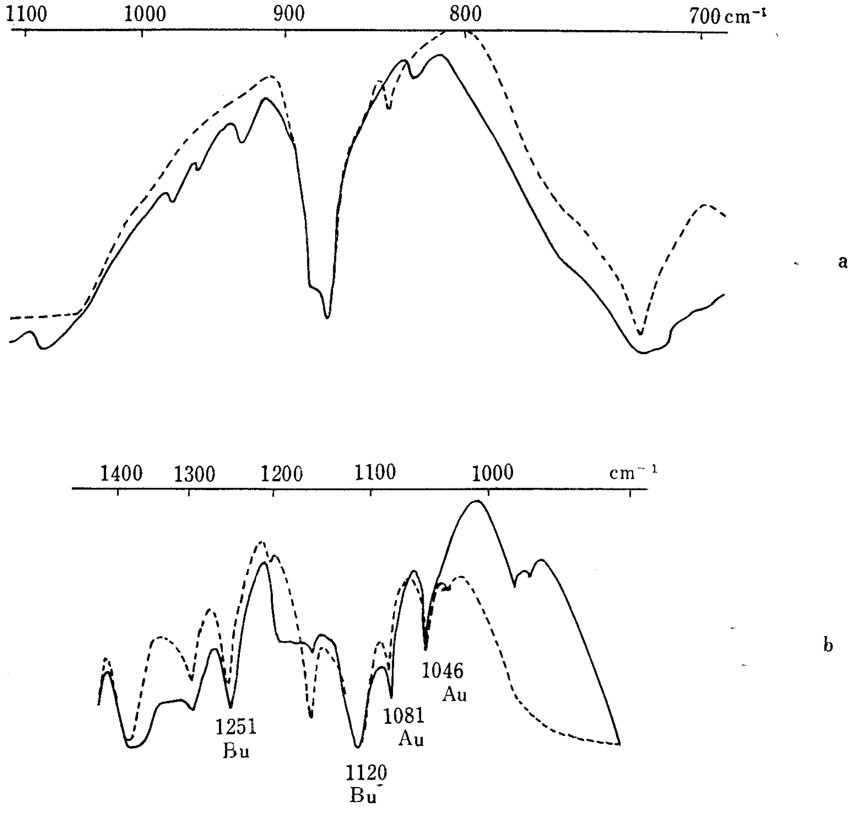

Fig. 4. Infrared Spectra of Dioxane Thiourea-d_4 Adduct

a : dioxane thiourea adduct
b : dioxane thiourea-d_4 adduct
—— and - - -, with the electric vector perpendicular and parallel, respectively, to the c-axis

stretching)[17] were attributed to the guest molecule. Both bands exhibited little dichroism as shown in Fig. 4. It was presumed, therefore, that dioxane was trapped rather loosely or at random. But the presumption based on the dichroism of only two bands might be in error. Moreover, these bands are too strong for precise dichroic measurements. By the use of thiourea-d_4 as a host molecule, however, additional four bands were observed; a strong band at 1120 cm^{-1} and three medium-intensity bands at 1251, 1081, and 1046 cm^{-1}. The dichroism of these four bands was again found to be little. Accordingly the above presumption was ascertained.

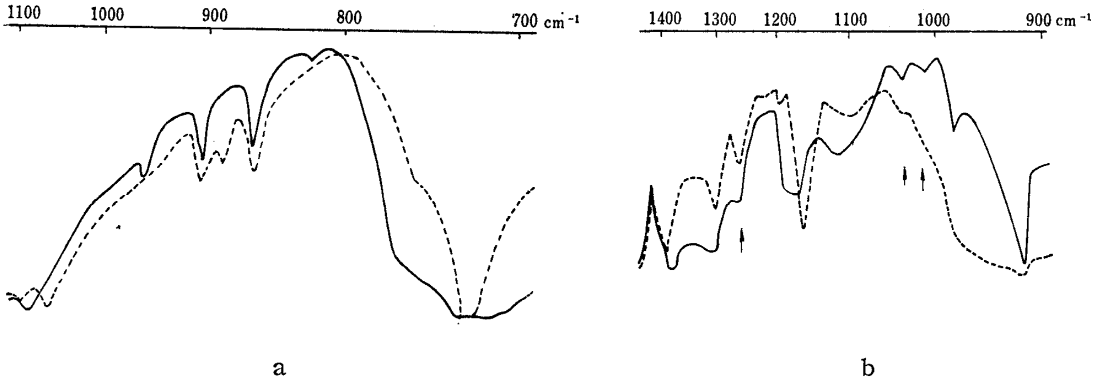

Fig. 5. Infrared Spectra of Cyclohexane Thiourea and Thiourea-d_4 Adduct

a : cyclohexane thiourea adduct
b : cyclohexane thiourea-d_4 adduct
—— and - - -, with the electric vector perpendicular and parallel, respectively, to the c-axis

In the spectra of cyclohexane-thiourea adduct, the bands of cyclohexane were located at 901 and 862 cm^{-1}, either of which exhibited no dichroism. Three new bands in the thiourea-d_4 adduct are indicated by arrows in Fig. 5 (b). Unfortunately, their dichroism was not clear because of weak intensity. Nevertheless, the cyclohexane molecules in thiourea channels appear to lie at random or freely.

In contrast to the above results, it may be recalled that absorption bands of cyclopentanone[10] and cyclohexanone[12] have shown strong dichroism when the molecules were trapped in thiourea channels. Evidently the size and shape of guest molecules are not the only factor which determine the orientation or mobility of the molecules; the variations in the molecular size and shape of cyclopentanone, cyclohexanone, dioxane, and cyclohexane are not significant as compared with space provided in the thiourea channels. The strong electric dipole of the carbonyl groups in cyclohexanone and cyclopentanone appears to be an important factor for determining the orientation of these molecules in thiourea channels. Similar phenomena were also found by Nakajima,[2] who showed that the benzene molecule, trapped in dicyanamminenickel (II) clathrates could rotate about its sixfold axis at room temperature, whereas pyridine, pyrrole or thiophene molecules did not move in the cavity up to the melting point of the clathrates. He attributed this restriction of rotational motion to the electric dipole interaction between trapped molecules. Such electric dipole interaction, whether it operates among guest molecules or between guest and host molecules, may well be stabilizing the inclusion compounds in addition to van der Waals' forces. In fact, tetrahydrofuran forms stabler adducts with thiourea than dioxane does, although the size of the former molecule is undoubtedly smaller than that of the latter.

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17) F.E. Malherbe, H.J. Bernstein: J. Am. Chem. Soc., **74**, 4408 (1952).

The authors express their deep thanks to Dr. H. Mima for his kind encouragement in these studies, and to Takeda Chemical Industries, Ltd. for permission to publish this report.

Summary

Infrared spectroscopic methods were applied for investigations on the states of molecules trapped in thiourea-d_4 channels. It was found that, in the spectra of thiourea-d_4 adducts, absorption bands due to guest molecules could be observed in the regions between 2800 and 2600 cm^{-1}, 2200 and 1600 cm^{-1}, 1300 and 990 cm^{-1} and 790 and 680 cm^{-1}. Thus, nearly all absorption bands of guest molecules were observed in sodium chloride region by the use of both thiourea and thiourea-d_4 adducts, except the regions where CH_2 stretching and bending vibrations absorb. As an example, bands due to the axial isomers of halogenated cyclohexanes were determined by this technique. Relatively free rotation or random orientation of dioxane or cyclohexane molecules in thiourea channels was also supported by the present work. From the comparison with the orientation of cyclopentanone or cyclohexanone molecule in thiourea channels, it is suggested that electric dipole forces would operate to orientate guest molecules in channels.

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UDC 581.19 : 582.34 : 543.544

42. **Satoru Kuwada and Masatake Hori** : Supplement of the Notice on the Formation of 6-Methyl-7-hydroxyribolumazine. (Application of Chromatography. XLVI.*[1]).

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In order to investigate unexamined ingredients of the mycelium of *Eremothecium ashbyii* by thin layer chromatography (TLC), the fresh mycelium of the microorganism was extracted with water at 80° for 15 minutes. The extract was concentrated at 45~50° *in vacuo* and an aqueous solution of the residue was applied on a thin layer of alumina or silica gel G and developed with such solvents as dimethylformamide or butanol series. As a result it was confirmed that separation was most effective in a combination of silica gel G and butanol-ethanol-water (50:15:35). Investigation of the chromatogram, however, revealed that while the spots of riboflavin (V. B_2) and 6-methyl-7-hydroxyribolumazine (V Comp.) were separated clearly, 6,7-dimethylribolumazine (G Comp.) was considerably tailed and the green fluorescence was very faint. Just when the authors were about to investigate the cause, Uehara[1] reported at the Vitamin Committee that the carbon atom (=CH-) at the position 2 and 8 of purine compounds such as hypoxanthine and adenine is converted to $\overset{\text{OH}}{\overset{|}{=\text{C}-}}$ by photochemical oxidation in the presence of V. B_2, namely, uric acid is produced from hypoxanthine. It seemed that V Comp. was not originally present in the mycelium but produced from G Comp. in the

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*[1] Part XLV : This Bulletin, **11**, 23 (1963).

*[2] Juso-nishino-cho, Higashiyodogawa-ku, Osaka (桑田 智, 堀 正剛).

[1] K. Uehara, Y. Okada : Vitamins (Kyoto), **27**, 321 (1963).