[Chem. Pharm. Bull. 11 (8) 990 ~ 999]

UDC 615.779.931-011

170. Shigeharu Inouye: Molecular Complexes of Tetracycline Acid Salts. V.[1] Infrared Spectra of Tetracycline Sulfate-Complexes.

(*Central Research Laboratories of Meiji Seika Kaisha Ltd.*[*1])

On the basis of an X-ray investigation about tetracycline salt-complexes,[1] a clathrate-like structure was proposed for a series of isomorphous salt-complexes designated as the *dα* form, which has monoclinic symmetry, space group $P2_1$, with $a=15.8$, $b=10.5$, $c=17.4$ Å and $\beta=109°$. The crystal structure consists of two distinct groups per unit cell, each group containing two tetracycline molecules, one sulfate anion or fluosilicate anion, one complexing agent and about ten water molecules. Upon partial dehydration, some of the sulfate-complexes of the *dα* form change into the other isomorphous series of sulfate-complexes named the *hγ* form, which is also monoclinic, space group $P2_1$ or $P2$, $a=16.0$, $b=10.5$, $c=14.7$ Å and $\beta=102°$. An asymmetric unit is made up of the same composition as that of the *dα* form, except it has about six water molecules instead of ten. Besides them, two other crystal forms, the *dβ* (decahydrates) and *hδ* (hexahydrates) forms, were described as the polymorphic modifications of the *dα* (decahydrates) and *hγ* (hexahydrates) forms, respectively.

In addition to the X-ray investigation, an infrared investigation on the salt-complexes was undertaken to provide additional structural information, particularly in relation to the binding states of crystal water, complexing agent, and salt anion in the crystals. Though the vibrational spectrum in a crystalline state is usually complicated owing to various crystal field effects, a comparison among the spectra of a series of isomorphous crystals may be made much simpler, because certain field effects such as correlation field effect[2] can be expected to be nearly equal to each other. Most of this work, therefore, is devoted to the examination of the spectra of the two isomorphous series of sulfate-complexes, that is, the *dα* crystals and their partial dehydrates, the *hγ* crystals.

Experimental

Preparation——The samples of tetracycline salt-complexes used in this work were prepared by the method reported in the preceding papers,[1,3] and their crystal types were confirmed by X-ray diffraction powder patterns. Deuterated analogues were obtained by the usual exchange reaction, *i.e.*, by recrystallization from D_2O. Tetracycline hydrochloride and tetracycline nitrate were crystallized from EtOH and H_2O respectively, and both without solvent of crystallization.

Measurement——IR absorption spectra were recorded in a range from 4000 to 600 cm^{-1} using a Koken 401 grating spectrometer. Wave numbers were calibrated employing polystyrene film as a standard. Since the sulfate-complexes of the *dα* type are extraordinarily unstable against exposure to dry air, the crystals were coated with nujol or hexachlorobutadiene immediately after drying between two filter papers and were measured without delay by employing the mull technique.

Considering unstableness of the *dα* crystals especially by both vacuum pumping and mechanical stress such as grinding, the KBr disk technique seems to be unsuitable for the measurement of the spectra. The spectra of the *dα* crystals taken with the disk technique, indeed, showed marked discrepancies when compared either with the spectra of the *dα* crystals or with the spectra of the *hγ* crystals taken with the mull techniques. Similarities were noted when compared with the spectra of amorphous sulfate-complexes which were prepared by dissolving crystalline sulfate-complexes in H_2O followed by immediate lyophilization.

[*1] Morooka, Yokohama (井上重治).

1) Part IV. S. Inouye, Y. Iitaka: Bull. Chem. Soc. Japan, in press (1963).

2) W. Vedder, D. F. Hornig: "Advances in Spectroscopy," 2, 189 (1961). Interscience Publishers, New York-London.

3) S. Inouye: This Bulletin, 9, 417 (1961).

Results and Discussion

Figs. 1 and 2 show the infrared absorption spectra of the two different kinds of the *dα* type salt-complexes, namely, tetracycline sulfate-dicyanodiamide complex and -oxalic acid complex, and tetracycline fluosilicate-dicyanodiamide complex and -oxalic acid complex, respectively. Practically no difference was found in the spectra of each set of the salt-complexes, with the exception of the bands caused by the complexing agents. The spectra of other *dα* type sulfate-complexes and fluosilicate-complexes examined were also found to be almost identical with those shown in Figs. 1 and 2, respectively. Furthermore, the *dα* type sulfate-complexes differ only little in spectra from the corresponding *dα* type fluosilicate-complexes. A major difference lies in intensification of the band near 1100 cm^{-1} of the former, and of the band near 745 cm^{-1} of the latter. These differences appear to be ascribed only to those of the respective salt anions.*[2] On the other hand, the spectra of the *dα* crystals, when compared with

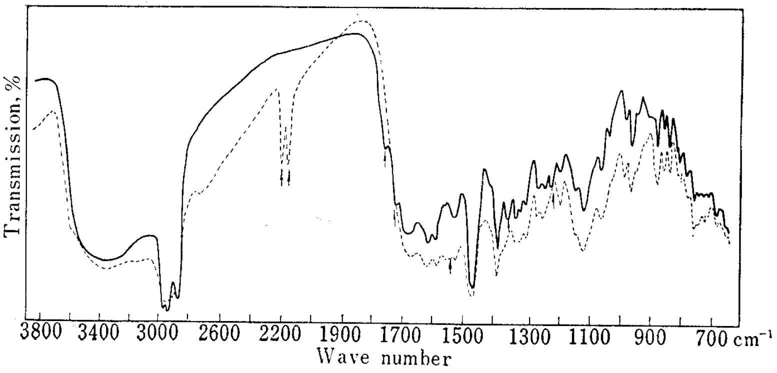

Fig. 1. Infrared Absorption Spectra of *dα* Type Sulfate-Complexes of Tetracycline in Nujol

—— : sulfate-oxalic acid complex
------ : sulfate-dicyanodiamide complex
An arrow indicates the band due to complexing agents.

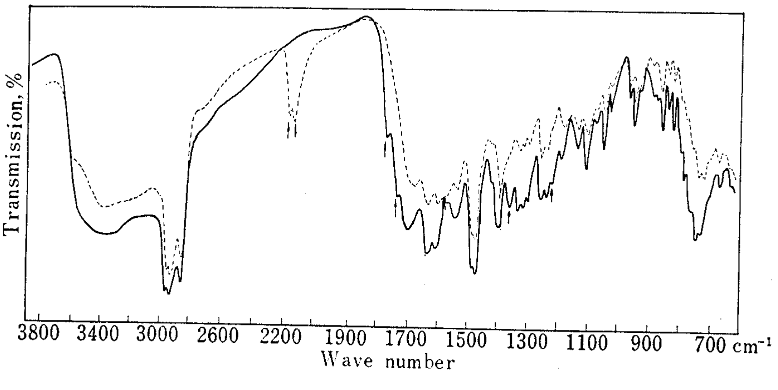

Fig. 2. Infrared Absorption Spectra of *dα* Type Fluosilicate-Complexes of Tetracycline in Nujol

—— : fluosilicate-oxalic acid complex
------ : fluosilicate-dicyanodiamide complex
An arrow indicates the band due to complexing agents.

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*[2] An strong band near 1100 cm^{-1} in the sulfate-complexes can be attributable to the ν_3 mode (F_2) of the tetrahedral sulfate anion. The assignment will be discussed later in detail. An strong band near 745 cm^{-1} which was observed in all the fluosilicate-complexes, can be assigned to the ν_3 mode ($F_{1\mu}$) of the octahedral fluosilicate anion. Potassium fluosilicate and calcium fluosilicate dihydrate both show a similar absorption at 745 cm^{-1} with high intensity.

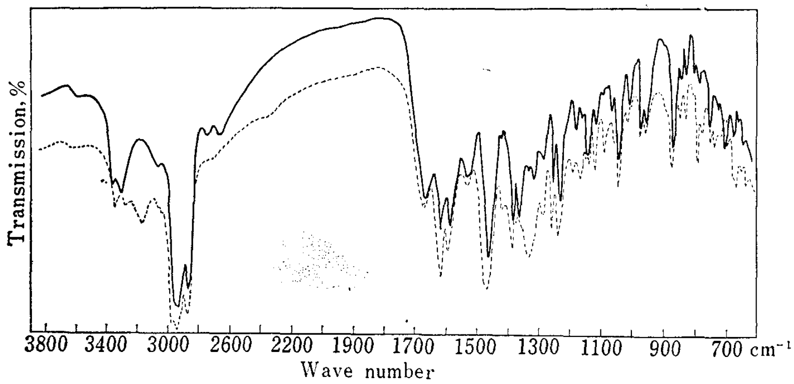

Fig. 3. Infrared Absorption Spectra of Tetracycline Hydrochloride and Tetracycline Nitrate in Nujol

——: hydrochloride ------: nitrate

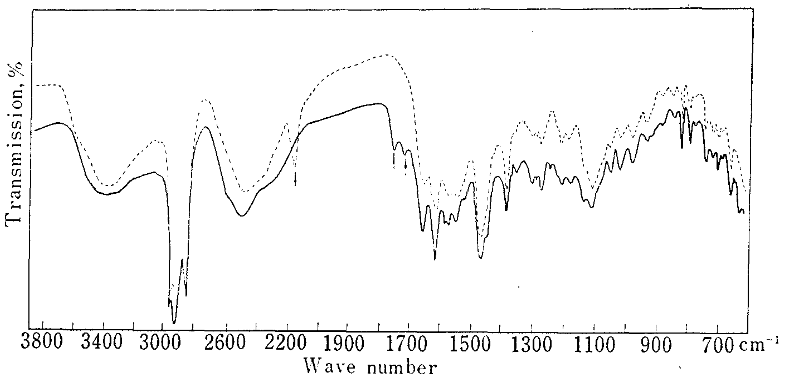

Fig. 4. Infrared Absorption Spectra of Deuterated *dα* Type Sulfate-Complexes of Tetracycline in Nujol

——: deuterated sulfate-oxalic acid complex
------: deuterated sulfate-dicyanodiamide complex
An arrow indicates the band due to complexing agents.

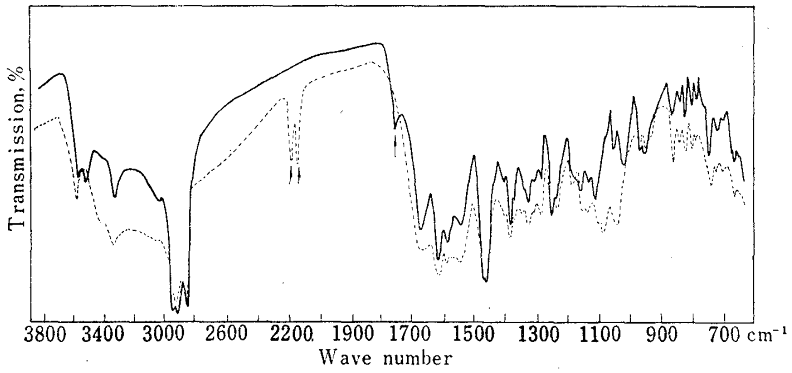

Fig. 5. Infrared Absorption Spectra of *hγ* Type Sulfate-Complexes of Tetracycline in Nujol

——: sulfate-oxalic. acid complex
------: sulfate-dicyanodiamide complex
An arrow indicates the band due to complexing agents.

those of tetracycline hydrochloride and tetracycline nitrate illustrated in Fig. 3, exhibit many differences in the whole region.

The resemblance of the spectra among the *dα* crystals was further observed in the case of the deuterated analogues, as shown, for example, in Fig. 4, though the spectra themselves changed markedly upon deuteration. Such spectral similarities observed among the *dα* crystals undoubtedly correspond to the similarities in their crystal structure, in agreement with X-ray evidence.1)

It was observed, however, that when the *dα* type sulfate-complexes changed into the *hγ* type crystals, remarkable alterations in spectra were accompanied with the partial dehydration as shown in Fig. 5. These spectral changes were noted primarily in the three regions, from which some structural information about the states of crystal water, complexing agent, and sulfate anion was derived as described below.

Crystal Water——In Table I the bands arising from the crystal water of the nine different sulfate-complexes are listed. The spectra of the *dα* crystals are characterized by a very strong broad absorption between 3600 and 3100 cm^{-1}, centred near 3350 cm^{-1}, which on deuteration is displaced to 2500 cm^{-1} with the similar broadness (Fig. 4). The broad band, however, disappears on partial dehydration, and two sharp bands appear at 3590 and 3330 cm^{-1} (Fig. 5). In some of the spectra, additional bands around 3540 and 3440 cm^{-1} are also found. The 3350 cm^{-1} band in the original *dα* crystals is undoubtedly associated with the O-H stretching mode with moderately strong hydrogen bond. Furthermore, its unusual broadness seems to imply the liquid-like character of the crystal water, rather than the unresolved superposition of many bands occurring from rigid periodic arrangement, as the similar broadness is observed with freely moving water.[*3] Indeed, the ready escape of crystal water from the *dα* crystals was shown by the observation that water evolution in the *dα* crystals occured at far lower temperature (50∼70°) than in the *hγ* crystals (125∼150°), which was measured by a thermal analysis.[*4] The sharp band at 3590 cm^{-1} in the *hγ* crystals disappears by further drying *in vacuo* at 80°, suggesting the presence of little hydrogen-bonded crystal water.

Table I. Infrared Frequencies (cm^{-1}) due largely to Crystal Water (3600∼3100 cm^{-1})

Tetracycline sulfate-complex	*dα* Crystal	*hγ* Crystal			
Oxalic acid complex	3350	3590	3545		3340
Formic acid complex	3340	3590	3520	3440	3330
Glycolic acid complex	3380	3590	(3540)		3340
Malonic acid complex	3340	3590	3510		3340
Fumaric acid complex	3360	3590	(3535)	3445	3335
Dicyanodiamide complex	3350	3590		(3430)	3330
Hydantoin complex	3350	3590			3330
Water complex	3340	3590			3340
Pyrazine complex	3340	3585			3330

( ) : a sideband occurring as a shoulder

Complexing Agent——The $C\equiv N$ and $C=O$ stretching frequencies of the five complexing agents in the sulfate-complexes are listed in Table II. Unfortunately, many of the other bands due to the complexing agents cannot be recognized owing to masking by strong tetracycline bands. It should be noted from Table II that, in contrast to the bands due to hydantoin and dicyanodiamide in which the band positions are nearly the same in both crystal types, the carbonyl frequencies of the three carboxylic acids

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[*3] As judged from the unusual broadness of O-H band around 3400 cm^{-1}, Sugiura suggested random distribution of crystal water in cordierite. (K. Sugiura : Bull. Tokyo Inst. Technol., Ser. B, 1 (1959)).

[*4] Detailed experimental data on the thermal analysis of the salt-complexes will be published elsewhere.

Table II. Infrared Frequencies (cm^{-1}) due to Complexing Agent (2600～1700 cm^{-1})

Tetracycline sulfate-complex	*dα* Crystal		*hγ* Crystal	
Oxalic acid complex	1750	1710	1754	
Glycolic acid complex	1742	(1720)		(1720)
Malonic acid complex	1727		1780$^{a)}$	(1735)
Dicyanodiamide complex	2190	2150	2190	2150
Hydantoin complex	1754	1712	1750	1710

( ): a sideband occurring as a shoulder
a) a weak band

vary with the dehydration. The spectrum of the *dα* type sulfate-oxalic acid complex shows two sharp bands at 1750 and 1710 cm^{-1}, which may be attributable to the carbonyl vibrations of two undissociated carboxylic acid groups. Upon partial dehydration, however, the lower band is missed, while the higher band remains nearly unaltered. The similar spectral changes that imply the band shifts towards lower wave numbers were observed also in the cases of sulfate-glycolic acid and -malonic acid complexes. Though the observed changes of the carbonyl frequencies reflect changes of hydrogen bonding involving carboxylic acid groups, spectral data alone are insufficient to warrent any conclusions on the binding states of the carboxylic acids in the *dα* crystals. But in the case of *hγ* crystals, it is suggested in combination with the X-ray evidence that a complexing agent would take preferably a monomeric form, because the occurrence of interaction between sulfate anion and various complexing agents in an almost constant unit cell, as discussed later, is impossible if a complexing agent is dimeric or polymeric.

Table III. Infrared Frequencies (cm^{-1}) due largely to Sulfate Anion (1170～950 cm^{-1})

Tetracycline sulfate-complex	*dα* Crystal ν_3		*hγ* Crystal ν_3					ν_1
Oxalic acid complex		1100	1156	1110	1014			946
Formic acid complex		1100	1160	1100	1020			948
Glycolic acid complex		1095	1152	1100	1020			962
Malonic acid complex		1106	1140	1104	1086	1020		964
Fumaric acid complex		1098	1134	1097	1080	1020		968
Dicyanodiamide complex		1100	1150	(1137)	(1100)	1080	1035	
Hydantoin complex	1100	(1085)	1140	1082	1040			
Water complex		1105	1140	1106	1084	1040		
Pyrazine complex		1106		1104	(1086)			

( ): a sideband occurring as a shoulder

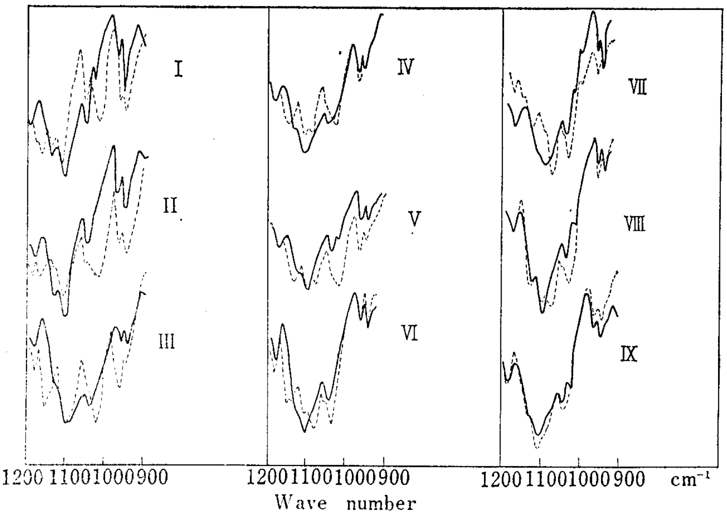

Fig. 6. Infrared Absorption Spectra due largely to Sulfate Anion in *dα* Crystals (——) and *hγ* Crystals (------)

I : sulfate-oxalic acid complex
II : sulfate-formic acid complex
III : sulfate-glycolic acid complex
IV : sulfate-malonic acid complex
V : sulfate-fumaric acid complex
VI : sulfate-dicyanodiamide complex
VII : sulfate-hydantoin complex
VIII : sulfate-water complex
IX : sulfate-pyrazine complex

Sulfate Anion——Table III and Fig. 6 indicate the spectra of the nine different kinds of sulfate-complexes in the region 1200~900 cm^{-1}. A strong broad band near 1100 cm^{-1}, which is characteristic of all the *dα* crystals, can be assigned as the triply degenerated stretching vibration (υ_3) of sulfate anion from the following considerations: a) The origin of this band should be ascribed to the sulfate vibration, since the band of this intensity cannot be observed in the spectra of the isomorphous fluosilicate-complexes ⌊*a*, and also in the spectra of hydrochloride-complexes ⌊*b* and nitrate-complexes ⌊*c*. b) Though the latter three different kinds of salt-complexes (⌊*a*, ⌊*b*, ⌊*c* in a)) are not all the same in crystal structure,[1] their spectra are very similar in the region 1300~800 cm^{-1} as shown, for example, in Fig. 7 (curves IV, V, VIII, IX, X, XI, XII, XV, XVI, XVII, XVIII). Since these bands are little affected by the change of the components as well as the change of a degree of hydration, it is evident that most of the bands in this region come from the vibration of the tetracycline molecules in the salt-complexes. Moreover, they seem to be insensitive to the crystal structure modification among the salt-complexes, but definitely differ from the bands of the simple salts or hydrated free base of tetracycline as seen in Fig. 7 (curves I, II, III). In this connection, it is further noted from Fig. 7 that, in spite of the apparent divergence in spectra between sulfate-complexes and the other salt-complexes, the peak positions in the former *dα* crystals are in good accord with those in the latter, and the spectra of the former *hγ* crystals are also indistinguishable with those of the latter except in the region 1150~950 cm^{-1}. It is reasonable, therefore, to assume that the absorption bands of the tetracycline molecules, which are common to the other salt-complexes, also exist unchanged in the spectra of the both types of sulfate-complexes. Then we can obtain a sulfate band more clearly by subtraction of the spectra of either fluosilicate-complexes or hydrochloride-complexes or nitrate-complexes from the spectra of the corresponding sulfate-complexes. The resulting difference spectra for the *dα* crystals are shown graphically by curves VI−IV, XIII−XI, and XIX−XVII in Fig. 7, where the absorption due to tetracycline and complexing agents have been eliminated. Each of them shows an almost symmetrical peak at about 1100 cm^{-1}. Considering the spectral similarity in the *dα* crystals shown in Fig. 6, there seems little doubt that the other six *dα* type sulfate-complexes will show the similar curves as a sulfate band. Since the υ_3 mode of the freely rotating sulfate anion similarly absorbs at 1100 cm^{-1}, the above sulfate band can be reasonably assigned to υ_3 (F_2).[*5] c) Similar trend is seen in the deuterated sulfate-complexes of the *dα* form. Curve XXIII−XXI in Fig. 7 represents the band due to sulfate anion as obtained by subtracting the spectrum of the deuterated fluosilicate-complex (XXI) from that of the deuterated sulfate-complex (XXIII). Again an almost symmetrical band was obtained as the sulfate υ_3 band. From this it is obvious that, though deuteration produces marked change in the spectra due to tetracycline and complexing agents, the band due to sulfate anion remains largely unaltered after deuteration. These results indicate that the distorting crystal field effect of lower site symmetry (C_1) on the tetrahedral sulfate anion (Td) is insignificant in the *dα* crystals.

In the corresponding spectra of the *hγ* crystals, however, several resolved sharp bands, in place of one broad band, were found by the difference spectra. Four examples are shown by curves VII−V, XIV−XII, XX−XVIII, and XXIV−XXII in Fig. 7. Differing from the *dα* crystals in which the sulfate band is little affected by deuteration, there is a slight difference in the sulfate curve between *hγ* type sulfate-hydantoin complex

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[*5] Of the fundamentals of the sulfate anion having Td symmetry, only ν_3 and ν_4 are infrared active, which absorb in solution at 1104 and 613 cm^{-1}, respectively. While the former is strong enough to permit the identification of sulfate, the latter is medium, but difficult to recognize particularly when other strong absorptions overlap.

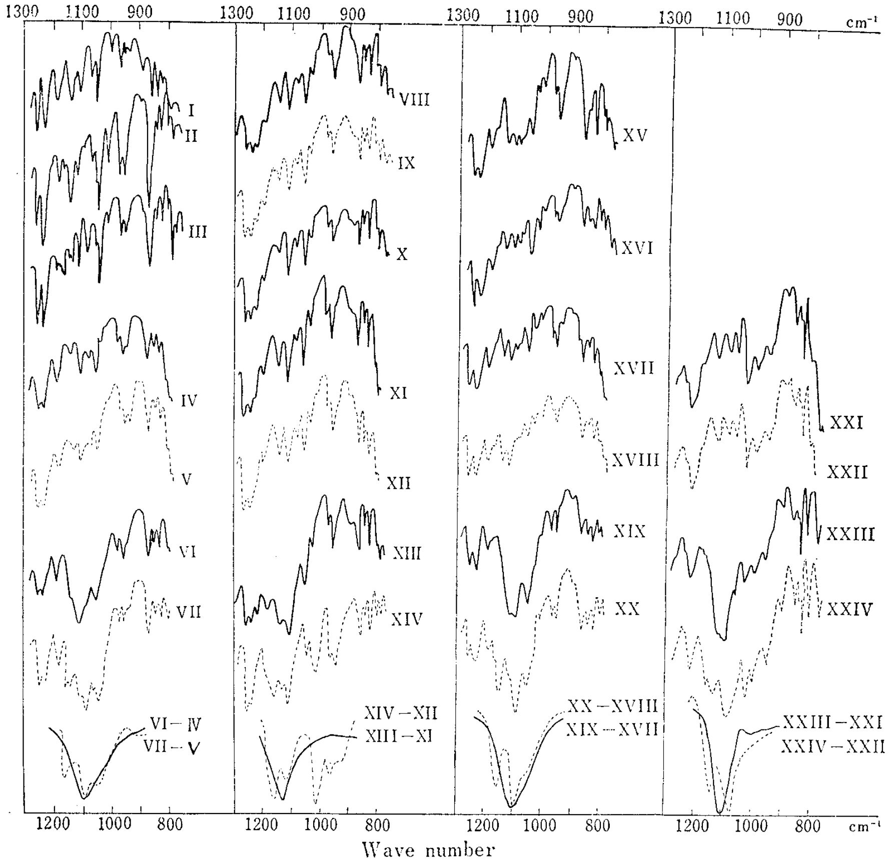

Fig. 7. Infrared Absorption Spectra of Various Salts and Salt-Complexes of Tetracycline in Nujol

I : tetracycline base hexahydrate
II : hydrochloride
III : nitrate
IV : fluosilicate-dicyanodiamide complex decahydrate
V : fluosilicate-dicyanodiamide complex hexahydrate
VI : sulfate-dicyanodiamide complex decahydrate
VII : sulfate-dicyanodiamide complex hexahydrate
VIII : hydrochloride-oxalic acid complex decahydrate
IX : hydrochloride-oxalic acid complex hexahydrate
X : nitrate-oxalic acid complex hexahydrate
XI : fluosilicate-oxalic acid complex decahydrate
XII : fluosilicate-oxalic acid complex hexahydrate
XIII : sulfate-oxalic acid complex decahydrate
XIV : sulfate-oxalic acid complex hexahydrate
XV : hydrochloride-hydantoin complex hexahydrate
XVI : nitrate-hydantoin complex hexahydrate
XVII : fluosilicate-hydantoin complex decahydrate
XVIII : fluosilicate-hydantoin complex hexahydrate
XIX : sulfate-hydantoin complex decahydrate
XX : sulfate-hydantoin complex hexahydrate
XXI : deuterated fluosilicate-hydantoin complex decahydrate
XXII : deuterated fluosilicate-hydantoin complex hexahydrate
XXIII : deuterated sulfate-hydantoin complex decahydrate
XXIV : deuterated sulfate-hydantoin complex hexahydrate

An arrow indicates the band due to complexing agents.

(XX—XVIII) and its deuteration counterpart (XXIV—XXII). It is noted, moreover, from Table III and Figs. 6 and 7 that the band splitting among the isomorphous $h\gamma$ crystals are no longer equal in magnitude, but larger for the sulfate-oxalic acid complex and -formic acid complex and smaller for the sulfate-pyrazine complex.

In view of the large intensities of these splitted bands, it dose not seem likely that some of them are overtone or combination bands associated with lattice vibration. Hence, they are reasonably ascribed to the splitting of the sulfate band mentioned above. The splitting arising from intermolecular coupling in the crystalline state is usually small. Therefore, we can expect for such large splitting as a result of the mutual coupling only when two sulfate anions in the unit cell approach each other enough to permit an extraordinarily strong interaction.[4] But if so, this cannot account for the different magnitude in splitting observed in the isomorphous members, because the distance between two sulfate anions in the $h\gamma$ type unit cell is assumed to be nearly constant, even though various complexing agents of different molecular size are enclosed.[1] Thus, the fact that the splitting is entirely specific to the complexing agent favours the interpretation that the splitting of the degenerate ν_3 mode, together with the probable appearance of the forbidden ν_1 mode,[*6] is caused by the lowering of Td symmetry by the perturbation of the complexing agents.

The difference in the splitting magnitude of the sulfate band among the $h\gamma$ crystals can be explained as follows. It was reported in the case of sulfato-metallic complexes[5] that the stronger the metal-sulfate bond is, the larger the splitting of the degenerate mode of sulfate. If the approach of a complexing agent to a sulfate anion is permitted in the dehydration process under consideration, the similar explanation may be applicable : the more acidic the complexing agent is, the stronger the hydrogen bonding to sulfate anion, and in consequence the larger the splitting of the degenerate mode. In agreement with this expectation there is a rough correlation between the acidity of the complexing agent and the magnitude of splitting. The splitting increases in magnitude in the following order. With the increasing intensities of the Raman active vibration (ν_1) : sulfate-pyrazine, -water, -hydantoin, -dicyanodiamide, -fumaric acid, -malonic acid, -glycolic acid, -formic acid, and -oxalic acid complexes. Sulfate-oxalic acid complex, for which the complexing agent might reasonably be expected to form the strongest hydrogen bond, exhibits the largest splitting, whereas splitting is no longer apparent in the spectrum of sulfate-pyrazine complex, in consistent with the fact that pyrazine has no active hydrogen atom for hydrogen bonding. In this connection, it is interesting to note that the shifts of the carbonyl bands of the carboxylic acids towards lower frequencies, which were observed with partial dehydration, suggest the stronger hydrogen bonding or dissociation of the carboxylic acid groups.

As a result of the removal of the degeneracy of ν_3, the splitting magnitude in the case of sulfate-water complex, of which the widest separation is 100 cm^{-1}, is comparable to that observed in copper sulfate monohydrate (80 cm^{-1}),[6] while the larger separation in the case of sulfate-oxalic acid complex (140 cm^{-1}) is comparable to those of the sulfato-cobalt coordination compounds (115 cm^{-1}).[5] Since the hydrogensulfate of tetracycline,

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[*6] When Td symmetry is destroyed by perturbation, totally symmetric vibration, ν_1, which occurs at 981 cm^{-1} in the Raman line, becomes infrared active. Though ν_1, as compared with ν_3, is weaker in general in the infrared spectra, there remains somewhat ambiguity as to the assignment of the splitted bands of the region 1050～1000 cm^{-1} either to ν_3 or to ν_1 by infrared evidence alone. A definite assignment may be possible with the aid of depolarization measurement in the Raman lines.

4) M. Tsuboi : "Sekigaisen Kyushu Supekutoru" (Infrared Absorption Spectra) **6**, 55 (1958), Nankodo, Tokyo.

5) K. Nakamoto, J. Fujita, S. Tanaka, M. Kobayashi : J. Amer. Chem. Soc., **79**, 4904 (1957).

6) I. Gamo : Bull. Chem. Soc. Japan, **34**, 764 (1961).

which corresponds to the extreme case of hydrogen bonding to sulfate, shows the more marked separation (180 cm^{-1}),³⁾ it seems to be possible that the strong hydrogen bonding between a sulfate and a complexing agent brings about the large splitting of ν_3.

As discussed previously,¹⁾ the escape of about eight molecules of water from the $d\alpha$ type unit cell causes a decrease in the c axis length (2.7 Å) and the β angle (7°), with negligible alterations in the a and b axes parameters. Since such splitting of the sulfate band could not be recognized in the $d\alpha$ crystals, the splitting seen in the $h\gamma$ crystals seems to indicate that the rearrangement of the crystal lattice from the $d\alpha$ form to the $h\gamma$ might occur in such a manner as to result the approach of a complexing agent towards a sulfate anion roughly along the c axis.

Polymorphism——Finally, a brief mention must be made of spectral changes caused by polymorphism. In the case of the $d\alpha$ and $d\alpha$ crystals, where X-ray evidence showed a similar molecular packing scheme between the two forms, the differences in spectra are extremely small, the only distinguishing absorption being at 1140 cm^{-1} in the former and at 1020 cm^{-1} in the latter. Somewhat larger divergences, however, were observed between the spectra of the dimorphic $h\gamma$ and $h\delta$ forms. Among the differences, which were noted in the three regions mentioned above, the largest difference was observed with the sulfate absorption. As seen in Fig. 8, the magnitude in splitting of the sulfate ν_3 band in the $h\delta$ crystals is generally not so remarkable as in the $h\gamma$ crystals. This may indicate that an interaction between a complexing agent and a sulfate anion is weaker in the former than in the latter.

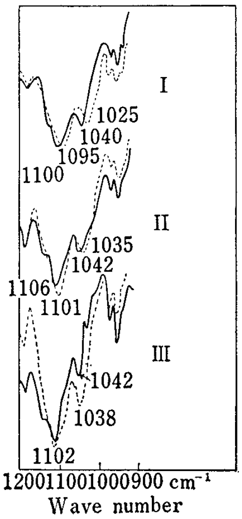

Fig. 8. Infrared Absorption Spectra due largely to Sulfate Anion in $d\alpha$ Crystals (——) and $h\delta$ Crystals (------)

I : Sulfate-monochloroacetic acid complex
II : Sulfate-propionic acid complex
III : Sulfate-thioacetic acid complex

The author wishes to express his hearty thanks to Prof. T. Okamoto, Prof. M. Tsuboi, and Associated Prof. Y. Iitaka of the University of Tokyo, and to Dr. H. Ogawa of this laboratory for their kind guidance and encouragement throughout this work.

Summary

1. Structural information concerning to the binding states of crystal water, complexing agent and sulfate anion in the $d\alpha$ and $h\gamma$ crystals are deduced from the spectral changes accompanied with partial dehydration, together with X-ray evidence. The findings include the mobility or statistical disorderness of crystal water in the $d\alpha$ form, and the presences of little bonded water and a monomeric complexing agent in the $h\gamma$ form.

2. From the intense band near 1100 cm^{-1} observed in all the $d\alpha$ crystals, it is concluded that Td symmetry of sulfate anion still holds in the $d\alpha$ form. The different magnitude in splitting of the sulfate band among the $h\gamma$ crystals is interpreted in terms of the removal of vibrational degeneracy of ν_3, as a result of interaction of a sulfate anion with a complexing agent.

3. The spectral differences arising from polymorphism are shown in the cases between the $d\alpha$ and $d\beta$ forms and between the $h\gamma$ and $h\delta$ forms.

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171. **Kozo Nagano, Hisashi Kinoshita, and Zenzo Tamura** : Metal Complexes of Isonicotinoylhydrazine and Related Compounds. III.*1 Consecutive Formation Constants for Various Metal Ions by the pH Titration Method.

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In order to determine formation constants of metal complexes, pH measurements have been frequently used as the most accurate and reliable method. Bjerrum devised a comparatively simple method of determining consecutive formation constants for metal amine complexes.1) Schwarzenbach provided the more general method of analysis considering the existence of "Hydrogenkomplexe" and "Mehrkernige Komplexe" and using some approximations.2) But his method was not so useful in analysing such equilibria concerning various species of complexes in ligand excess solutions as in the case of isonicotinoylhydrazine (INH). The authors improved the method more complete in order to determine consecutive formation constants of metal complexes of

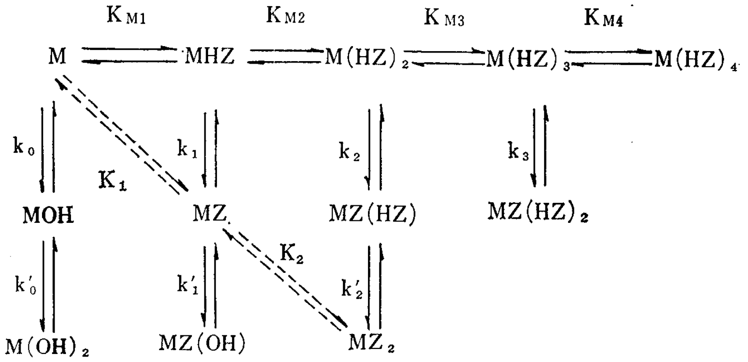

Chart 1.

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*1 A part of this work was lectured by M. Ishidate in Feigl Anniversary Symposium, Birmingham, England, 1962. Part II : This Bulletin, 11, 797 (1963).

*2 Motofuji-cho, Bunkyo-ku, Tokyo (長野晃三, 木下 恒, 田村善蔵).

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2) G. Schwarzenbach : Helv. Chim. Acta, 33, 947 (1950).