X-Ray Diffraction Studies of the Molecular Complexes of Tetracycline Salts

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(Received April 22, 1963)

In addition to the usual 1 : 1 type of molecular compounds formed by an antibiotic tetracycline with such other substancs as γ-butyrolactone,[1] urea,[2] or methanol,[3] tetracycline sulfate forms a series of complexes with the general formula: [2 tetracycline] sulfate·complexing agent·10 water, where "complexing agent" denotes a second molecule, such as an aliphatic carboxylic acid or a derivative of urea, having a rather smaller molecular volume. In a dry atmosphere, however, the decahydrate readily loses 4 molec. of water to form a relatively stable hexahydrate, while the crystal lattices are simultaneously rearranged. The latter dehydrated sulfate-complexes have been the subject of previous investigations,[4,5] and from the close resemblance of their X-ray diffraction powder patterns, a clathrate-like structure has been suggested for them. The formation of similar sulfate-complexes has been observed with oxytetracycline, but not with chlorotetracycline.[6]

This communication is concerned with a crystallographic investigation of the decahydrated sulfate-complexes, together with a classification of the powder patterns of various salt-complexes, including fluosilicate-, hydrochloride-, and nitrate-complexes, as well as of sulfate-complexes.

Experimental

Preparation.—Neutral hydrated tetracycline or oxytetracycline (1 mmol.) is dissolved in water (3~5 ml.) by the addition of strong acid (1~1.5 mequiv.). The complexing agent (2.5 mmol.) is dissolved in the solution under gentle heating. Removing unsoluble compounds by filtration, the solution is cooled to obtain the salt-complexes as crystallites. When guanidine sulfate, dimethylglyoxime or glyoxal, for example, is added to the solution as a complexing agent, crystals are obtained in which water molecules are enclosed in place of guanidine sulfate, etc.

Most of the salt-complexes were obtained as fine crystalline powders. However, when the supersaturated solution was kept in a refrigerator without shock for several days, some of the sulfate-complexes grew in a form of needles elongated along the b-axis, showing {001} as the principal face and often twinned on {001}. The single crystals are yellow in color and show a marked pleochroism.

When the salt anion is sulfate or fluosilicate, the salt-complexes usually crystallize as decahydrates. Hexahydrates of these salt-complexes are prepared by storing the original decahydrates over silica gel at room temperature overnight. Most of the hydrochloride- and nitrate-complexes, as well as a few of the sulfate- and fluosilicate-complexes, crystallize from an aqueous solution directly as hexahydrates. In Table I, the hexahydrates that crystallize directly are distinguished by an asterisk.

Analysis.—The chemical analyses of the composition of the salt-complexes have been reported in previous papers.[4-7] The specific gravity of a single crystal was determined by flotation in a mixture of carbon tetrachloride and chloroform at 20°C. The values given in Table II, however, are not expected to be more accurate than ± 0.02, since the crystals were subjected to dehydration.

X-Ray Measurement.—X-Ray diffraction powder patterns were taken by means of CuK_α radiation using a Norelco diffractometer. The powder patterns of the decahydrates were measured using undried specimens, for they are readily converted into partially dehydrated forms when exposed to dried air.

Single crystal X-ray photographs of oscillation, rotation, and Weissenberg were taken by means of CuK_α radiation. The unit cell parameters recorded in Table II were calculated mainly from the oscillation and (*h*0*l*) Weissenberg photographs about the b-axis. The mean errors for the monoclinic cell dimensions are *a*, ± 0.04; *b*, ± 0.02; *c*, ± 0.04 Å, and β, $\pm 0.2°$, whilst for orthorhombic cells, they are ± 0.04, ± 0.03, and ± 0.05 Å for the a, b, and c-axes respectively. In the case of the dehydrated sulfate-complexes, the errors may exceed the above values, since the reflections consist of diffuse spots. A comparison among the intensities of corresponding X-ray reflections was made mainly on the (*h*0*l*) and (*h*1*l*) Weissenberg photographs for the 11 different salt-complexes of the *d*α form shown in Table II.

1) E. Gans and T. Higuchi, *J. Am. Pharm. Assoc.*, **46**, 587 (1957).
2) L. L. Smith, S. A. Muller, M. Marx, R. Winterbottom and A. P. Doerschuk, *J. Org. Chem.*, **23**, 721 (1958).
3) A. Soder and W. Siedel, Japanese Pat. 34-8746 (1959).
4) H. Ogawa and S. Inouye, *Bull. Agr. Chem. Soc. Japan*, **24**, 657 (1960).
5) S. Inouye, *Chem. Pharm. Bull.*, **9**, 417 (1961).
6) S. Inouye and H. Ogawa, *Agr. Biol. Chem.*, **25**, 333 (1961).
7) S. Inouye, Thesis, The University of Tokyo (1962).

TABLE I. CLASSIFICATION OF SALT-COMPLEXES OF TETRACYCLINE (TC) AND OXYTETRACYCLINE (OTC) ACCORDING TO X-RAY DIFFRACTION POWDER PATTERNS

Decahydrate

Salt-complex of the *dα* Form

TC Sulfate-complex
Acetic acid
Acrylic acid
Aspartic acid
Cyanamide
Dicyanodiamide
Formic acid
Fumaric acid
Glycolic acid
Hydantoin
Malonic acid
Methyl hydrogenoxalate
Monobromoacetic acid
Monochloroacetic acid
Oxalic acid
Propionic acid
Pyrazine
Succinimide
Thioacetic acid
Water

TC Fluosilicate-complex
Acetic acid
Dicyanodiamide
Formamide
Formic acid
Hydantoin
Monochloroacetic acid
Succinimide

OTC Sulfate-complex
Acetic acid
Monochloroacetic acid
Propionic acid

Salt-complex of the *dβ* Form

TC Sulfate-complex
Ascorbic acid
Glutaric acid
Malonic acid
Parabanic acid

Other Salt-complex

TC Sulfate-complex
Dimethyl carbonate
Pyruvic acid

TC Hydrochloride-complex
Oxalic acid

TC Fluosilicate-complex
Oxalic acid

OTC Sulfate-complex
Fumaric acid
Oxalic acid
Succinic acid

Hexahydrate

Salt-complex of the *hγ* Form

TC Sulfate-complex
Acetic acid
Aspartic acid
Cyanamide
Dicyanodiamide
Formic acid
Fumaric acid
Glycolic acid
Hydantoin
Malonic acid
Monochloroacetic acid
Oxalic acid
Pyrazine
Water

OTC Sulfate-complex
Acetic acid
Monochloroacetic acid
Oxalic acid

Salt-complex of the *hδ* Form

TC Sulfate-complex
Acetic acid
Acrylic acid
Ascorbic acid
Biuret*
Dimethyl carbonate
Formamide*
Glutaric acid
Malonic acid
Methyl hydrogenoxalate
Monobromoacetic acid
Monochloroacetic acid
Propionic acid
Pyruvic acid
Thioacetic acid
Thioglycolic acid

Urethan*

TC Hydrochloride-complex
Oxalic acid
Pyrazine*
Succinimide*

TC Nitrate-complex
Biuret*
Oxalic acid*

TC Fluosilicate-complex
Acetic acid

TC Hydrobromide-complex
Oxalic acid*

OTC Hydrochloride-complex
Pyrazine

Other Salt-complex

TC Sulfate-complex
Parabanic acid

TC Hydrochloride-complex
Hydantoin*
Parabanic acid*

TC Nitrate-complex
Hydantoin*
Parabanic acid*
Succinimide*

TC Fluosilicate-complex
Biuret*
Dicyanodiamide
Formamide
Formic acid
Hydantoin
Urethan*

* The salt-complex that crystallizes directly as a hexahydrate.

Results and Discussion

Table I shows a classification of the salt-complexes according to their powder patterns. Many of the salt-complexes belong to one of four distinct groups, that is, the *dα*, *dβ*, *hγ*, *hδ* forms, in which *dα* and *dβ* are the crystal forms found in decahydrates, and *hγ* and *hδ* are those found in hexahydrates. Figure 1 illustrates their typical powder patterns. Besides these, several other variations of the above forms are also recognized; these are shown in Table I as "other salt-complex," but they are not mentioned further in this paper.

The general characters of each of these four forms are set out below.

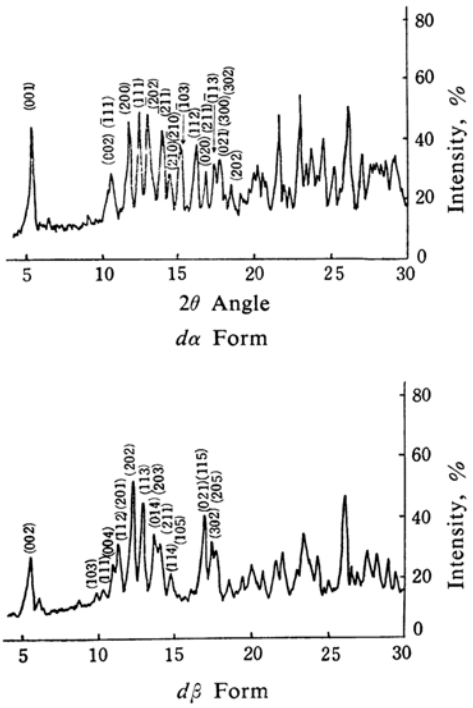
The *dα* Form.—*Sulfate-complexes.*—To this group belong most of the sulfate-complexes of decahydrate, namely, 19 members so far examined. Since all of them show striking

TABLE II. UNIT CELLS AND SPECIFIC GRAVITIES OF SALT-COMPLEXES

Tetracycline salt-complex	Form	System	a, Å	b, Å	c, Å	β	Specific gravity	
							Calcd.	Found
Sulfate-acetic acid	$d\alpha$	Monoclinic	15.87	10.50	17.36	109°00′	1.49	1.49
Sulfate-aspartic acid	$d\alpha$	Mcnoclinic	15.82	10.50	17.32	109°12′	1.49[d]	1.49
Sulfate-fumaric acid	$d\alpha$	Monoclinic	15.81	10.50	17.34	108°36′	1.56[e]	1.52
Sulfate-malonic acid	$d\alpha$	Monoclinic	15.93	10.50	17.50	109°12′		
Sulfate-monobromoacetic acid	$d\alpha$	Monoclinic	15.79	10.49	17.46	108°40′	1.58	1.55
Sulfate-monochloroacetic acid	$d\alpha$	Monoclinic	15.90	10.50	17.38	108°48′	1.525	1.52
Sulfate-oxalic acid	$d\alpha$	Monoclinic	15.72	10.50	17.44	109°06′	1.555	1.53
Sulfate-propionic acid	$d\alpha$	Monoclinic	15.72	10.49	17.40	108°24′	1.51	1.49
Sulfate-water[a]	$d\alpha$	Monoclinic	15.90	10.50	17.35	109°12′	1.46	1.47
Sulfate-water[b]	$d\alpha$	Monoclinic	15.82	10.49	17.31	109°00′	1.46	1.47
Fluosilicate-monochloroacetic acid	$d\alpha$	Monoclinic	15.91	10.49	17.50	109°00′		
Oxytetracycline sulfate-acetic acid	$d\alpha$	Monoclinic	15.79	10.50	17.40	108°12′	1.52	1.515
Sulfate-glutaric acid	$d\beta$	Orthorhombic	16.05	10.50	32.40	90°00′		
Sulfate-malonic acid	$d\beta$	Orthorhombic	15.97	10.53	32.13	90°00′	1.55	1.52
Sulfate-oxalic acid[c]	$h\gamma$	Monoclinic	16.02	10.50	14.69	102°		
Sulfate-water[c]	$h\gamma$	Monoclinic	16.00	10.50	14.83	102°		

a) The dodecahydrate crystallized in the presence of guanidine sulfate.
b) The dodecahydrate crystallized in the presence of dimethylglyoxime.
c) The hexahydrate prepared by the partial dehydration of the decahydrate.
d) Calculated for $2C_{22}H_{24}O_8N_2$-H_2SO_4-2/5$C_4H_7O_4N$-10H_2O.
e) Calculated for $2C_{22}H_{24}O_8N_2$-H_2SO_4-4/5$C_4H_4O_4$-10H_2O.

similarities in their powder patterns, it was assumed that they possess essentially the same unit cell dimensions and symmetry. In order to confirm this point, X-ray single crystal patterns of 9 different kinds of sulfate-complexes were examined; it was, indeed, found that the unit cell dimensions are nearly



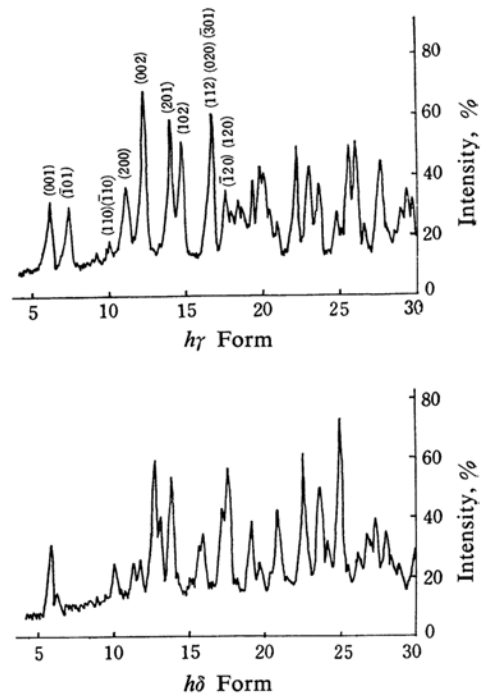


Fig. 1. X-Ray diffraction powder patterns of the four crystal forms.

the same in all of them, as Table II shows. Generally, the cell dimensions are in the range a=15.7~15.9, b=10.5, c=17.3~17.5 Å, and β=108~109°. Taking into consideration the optically-active character of the tetracycline

melecule coupled with the systematic absence, $(0\,k\,0)$ when k is odd, the space group of the $d\alpha$ form was determined as $P2_1$. In nearly every case, there are [2 molec. of tetracycline], one sulfate, one molecule of a complexing agent, and 10 molec. of water in each asymmetric unit, as calculated from the cell volume and the density given in Table II.

Fluosilicate-complexes. — Seven of the fluosilicate-complexes so far examined gave powder patterns very similar to those of the corresponding sulfate-complexes. The isomorphous series of sulfate-complexes, therefore, seems to be extended to include the fluosilicate-complexes. In fact, the monoclinic cell parameters of the fluosilicate-monochloroacetic acid complex were found to be $a=15.91$, $b=10.49$, $c=17.50$ Å and $\beta=109°00'$, figures are indistinguishable from those of its sulfate-complex.

Oxytetracycline Sulfate-complexes.—In addition to the oxytetracycline sulfate-acetic acid complex, whose cell parameters were $a=15.79$, $b=10.50$, $c=17.40$ Å, and $\beta=108°12'$, two more members (sulfate-propionic acid and -monochloroacetic acid complexes) are found to belong to the $d\alpha$ form.

The above results, together with the similar compositions of the salt-complexes, indicate that the molecular packings within the isomorphous salt-complexes of the $d\alpha$ form are very similar. No influence of the complexing agent was observed in determining the crystal structure. Molecules so chemically diverse as water, formic acid, oxalic acid, hydantoin, and dicyanodiamide are considered to be interchangeable in the $d\alpha$-type unit cell without any considerable enlargement or contraction of the cell dimensions.

In the isomorphous molecular compounds, which contained such a variety of chemical compounds, it does not seem likely that the binding force arises primarily from ion-dipole interactions as in the case of the tetraethylammonium bromide succinimide complex.[8] The formation of such a co-ordination compound is subjected to restriction not only by a charge distribution of the complexing agents but also by dimensional factors, so that even chemically-analogous substances may not form similar co-ordination compounds.[9] Therefore, it seems reasonable that the crystal strcutures of the $d\alpha$ form consist of an almost identical framework of the tetracycline (or oxytetracycline) molecules enclosing spaces capable of containing a complexing agent, just as those observed in inclusion compounds.

Further sppport for this point comes from a consideration of the restricted size (50～120 Å^3) and shape (co-planarity) of the complexing agent, as has been discussed previously,[7] and particularly from a detailed comparison of the X-ray diffraction intensities as a whole. Among the $d\alpha$-type sulfate-complexes given in Table II, there is a notable intensity resemblance when due allowance is made for the differences in absorption effect. For example, the difference in intensity between the sulfate-monobromoacetic acid and -propinic acid complexes of tetracycline is less than the difference between two sulfate-acetic acid complexes of tetracycline and oxytetracycline. Since the crystal structures of these complexes are isomorphous, it is to be expected that the differences in intensity of the corresponding reflections are due largely to the different scattering contributions of the complexing agents. However, the above finding suggests that they make only a small contribution. This may be explained, as in the case of the β-quinol clathrate,[10] by the freedom of motion or the statistical disorderness of the complexing agents in the cavities.

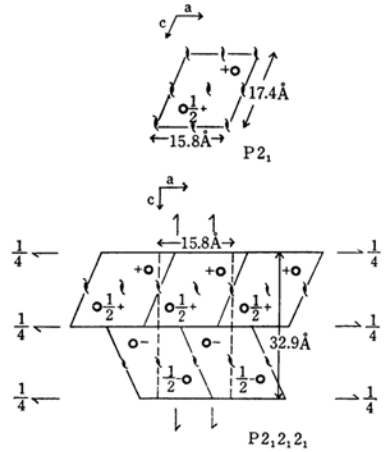


Fig. 2. Relationship between the $d\alpha$ type monoclinic cell ($P2_1$) and the $d\beta$ type orthorhombic cell ($P2_12_12_1$) (projection along [010]).

The $d\beta$ Form.—The $d\beta$ from is found in a few decahydrated sulfate-complexes, that is, sulfate-malonic acid, -glutaric acid, -parabanic acid and ascorbic acid complexes. Since sulfate-malonic acid complex also crystallizes in the $d\alpha$ form, the $d\beta$ form is evidently a polymorphic modification of the $d\alpha$ form. The crystals of the $d\beta$ form are orthorhombic, with the space group $P\,2_12_12_1$ and with cell dimensions of $a=16.0$, $b=10.5$, $c=32.1$～32.4 Å ; there are 4 units in a cell, each unit consisting of [2

8) H. M. Powell and E. Wait, *J. Chem. Soc.*, **1958**, 1866.
9) H. M. Powell, *J. Inorg. & Nuclear Chem.*, **8**, 546 (1958).
10) D. E. Palin and H. M. Powell, *J. Chem. Soc.*, **1947**, 208.

tetracycline] sulfate, one complexing agent and 10 water molecules. A comparison of these parameters with those of the $d\alpha$ form reveals that a and b are almost identical with those of the $d\alpha$ form, whilst c corresponds to $2\,c_{d\alpha}\sin\beta$. If two monoclinic cells of the $d\alpha$ form are twinned, as in Fig. 2, where the twinning axis is the twofold screw axis lying at $y=1/4$ or 3/4 in the (001), the newly-formed orthorhombic cell has cell dimensions and a space group which are nearly identical with those of the $d\beta$ form. It is, therefore, possible that the $d\beta$ form is really a monoclinic substance submicroscopically twinned, thus exhibiting orthorhombic symmetry.* If so, the molecular packing must be essentially the same in both the $d\alpha$ and $d\beta$ forms, in spite of the apparent differences in unit cell dimensions and symmetry.

In this connection, it is interesting to note that the monoclinic crystals of the $d\alpha$ form are often twinned on a plane parallel to (001), giving apparent orthorhombic symmetry on the $h0l$ reflections. However, a careful examination of the Weissenberg photographs shows that they are, in fact, made up of the superposition of the sets of reflections from each of the untwinned crystals. In this case, therefore, each component part of the twinned crystal is large enough compared with a coherent region of X-rays.

The $h\gamma$ Form.—The $h\gamma$ form is found in the hexahydrates produced by the partial dehydration of the decahydrated sulfate-complexes: about one-half of the $d\alpha$-type sulfate-complexes fall into the $h\gamma$ form on dehydration. When a single crystal of the $d\alpha$-type sulfate-oxalic acid or -water complex was kept in dried air for a few minutes, diffuse spots due to the partial dehydrates appeared on the X-ray photograph, superposed on the normal diffraction spots. From the oscillation and ($h0l$) Weissenberg photographs about the b-axis of the dehydrated crystals, the approximate monoclinic cell dimensions of the $h\gamma$ form were found to be $a=16.0$, $b=10.5$, $c=14.7$ Å and $\beta=102°$ (Table II). The reliability of the proposed parameters may be judged further from the indexing of the powder pattern shown in Table III, in which there is no significant discrepancy between the observed and calculated diffraction angles.

Since all the powder patterns belonging to the $h\gamma$ form are very similar, it is reasonable, as in the case of the $d\alpha$ form, to assume the existence of the same essential framework among the $h\gamma$-type sulfate-complexes. It is to be noted, however, that, unlike the $d\alpha$ and $h\delta$ forms, where other salt-complexes besides sulfate-complexes are isomorphous members, the formation of the $h\gamma$ form is limited to the sulfate-complexes, insofar as we have been able to determine. This suggests that a sulfate anion plays an important part in determining the common framework of the $h\gamma$ form.

Table III. Indexing and Comparison of the Observed and Calculated Diffraction 2θ Angles for the Powder Patterns of the $d\alpha$ and $h\gamma$ Forms

$d\alpha$ Form			$h\gamma$ Form		
hkl	Calcd.	Found	hkl	Calcd.	Found
001	5.35°	5.35°	001	6.1 °	6.1 °
002, $\bar{1}11$	10.7	10.7	$\bar{1}01$	7.4	7.3
			$\bar{1}10$	10.15	10.05
200	11.9	11.9	200	11.3	11.3
111	12.5	12.5	002	12.3	12.3
$\bar{2}02$	13.25	13.2	201	14.0	14.0
$\bar{2}11$	14.2	14.1	102	14.6	14.6
210	14.6	14.6	112, 020, $\bar{3}01$	16.9	16.8
$\bar{1}03$	15.3	15.3			
112	16.2	16.2	120	17.8	17.7
020	16.9	16.9			
$\bar{1}13$	17.5	17.5			

As may be seen from a comparison of the cell parameters of the $d\alpha$ and $h\gamma$ forms, it is apparent that the escape of 8 mol. of water from the the $d\alpha$-type unit cell causes a decrease in the c-axis length (2.7 Å) and in the β angle (7°). According to an infrared analysis,[11] the lattice deformation probably corresponds to the approach of a complexing agent to a sulfate anion. In contrast to the $d\alpha$ form, where the sulfate anion holds tetrahedral symmetry as a whole, in the $h\gamma$ form a splitting of the sulfate bands is observed, this can be reasonably interpreted as a lowering of the T_d symmetry caused by interaction between a complexing agent and a sulfate anion.

The $h\delta$ Form.—The $h\delta$ form is a polymorphic modification of the $h\gamma$ form, for some of the hexahydrates, for example, sulfate-acetic acid or -monochloroacetic acid complex, crystallize in both of these forms. Though its unit cell dimensions were not determined, many hexahydrates of the sulfate-, nitrate-, hydrochloride-, and hydrobromide-complexes belong to this group (Table I).

A particularly interesting phenomenon observed in this group is that the powder patterns of 4 different kinds of salt-complexes, namely, the hydrochloride-oxalic acid, hydrobromide-oxalic acid, nitrate-oxalic acid and

* Such submicroscopic twinning is often found in minerals (T. Ito, "X-Ray Studies on Polymorphism," Maruzen, Tokyo (1950)).

11) S. Inouye, *Chem. Pharm. Bull.*, in press (1963).

sulfate-methyl hydrogenoxalate complexes, are quite similar to one another, suggesting the isomorphous replacement of Cl^-, Br^-, NO_3^- and SO^{2-} anions among these crystals.

Summary

An examination of the salt-complexes of tetracycline and oxytetracycline by the X-ray powder method has shown the presence of at least four different kinds of crystal structures, i. e., the *dα*, *dβ*, *hγ*, and *hδ* forms. Among them, the *dα* form has been studied in detail by the use of single crystals. The monoclinic unit cell dimensions of the *dα* crystals do not vary much either with a salt anion or with a complexing agent, and the intensities of the various reflections so far examined are very similar. On the basis of these findings, it is suggested that the crystal structures of the *dα*-type salt-complexes consist of an almost identical framework of tetracycline or oxytetracycline molecules which encloses spaces capable of containing complexing agents of various types. The orthorhombic *dβ* form is a polymorphic modification of the *dα* form, probably arising from submicroscopically polysynthetic twinning of the latter.

The authors wish to express their gratitude to Professor Toshihiko Okamoto and Professor Masamichi Tsuboi of the University of Tokyo, and to Dr. Hiroshi Ogawa of this laboratory, for their interest and encouragement. Thanks are also due to Dr. Kazuhiko Ohta and Messrs. Katsumi Hamano and Tomoya Ogawa for the use of their X-ray facilities.

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