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63. Shigeharu Inouye: Molecular Complexes of Tetracycline Acid Salts. II.*2 On the Unique Clathrate-like Complexes of Tetracycline Sulfate.

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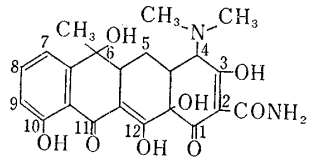
In the preceding paper, a series of oxalic acid complexes of tetracycline (I) acid salts and the effect of various salt anions on the composition and properties of the complexes were mentioned. In the present paper, the complex formation of tetracycline sulfate with other complexing agents and the effect of various complexing agents on the structure of tetracycline sulfate complexes are described.



(I)

Molecular complexes of tetracycline salts generally crystallize from aqueous medium. Among many tetracycline salt complexes, tetracycline sulfate complex was preferred for the detailed investigation of the salt complex, because the sulfate complex can be obtained with the best yield and many salt complexes have similar composition as illustrated in Tables I and III.

Table II presents typical compounds, among a large number and variety of substances investigated, with which tetracycline sulfate forms crystalline molecular complexes. Although the complexing agents have a wide variety of structure, certain characteristics can be detected. Many of the complexing agents are aliphatic substances having oxygen or nitrogen atom in the molecule. The complexes of aromatic substances do not crystallize in general.*3 The effective complexing agents have a rather smaller molecular weight (mean mol. wt., ca. 100) compared with that of tetracycline sulfate (mol. wt., 493). It was observed in the homologs of aliphatic carboxylic acids that a subsequent increase in the length of the alkyl group results in a great increase in solubility of sulfate complex. Some complexes of tetracycline sulfate were quantitatively obtained with the complexing agents that possess two carbonyl groups attached directly or separated by one nitrogen, such as α-dioxo (I) or carbimide (II) compounds. As summarized in Table III, general composition formula of tetracycline sulfate complexes is tetracycline-$\frac{1}{2}H_2SO_4$-$\frac{1}{2}$CA-xH_2O, where CA is a complexing agent and x is an integer of 2~4.

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*3 As an exception, *p*-nitrophenol forms an insoluble complex with tetracycline sulfate in a molecular ratio of 1:1, but this complex appears to be rather abnormal, differing from aliphatic complexes having molecular ratio of 2:1.

Table I. Comparison of Preparative Yield of Three Kinds of Salt Complexes

Tetracycline salt complex	Yield (%)	Tetracycline remaining in the mother liquor (γ/cc.)
Tetracycline sulfate-succinimide	95	4,000
Tetracycline silicofluorate-succinimide	95	4,600
Tetracycline hydrochloride-succinimide	68	12,000
Tetracycline sulfate-pyrazine	90	10,000
Tetracycline silicofluorate-pyrazine	76	18,000
Tetracycline hydrochloride-pyrazine	47	31,000

Table II. Molecular Weight of Various Complexing Agents and Preparative Yield of Tetracycline Sulfate Complexes

Structural type	Complexing agent	Mol. wt.	Yield of tetracycline sulfate complex (%)
R—C=O | R′—C=O (I) and its analogs	Glyoxal	58	70
	Oxamide	88	65
	Oxamic acid	89	90
	Oxalic acid	90	93
	Methyl hydrogenoxalate	104	85
	Rubeanic acid	120	52
	Ascorbic acid	176	88
	Dimethylglyoxime	116	52
	Pyrazine	80	90
	Pyrazinamide	123	46
R—C=O | NH | R′—C=O (II) and its analogs	Succinimide	99	95
	Hydantoin	100	95
	Acetylurea	102	37
	Parabanic acid	114	77
	Biuret	121	91
	Thiouracil	128	50
	Barbituric acid	128	60
	Allantoin	158	63
	Maleic hydrazide	112	88
R—C=O | CH_2 | R′—C=O (III) and its analogs	Pyruvic acid	88	82
	Malonic acid	104	55
	Fumaric acid	116	65
	Succinic acid	118	70
	Glutaric acid	132	25
	Acetylacetone	100	35
	Aspartic acid	133	60
R—O | R′—C=O (IV) or R—NH | R′—C=O (V) and its analogs	Formamide	45	80
	Formic acid	46	60
	Acetic acid	60	75
	Acrylic acid	72	70
	Propionic acid	74	73
	Glycine	75	75
	Glycolic acid	76	70
	Thioacetic acid	76	75
	Urethan	89	84
	Dimethyl carbonate	90	90
	Thioglycolic acid	92	80
	Chloroacetic acid	95	60
	Bromoacetic acid	139	60
R—NH | R′—C=NH (VI) and its analogs	Cyanamide	42	70
	Dicyanodiamide	84	75
	Cyanuric acid	129	70
Others	*p*-Nitrophenol	139	90

TABLE III. Analyses of the Composition of Tetracycline Sulfate Complexes and Other Salt Complexes

Tetracycline complex[a]	m.p. (°C)	Molecular formula	C (%)		H (%)		N (%)		Acid equiv.		H_2O (%)	
			C	F	C	F	C	F	C	F	C	F
Sulfate-acetic acid	79～ 81	Tetracycline-½H_2SO_4-½$C_2H_4O_2$-2H_2O							1.5	1.4	6.4	5.0
Sulfate-ascorbic acid	99～103	Tetracycline-½H_2SO_4-½$C_6H_8O_6$-2H_2O	48.6	48.1	5.4	5.3	4.5	3.95			9.9[d]	10.7
Sulfate-ascorbic acid[b]		Tetracycline-½H_2SO_4-½$C_6H_8O_6$-2H_2O	48.6	48.4	5.4	5.5	4.5	4.35	1.5	1.5		
Sulfate-biuret[e]	117～119[c]	Tetracycline-½H_2SO_4-½$C_2H_5O_2N_3$-3H_2O	46.0	44.8	5.6	5.5	8.2	8.5	1.0	1.0	9.0	9.2
Sulfate-cyanamide	80～ 82	Tetracycline-½H_2SO_4-¾$C_2H_2N_2$-2H_2O	48.7	48.6	5.5	5.6	8.7	8.9	1.0	1.0	6.4	5.4
Sulfate-dicyanodiamide	172～173[c]	Tetracycline-½H_2SO_4-½$C_2H_4N_4$-2H_2O	48.5	48.5	5.5	5.3	9.8	9.3	1.0	1.0	6.3	8.1
Sulfate-dicyanodiamide[b]		Tetracycline-½H_2SO_4-½$C_2H_4N_4$-H_2O	49.9	49.9	5.3	5.1	10.1	9.3	1.0	1.0		
Sulfate-formamide	120～122[c]	Tetracycline-½H_2SO_4-½CH_3ON-3H_2O[f]	47.5	47.5	5.7	5.5	6.1	7.1	1.0	1.0	9.5	10.5
Sulfate-formamide[b]		Tetracycline-½H_2SO_4-½CH_3ON-H_2O	50.6	50.0	5.4	5.2	6.6	6.5	1.0	1.0		
Sulfate-formic acid	156～161[c]	Tetracycline-½H_2SO_4-½CH_2O_2-3H_2O	47.3	47.9	5.6	5.3	4.9	4.55	1.5	1.5		
Sulfate-formic acid[b]		Tetracycline-½H_2SO_4-1/10CH_2O_2-1.5H_2O	50.5	50.9	5.4	5.5	5.3	4.8	1.1	1.1		
Sulfate-fumaric acid	171～173[c]	Tetracycline-½H_2SO_4-½$C_4H_4O_4$-2H_2O							2.0	1.9	6.1	6.0
Sulfate-glutaric acid	83～ 89	Tetracycline-½H_2SO_4-½$C_5H_8O_4$-2H_2O							2.0	2.0	6.0	6.2
Sulfate-hydantoin		Tetracycline-½H_2SO_4-½$C_3H_4O_2N_2$-2H_2O	48.8	49.4	5.4	5.55	7.25	7.1	1.0	1.0		
Sulfate-malonic acid	79～ 81	Tetracycline-½H_2SO_4-½$C_3H_4O_4$-2H_2O							2.0	2.0	6.2	5.4
Sulfate-bromoacetic acid	94～100	Tetracycline-½H_2SO_4-½$C_2H_3O_2Br$-2H_2O							1.5	1.4	6.0	6.25
Sulfate-chloroacetic acid	80～ 85	Tetracycline-½H_2SO_4-½$C_2H_3O_2Cl$-2H_2O							1.5	1.55	6.2	6.3
Sulfate-methyl hydrogenoxalate	107～109[c]	Tetracycline-½H_2SO_4-½$C_3H_4O_4$-4H_2O	45.8	45.2	5.7	5.6	4.5	4.25	1.5	1.5[g]	11.65	11.95
Sulfate-*p*-nitrophenol	74～ 76	Tetracycline-½H_2SO_4-$C_6H_5O_3N$-H_2O[h]	51.6	52.1	4.95	4.7	6.5	6.05	1.1	1.1	3.3	5.7
Sulfate-*p*-nitrophenol[b]		Tetracycline-½H_2SO_4-$C_6H_5O_3N$	53.1	53.0	4.8	4.6	6.6	6.3	1.1	1.0		
Sulfate-propionic acid	78～ 80	Tetracycline-½H_2SO_4-½$C_3H_6O_2$-2H_2O							1.5	1.4	6.4	6.4
Sulfate-pyrazine	161～167[c]	Tetracycline-½H_2SO_4-½$C_4H_4N_2$-3H_2O[i]	49.1	49.3	5.7	5.4	7.2	7.4	1.0	1.0		
Sulfate-pyrazine[b]		Tetracycline-½H_2SO_4-¼$C_4H_4N_2$-1.5H_2O	51.2	51.4	5.3	5.8	6.5	6.7			5.0	5.3
Hydrochloride-pyrazine	192～198[c]	Tetracycline-HCl-½$C_4H_4N_2$-2H_2O[j]	51.7	51.4	5.6	5.1	7.5	7.0	1.0	1.0	6.45	7.1
Silicofluorate-pyrazine	196～198[c]	Tetracycline-½H_2SiF_6-½$C_4H_4N_2$-4H_2O	45.9	45.6	5.6	5.5	6.7	6.9	3.0	3.0		
Sulfate-pyruvic acid	92～ 95	Tetracycline-½H_2SO_4-½$C_3H_4O_3$-2H_2O	49.2	49.5	5.4	6.0	4.9	4.2	1.5	1.4		
Sulfate-pyruvic acid[b]		Tetracycline-½H_2SO_4-¼$C_3H_4O_3$-½H_2O	52.0	52.0	5.2	5.3	5.3	4.8	1.25	1.25		
Sulfate-succinic acid	93～ 94	Tetracycline-½H_2SO_4-½$C_4H_6O_4$-2H_2O							2.0	1.9	6.1	5.4
Sulfate-succinimide		Tetracycline-½H_2SO_4-½$C_4H_5O_2N$-4H_2O[k]	46.8	47.5	5.8	5.9	5.7	5.3	1.0	1.05		
Hydrochloride-succinimide	145～146[c]	Tetracycline-HCl-½$C_4H_5O_2N$-2H_2O[l]	50.8	50.3	5.6	5.7	6.2	6.1	1.0	1.0	6.35	7.4
Silicofluorate-succinimide	160～164[c]	Tetracycline-½H_2SiF_6-½$C_4H_5O_2N$-4H_2O	45.2	44.5	5.6	5.5	5.5	5.4	3.0	3.0	11.8	11.8
Sulfate-thioglycollic acid	79～ 82	Tetracycline-½H_2SO_4-½$C_2H_4O_2S$-2H_2O	48.0	48.0	5.4	5.6	4.9	4.5	1.5	1.4		
Sulfate-thioglycollic acid[b]		Tetracycline-½H_2SO_4-½$C_2H_4O_2S$	51.2	51.4	5.0	5.3	5.2	4.6	1.5	1.4		
Sulfate-urethan	101～102	Tetracycline-½H_2SO_4-¼$C_3H_7O_2N$-3H_2O	48.0	47.8	5.8	5.1	5.5	5.3	1.0	1.0	11.3	10.0
Sulfate-urethan[b]		Tetracycline-½H_2SO_4-¼$C_3H_7O_2N$-H_2O	51.1	51.75	5.4	5.5	5.9	5.8	1.0	0.9		

a) Samples were dried *in vacuo* at room temperature (20～30°) over KOH overnight, unless otherwise stated. *b*) Dried *in vacuo* at 75° over P_2O_5 for 7.5 hr. *c*) With decomposition. *d*) Ascorbic acid consumed ca. 1.5 moles of the Karl Fischer reagent. *e*) Crystallized from hydr. EtOH. *f*) Or calcd. for tetracycline-½H_2SO_4-CH_3ON-3H_2O : C, 46.6; H, 5.8; N, 7.1. *g*) Saponification equivalent : Calcd. 3.0. Found, 3.0. *h*) Molecular ratio of this complex was determined by the ultraviolet absorbance in 0.1*N* H_2SO_4. *i*) S content : Calcd. 2.7. Found, 3.7. *j*) Cl content : Calcd. 7.1. Found, 6.4. *k*) S content : Calcd. 2.6. Found, 3.0. *l*) Cl content : Calcd. 6.3. Found, 6.4.

Many complexes are unstable upon heating at 75° *in vacuo* and a part of complexing agent and crystal water are lost by this treatment (Table III). By examination with X-ray powder diffraction, it was found that the partial loss of complexing agent and crystal water from the formamide or pyrazine complex is accompanied with alteration in the lattice structure of these complexes.

Solubilities of sulfate complexes are affected by the complexing agents, as shown in Table IV.

Table IV. Solubility of Several Tetracycline Sulfate Complexes in 0.01*N* H_2SO_4 at 0°

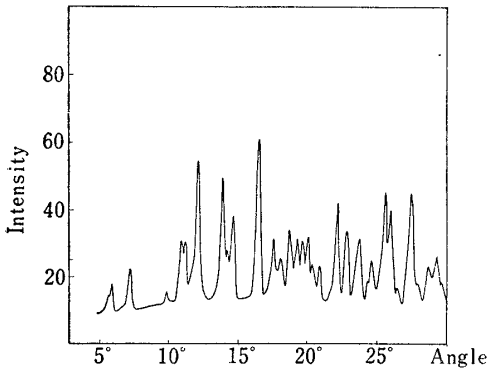
Complex	Solubility (γ/cc.)	Complex	Solubility (γ/cc.)
Tetracycline sulfate-ascorbic acid	20,000	Tetracycline sulfate-oxamic acid	16,000
Tetracycline sulfate-dicyanodiamide	19,000	Tetracycline sulfate-pyruvic acid	22,000
Tetracycline sulfate-formamide	24,000	Tetracycline sulfate-succinimide	3,750
Tetracycline sulfate-formic acid	29,500	Tetracycline sulfate-thioglycollic acid	21,900
Tetracycline sulfate-glycine	17,500	Tetracycline sulfate-urethan	28,000
Tetracycline sulfate-hydantoin	2,600	Tetracycline hydrogensulfate	150,000
Tetracycline sulfate-*p*-nitrophenol	7,250	Tetracycline sulfate	19,000
Tetracycline sulfate-oxalic acid	13,000		

Infrared spectra of sulfate complexes were generally additive ones of the bands of three components (tetracycline cation, sulfate anion, and complexing agent), and no particular band or noticeable spectral change relating to complex formation was recognized.

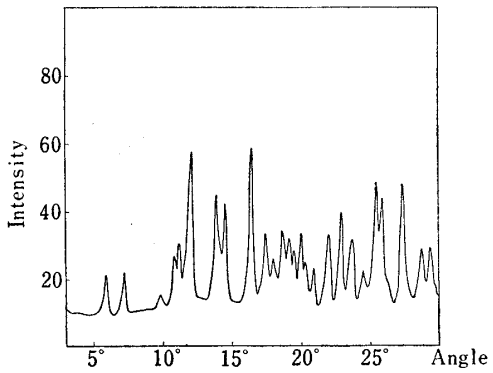
Comparison of X-ray powder diffraction patterns of various salt complexes clearly shows that the packing of tetracycline salt complexes are markedly influenced by the salt anions; the lattice structure of tetracycline sulfate complexes is different from those of hydrochloride complexes and silicofluorate complexes, even when these three salt complexes have the same complexing agent. On the other hand, effect of the complexing agent upon the lattice structures of complexes is small. As demonstrated in Fig. 1, the diffraction patterns of many complexes were similar and for the most part, can be summarized into two diffraction types, in which the diffraction patterns are quite identical with each other. Since the close similarity of diffraction patterns indicates the resemblance of crystal structure, the similarity of diffractions in spite of a wide structural diversity of complexing agents suggests the presence of the essential structure of tetracycline sulfate in these complexes. Therefore, in subsequent experiment, an attempt was made to crystallize tetracycline sulfate.

The crystals of the desired tetracycline sulfate, however, could not be obtained by the usual methods of preparation. The crystals, when obtained from alcohols in the absence of a complexing agent, contain two equivalents of sulfuric acid and are not the expected tetracycline sulfate, but tetracycline hydrogensulfate. X-Ray powder diffraction pattern of this compound differs definitely from those of all sulfate complexes (Fig. 2). Tetracycline sulfate did not crystallize from 20% aqueous solution of tetracycline acidified with sulfuric acid, even after cooling for two weeks. However, by addition of a certain nitrogenous compound to this solution, the crystals of tetracycline sulfate were obtained. It was also found that X-ray powder diffraction pattern of tetracycline sulfate hydrate that crystallized in the presence of guanidine sulfate or hydrazoic acid was identical with the diffraction pattern of sulfate complexes (Fig. 3).

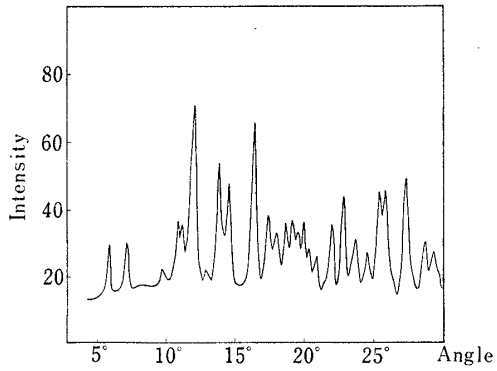
The formation of a sulfate complex can be recognized only in a crystal state, as evidenced by the characteristic solubility and by the X-ray powder diffraction pattern, but not in a solution. When dissolved in any solvent, tetracycline sulfate complexes are dissociated into its components or form complexes different from those in crystalline state. Ultraviolet spectrum of tetracycline in dilute sulfuric acid containing various quantities of complexing agents is almost similar to that of tetracycline hydrochloride. Paper chromatographic mobility and distribution coefficient of sulfate complexes, shown in Tables V



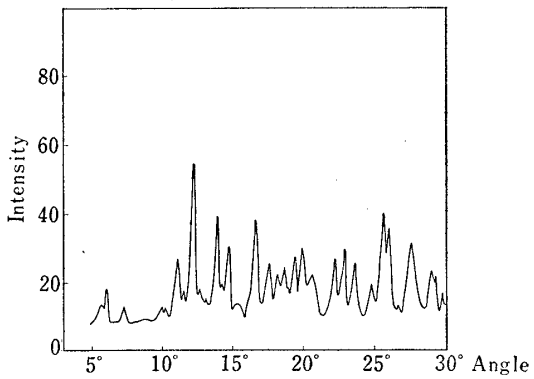
(a) Tetracycline Sulfate-Formic Acid Complex



(b) Tetracycline Sulfate-Dicyanodiamide Complex



(c) Tetracycline Sulfate-Cyanoamide Complex



(d) Tetracycline Sulfate-Fumaric Acid Complex

Fig. 1. X-Ray Powder Diffraction Curve

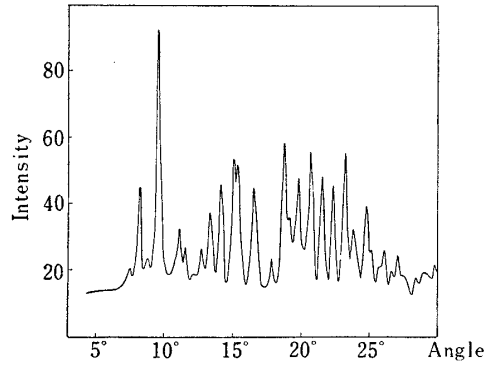


Fig. 2. X-Ray Powder Diffraction Curve of Tetracycline Hydrogensulfate-Monoethanolate Monohydrate

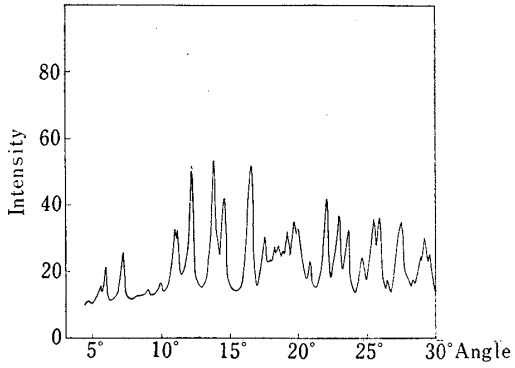


Fig. 3. X-Ray Powder Diffraction Curve of Tetracycline Sulfate Hydrate crystallized in the Presence of Guanidine Sulfate

and VI, are indistinguishable from those of tetracycline sulfate and variation caused by the acidic substances.

Discussion on the Binding Mode of Sulfate Complex

In some molecular compounds, such as those formed between aromatic hydrocarbons and certain polynitro compounds, the strong attraction between the components is responsible for the complex formation. Such strong interactions, however, would not be expected between tetracycline sulfate and the complexing agents, as suggested by the ready dissociation of the complexes in solution, by the partial loss of the complexing agents

Table V. Rf Values of Some Tetracycline Salts and Tetracycline Sulfate Complexes in Paper Chromatography[a]

Compound	Solvent[b] 1	2	3	4
Tetracycline sulfate-ascorbic acid	0.39	0.63[c]	0.58	0.89[c]
Tetracycline sulfate-dicyanodiamide	0.43	0.56[c]	0.63	0.71[c]
Tetracycline sulfate-hydantoin	0.41	0.56[c]	0.57	0.69[c]
Tetracycline sulfate-succinic acid	0.39	0.56[c]	0.65	0.70[c]
Tetracycline sulfate-succinimide	0.42	0.56[c]	—	0.70[c]
Tetracycline sulfate	0.45	0.58[d]	0.60	0.72[d]
Tetracycline hydrogensulfate	0.39	0.62[e]	0.56	0.78[e]
Tetracycline nitrate	0.44	0.57[e]	0.62	0.88[e]
Tetracycline hydrochloride	0.37	0.64[e]	0.61	0.78[e]

a) Filter paper, Toyō Roshi No. 50; ascending method. Indicator, ultraviolet lamp.
b) Solvent 1. BuOH-MeOH-H_2O=4:1:2.5 v/v
2. BuOH-MeOH-0.02*N* H_2SO_4=4:1:2.5 v/v
3. MeCOEt-dimethylformamide-H_2O=10:1:2.5 v/v
4. MeCOEt-dimethylformamide-0.02*N* H_2SO_4=10:1:2.5 v/v
c) Paper impregnated with 0.02*N* complexing agent in 0.02*N* H_2SO_4.
d) Paper impregnated with 0.02*N* H_2SO_4.
e) Paper impregnated with 0.04*N* strong acid.

Table VI. Distribution Coefficient of Tetracycline in the Presence of Complexing Agents between 0.02*N* Sulfuric Acid and Butanol at 29°

Complexing agent	K(organic phase/aqueous phase)	Complexing agent	K(organic phase/aqueous phase)
Blank	0.23, 0.22	Hydantoin	0.21
Acetic acid	0.235	Bromoacetic acid	0.25
Ascorbic acid	0.235	Oxalic acid	0.35
Cyanoacetic acid[a]	0.28	Succinimide	0.21
Dicyanodiamide	0.21	Urethan	0.22
Formamide	0.21	Hydrochloric acid	0.58[b]
Formic acid	0.23	Nitric acid	0.79[c]
Guanidine hydrochloride	0.43	Sulfuric acid	0.27
Guanidine sulfate	0.27		

a) The salt complex of this compound did not crystallize from aqueous solution.
b) Distribution coefficient between 0.02*N* HCl and BuOH.
c) Distribution coefficient between 0.02*N* HNO_3 and BuOH.

from the complexes by mild heating, and by the absence of characteristic infrared band of the complex indicating that the origin of the complex formation cannot be attributed to any forms of marked electron transfer. Accordingly, the formation of a molecular compound of tetracycline sulfate must be ascribed, at least partly, to other forms of interaction, such as a packing effect of crystal structure, for tetracycline sulfate complexes may not exist apart from the crystalline state.

Although the exact crystal structure of tetracycline sulfate complexes must await the elucidation by X-ray crystallographic analysis or other methods of direct determination, powder diffraction method clearly shows that sulfate complexes possess the essential structure*4 of tetracycline sulfate in these crystal structures. The lattice structure of sulfate is probably open, because, as the lattice formation of tetracycline sulfate was possible only by the co-presence of a third substance, such as amine sulfate or the complexing agent, and evidently these third substances might play an important rôle in the formation of a framework of tetracycline sulfate, it would be natural to consider the lattice structure as

*4 It is not uncertain that there is only one type of the common structure of tetracycline sulfate in all complexes. Formation of several different enclosures might be possible by a given enclosing component, or by a preparative condition.

enclosed spaces capable of holding these substances, just as were seen in the case of open structures of gas hydrate or β-quinol.*5

This structure in which the complexing agent is enclosed by a framework of tetracycline sulfate is that of the so-called clathrate.[1]

The clathrate-like structure of complexes is in agreement with the character of the complexing agents. The complexing characteristics of the weak crystallizing tendencies of aromatics and of the effective complexing of the compounds having smaller molecular weight (less than one-fourth of that of tetracycline), can easily be explained by the assumption that the enclosable molecules are limited to those which can fit into the restricted spaces (size and shape) constructed by tetracycline sulfate hydrates. As was described in the subsequent paper,[2] similar adducts were formed by the structurally related 5-hydroxytetracycline. The persistence of similar complex formation in a group of related compounds is observed in many clathrates.

The open frameworks of tetracycline salts in salt complexes*6 are closely related to the inserted salt anions and crystal water, because the formation of similar complexes did not occur with neutral tetracycline base[3] and the lattice arrangement of the complexes is definitely affected by salt anions. Since tetracycline sulfate complex can be obtained with more ease than other salt complexes, sulfate anion is particularly suitable for the open structure. As for crystal water, at least two moles of hydrated water were found in all the crystalline complexes and dehydration caused the collapse of lattice structure. It would therefore appear that the water molecules attached to the complex are oriented or bound in such a manner as to render the formation of open structure favorable.

Since the polar characters of tetracycline and of crystal water are undoubtedly favorable for inter- and intra-molecular hydrogen bonding, it seems reasonable that hydrogen bonding formation is playing a prominent, though not an exclusive, part in the formation of these clathrate-like structures, because the organic crystal structures are generally found to adjust themselves so that maximum hydrogen bonding is observed.[4]

Experimental*7

General Method for Preparation of Tetracycline Sulfate Complexes——Neutral hydrated tetracycline (500 mg.) is dissolved in H_2O (2.5 cc.) by addition of 50% H_2SO_4 (0.2~0.3 cc.). The complexing agent (ca. 250 mg.) is added to this solution and dissolved by heating to a suitable temperature, if necessary. The solution is filtered immediately and cooling of the solution (4°)*8 causes the complex to crystallize. The crystals are collected, washed with a small amount of cold H_2O, and dried *in*

*5 In the absence of the enclosed molecules, water and hydroquinone form a more compact ordinary ice and α-quinol respectively (H. M. Powell : J. Chem. Soc., **1954**, 2658).

*6 The framework of tetracycline salts in other salt complexes and tetracycline hydrochloride trihydrate that crystallizes in the presence of amine hydrochloride can also be considered to be open.

*7 The instrumental procedures and quantitative daterminations were essentially the same as in the previous work.

*8 Crystallization of complexes must be carried out at a constant temperature, since tetracyclines hydrochloride often showed polymorphism by crystallization at different temperatures (P. Gailliot, J. Gaudechon : Ind. chim. belge, **20**, spec. No. 506 (1955) (C. A., **50**, 7399 (1956)); J. S. Buckley, C. Stephens, R. L. Wagner : U. S. Pat. 2,867,661 (1959).

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4) S. Seki, H. Chihara, K. Suzuki : Suiso Ketsugô (Hydrogen Bonding), 20 (1956). Iwanami Shoten, Tokyo; J. M. Robertson : "Determination of Organic Structures by Physical Methods," 495 (1955). Academic Press Inc., New York.

vacuo at room temperature over KOH overnight. The yield varies from about 250 mg. to 550 mg.

Many of the complexes can be recrystallized with some loss from H_2O containing a small amount of H_2SO_4 and complexing agent. Recrystallization from weak acidic solution often gives a mixture of complex and neutral tetracycline due to the dissociation of tetracycline sulfate. When the complexing agent is an amphoteric substance such as glycine, the addition of excess amount of strong acid to prevent co-precipitation of neutral tetracycline is particularly required. The complexes of tetracycline hydrochloride and tetracycline-silicofluorate were prepared in a similar way.

Hydrated Tetracycline Sulfate——Neutral tetracycline hydrate (1.0 g.) was dissolved in H_2O (6 cc.) and acidified with 50% H_2SO_4 (0.25 cc.). To this solution, guanidine sulfate (500 mg.) was added. On cooling overnight at 4°, the solution deposited crystals which were collected, washed with a small amount of cold H_2O, and carefully dried at room temperature. m.p. 178~184° (decomp.). Yield, 937 mg., 900 γ/mg. (microbiological), 860 γ/mg. (photometric).

The same compound was obtained by adding NaN_3 in place of guanidine sulfate. Yield, 75%. The two specimens gave identical infrared spectra and X-ray powder diffraction patterns. Tetracycline sulfate was also obtained by addition of hydrazine sulfate. IR : ν_{max}^{KBr} 1100 cm^{-1} (SO_4^{2-}). No band corresponding to azide or guanidine group was detected.

X-Ray powder diffraction pattern shown in Fig. 3 has the following data.

d, Å	I/I_1	d, Å	I/I_1	d, Å	I/I_1
14.9	0.39	5.34	0.96	3.77	0.60
12.3	0.47	5.04	0.58	3.49	0.67
8.04	0.63	4.60	0.62	3.43	0.67
7.90	0.60	4.48	0.65	3.24	0.65
7.25	0.95	4.44	0.62	3.06	0.58
6.37	1.00	4.04	0.77		
6.07	0.79	3.87	0.69		

Anal. Calcd. for $C_{22}H_{24}O_8N_2 \cdot \frac{1}{2}H_2SO_4 \cdot 3.5H_2O$: C, 47.6; H, 5.8; N, 5.0; acid equiv., 1.0; H_2O, 11.3%. Found (guanidine sulfate) : C, 47.4; H, 5.5; N, 5.0; acid equiv., 0.9; H_2O; 10.0%. Found : (Hydrazoic acid) C, 47.7; H, 5.5; N, 4.9; Acid, 1.0 equiv.; H_2O; 10.4%. (Hydrazine sulfate) : N, 5.3%.

A sample was heated at 75° *in vacuo* for 8 hr. over P_2O_5, and analysed. *Anal.* Calcd. for $C_{22}H_{24}O_8N_2 \cdot \frac{1}{2}H_2SO_4 \cdot H_2O$: C, 51.7; H, 5.3; N, 5.5; S, 3.1; acid equiv., 1.0; H_2O, 3.5%. Found : C, 51.7; H, 5.4; N, 4.95, 5.5; S, 3.6; acid equiv., 1.0; H_2O, 3.5%.

Hydrated Tetracycline Hydrochloride——Tetracycline hydrochloride·$3H_2O$ was obtained by addition of guanidine hydrochloride or hydroxylamine hydrochloride to the aqueous solution of tetracycline sulfate, but not by addition of amine hydrochloride to the HCl solution of tetracycline. Yield employing guanidine hydrochloride : 30%; yield employing hydroxylamine hydrochloride : 50%. m.p. 203~205° (decomp.), 920 γ/mg. (microbiological). X-Ray powder diffraction pattern of this salt differed from that of ordinary tetracycline hydrochloride prepared from EtOH solution.

X-Ray powder diffraction data :

d, Å	I/I_1	d, Å	I/I_1	d, Å	I/I_1
14.49	0.34	5.01	0.45	3.85	0.36
10.05	0.62	4.77	0.34	3.71	0.67
8.35	0.83	4.40	0.43	3.47	0.43
6.92	1.00	4.31	0.74	3.21	0.45
6.56	0.48	4.15	0.49	3.10	0.42
6.42	0.50	4.10	0.70		
5.40	0.37	3.97	0.42		

Anal. Calcd. for $C_{22}H_{24}O_8N_2 \cdot HCl \cdot 3H_2O$: C, 49.4; H, 5.9; N, 5.2; Cl, 6.6; acid equiv., 1.0; H_2O, 10.35%. Found (guanidine hydrochloride) : C, 49.4; H, 6.1; N, 5.0; Cl, 7.8; acid equiv., 1.0; H_2O, 9.3%. (hydroxylamine hydrochloride)*9 : C, 49.1; H, 6.1; N, 4.7; Cl, 6.45; acid equiv., 1.0; H_2O, 9.9%.

Tetracycline Hydrogensulfate——Tetracycline hydrogensulfate·EtOH·H_2O was obtained by dissolving neutral tetracycline hydrate (6.0 g.) in EtOH (40 cc.) containing triethylamine (1.8 cc.), adding 3 cc. of 50% H_2SO_4, and keeping at room temperature for 5 hr. (yield, 50%), m.p. ca. 130~140° (decomp.), 830 γ/mg. (microbiological), and tetracycline hydrogensulfate·BuOH·H_2O from BuOH solution in place of EtOH. Yield, 80%, m.p. ca. 150~155° (decomp.), 820 γ/mg. (microbiological), 765 γ/mg. (photometric). IR ν_{max}^{KBr} cm^{-1} : 1229, 1182, 1047, 860 (HSO_4^-). X-Ray powder diffraction pattern of tetracycline

*9 Partial mixture (5%) of anhydrotetracycline was indicated by the ultraviolet absorbance of this salt.

hydrogensulfate·EtOH·H_2O is shown in Fig. 2. Comparison of the patterns of the two acid salts revealed a little difference, probably due to the difference of unit cell dimension.

Tetracycline hydrogensulfate·EtOH·H_2O. *Anal.* Calcd. for $C_{22}H_{24}O_8N_2 \cdot H_2SO_4 \cdot C_2H_5OH \cdot H_2O$: C, 47.5; H, 5.9; N, 4.6; acid equiv., 2.0; H_2O, 3.0%. Found : C, 47.1; H, 5.45; N, 4.0; acid equiv., 2.1; H_2O, 4.4%.

Tetracycline hydrogensulfate·BuOH·H_2O. *Anal.* Calcd. for $C_{22}H_{24}O_8N_2 \cdot H_2SO_4 \cdot C_4H_9OH \cdot H_2O$: C, 49.3; H, 6.0; N, 4.4; acid equiv., 2.0; H_2O, 2.8%. Found : C, 49.2; H, 5.9; N, 4.05; acid equiv., 2.0; H_2O, 4.3%.

Determination of Distribution Coefficient——A sample of neutral tetracycline was dissolved in 0.02*N* H_2SO_4 (previously equilibrated against the organic phase) in a concentration of 100 γ/cc. and equilibrated with BuOH (10 cc. of each phase), adding about 10 mg. of an appropriate complexing agent. Each phase was assayed spectrophotometrically.

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Summary

Tetracycline sulfate hydrate forms molecular addition complexes with a wide range of organic compounds containing electronegative atoms in the molecule. The complexes exist only in a solid state and to these complexes a clathrate-like structure is suggested, in which the clathrate-forming substance is tetracycline sulfate hydrate and the enclosed molecule is a complexing agent, from the following facts : The smaller molecular volume and the limiting size of the complexing agent for the complex formation, the molecular ratio of 2:1 for tetracycline sulfate and complexing agent, the similarity of crystal structures of the complexes, and the identity of the diffraction patterns of the complexes with that of tetracycline sulfate hydrate that crystallize only in the co-presence of certain nitrogenous compounds.

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