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1. Takeichi Sakaguchi, Masahiko Toma, Tomoko Yoshida, Hiroshi Omura, and Hisashi Takasu : Metal Chelate Compounds with Tetracycline Derivatives. VII.* The Structure of Tetracycline Chelates.

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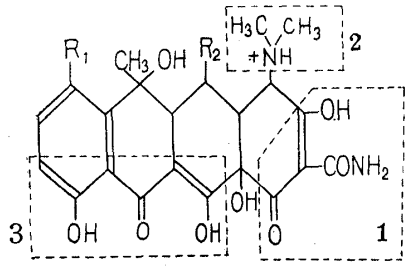
We have already determined that tetracyclines have a chelating group in their molecule, which is a phenolic β-diketone.[1]

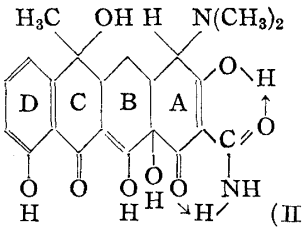
Stephens and others,[2] and Conover[3] recently observed that phenolic β-diketone was very important for chelate formation from the consideration of dissociation constants of tetracyclines. In the case of Aureomycin, pKa of groups 1, 2, and 3 is 3.30, 7.44, and 9.27, respectively.



(I)

(I) R_1=Cl, R_2=H Aureomycin (AM)
(I) R_1=R_2=H Achromycin (AchrM)
(I) R_1=H, R_2=OH Terramycin (TM)

Another theory, described by Hochstein,[4] involves the formation of a hydrogen bond structure in tetracyclines from the keto-enol system of a tricarbonylmethane group. This group also may be said to have a chelating ability.



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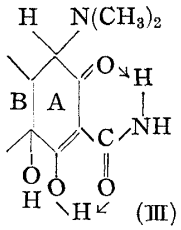

(III)

However, it cannot be likely that this group will have a chelating effect because of its high acidity, indicated by its pKa of 3.3.

The chelate formation of tetracyclines and metal ions was examined by the shift of their maximum absorbancy and pH drop of their chelates (Tables II and III, Figs. 1~4). The molar ratio of their chelates was determined in this experiment (Table I, Figs. 5 and 6), which was also described in the preceding paper.[1]

* Part VI : Yakugaku Zasshi, **78**, in press (1958).

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1) M. Ishidate, T. Sakaguchi : This Bulletin, **3**, 147(1955).
2) C.R. Stephens, K. Murai, K.J. Brunings, R.B. Woodward : J. Am. Chem. Soc., **78**, 4155(1956).
3) L.H. Conover : Nature, **177**, 1059(1956).
4) F.A. Hochstein : J. Am. Chem. Soc., **75**, 5455(1953).

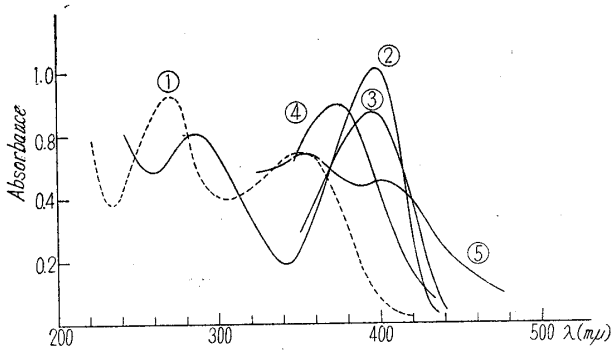


Fig. 1. Terramycin Chelate

① TM 20 γ/cc. (pH=2.0)
② Th (pH=4.6)
③ Zr (pH=5.0)
④ Al (pH=5.0)
⑤ UO_2 (pH=5.6)

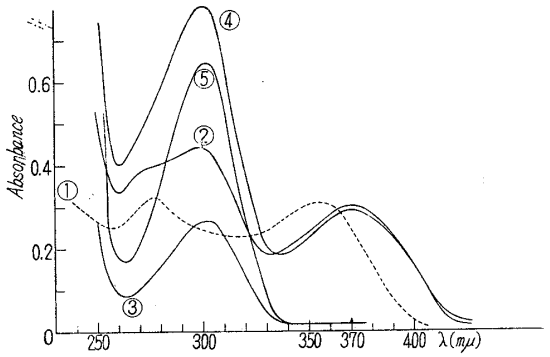


Fig. 2. Terramycin-Co Chelate

① TM 50 γ/cc. 2.0 cc. → water 10 cc. (10 γ/cc.)
② TM 50 γ/cc. 2.0 cc. + 1/100 *M* Co 2.0 cc. → 10 cc.
③ + 1/100 *M* Co 2.0 cc. → 10 cc.
④ TM 50 γ/cc. 2.0 cc. + 1/100 *M* Co 5.0 cc. → 10 cc.
⑤ + 1/100 *M* Co 5.0 cc. → 10 cc.
(none buffer)

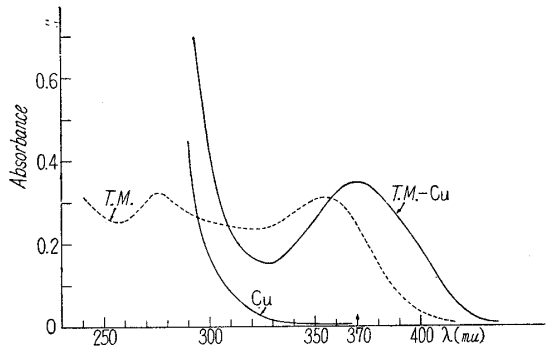


Fig. 3. Terramycin-Cu Chelate

50 γ/cc. TM 2.0 cc.
0.1 *M* Cu 2.0 cc.
buffer 2.0 cc.
} → 10 cc.

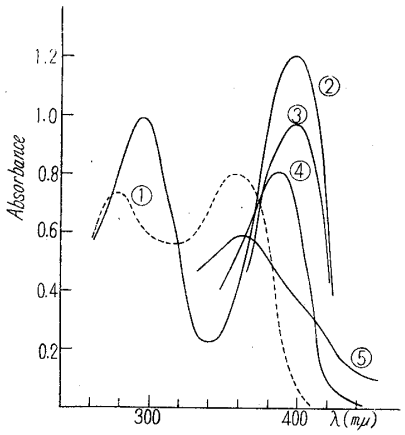


Fig. 4. Achromycin Chelate

① AchrM 25 γ/cc. (pH=4.4)
② Th
③ Zr
④ Al
⑤ UO_2

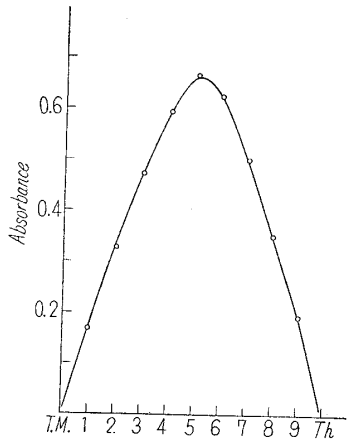


Fig. 5. Molar Ratio of Terramycin-Th Chelate (Continuous variation method)
pH 4.58 λ=395 mμ TM:Th=1:1

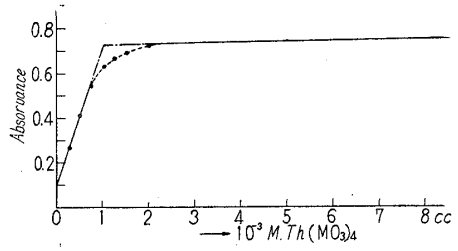


Fig. 6. Molar Ratio of Terramycin-Th Chelate
pH 4.58 λ=395 mμ TM=10^{-3}*M* 1 cc.

Table I. Composition of Aureomycin Metal Chelates (AM : Metal ratio)

	Photometric determination		pH method*
	Continuous variation	Molar ratio method	
Th	1 : 1	1 : 1	1 : 1
Zr	1 : 2	1 : 2	1 : 2
UO_2	1 : 1	1 : 1	1 : 1
Fe	1 : 2	1 : 2	1 : 2
Al	1 : 1	1 : 1	1 : 1
Zn	—	—	1 : 1
Cu	1 : 1	1 : 1 or 1 : 2	1 : 1 or 1 : 2
Co	1 : 1	1 : 1	1 : 1

* pH method means the ratio at maximum drop of pH.

Table II. pH Drop of Aureomycin Chelates

	pH before mix.	pH after mix.	Max. drop of pH	Ratio at max. drop of pH
AM	3.05	2.55	0.75	1 : 1
Th	3.45			
AM	4.10	3.80	0.47	1 : 2
Zr	4.35			
AM	2.80	2.30	0.30	1 : 2
Fe^{III}	2.50			
AM*	3.60	2.90	0.90	1 : 1
Al	4.00			
AM	5.80	4.55	1.40	1 : 1
Zn	6.00			
AM	2.93	2.36	0.45	1 : 1 or 1 : 2
Cu	2.70			
AM	5.95	4.96	1.37	1 : 1
Co	6.02			

$AM=5\times10^{-3}M$ Metal$=5\times10^{-3}M$ * $AM=5\times10^{-4}M$ Al$=5\times10^{-4}M$

Table III. pH Drop of Terramycin Chelates

	pH before mix.	pH after mix.	Max. drop of pH	Ratio of two components
TM	3.40	2.52	0.46	1 : 1
Th	2.98			
TM	3.40	2.68	no	
Zr	2.40			
TM	3.40	2.76	0.06	1 : 1
Cu	2.82			
TM	3.40	2.98	0.42	1 : 1
Al	3.80			
TM	3.40	3.55	no	
Mg	5.12			
TM	3.40	3.58	no	
Ca	5.66			

$TM=5\times10^{-3}M$ Th$=5\times10^{-3}M$

The molar ratio of zirconium chelate of Aureomycin, Anhydroaureomycin, Terramycin, and Achromycin was found to be 1:2 (Figs. 7 and 8), but the molar ratio of tetracycline chelates of thorium to other metals was always 1:1 (Fig. 5). Therefore, it will be noticed that chelation must involve the 10—11 or rather the 11—12 enol system in the case of thorium, but this effect must further involve the 12—1, besides 10—11, in the case of zirconium, supposing A and B rings to be almost in the same plane.

Further experiments on the Anhydroaureomycin chelates will help to explain these

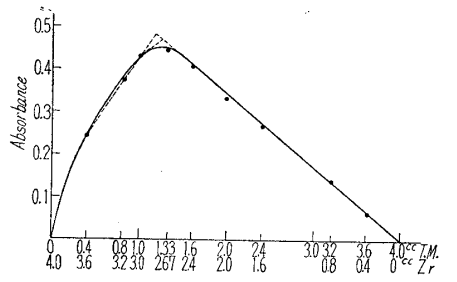


Fig 7. Terramycin-Zr Molar Ratio
(pH 5.0 λ=395)
TM : Zr=1:2

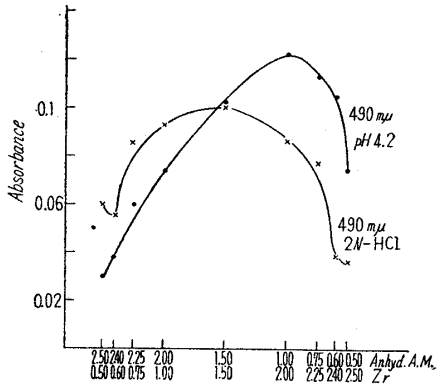
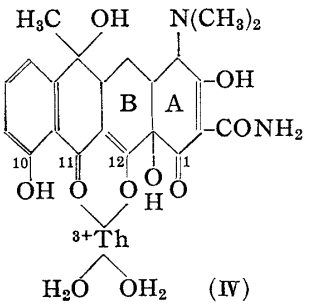
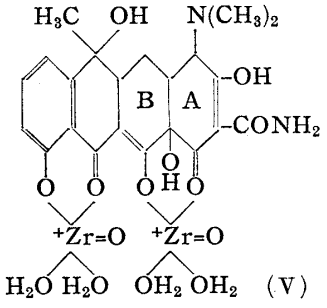


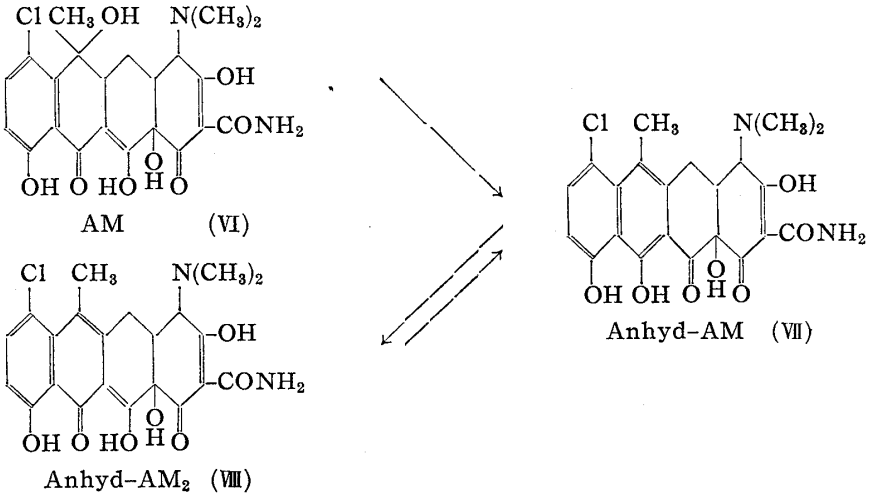
Fig. 8. Anhydroaureomycin-Zr Molar Ratio





circumstances. Anhydroaureomycin (Anhyd-AM) (VII), which is prepared by warming with hydrochloric acid, has only one chelating group. This structure of Anhyd-AM will satisfy the formation of thorium and zirconium chelates with 1:1 molar ratio, but cannot explain the formation of zirconium chelate with 1:2 ratio. It is necessary, therefore, either to consider the isomeric structure (VIII) of Anhydroaureomycin (Anhyd-AM_2) or to accept the chelating ability of the tricarbonylmethane system.

First, we shall consider the isomeric structure of anhydrotetracycline by shifting the hydroxyl group at 11 to 12-position.



According to this assumed isomeric structure, the formation of the various chelates of 1:2 molar ratio can be explained. We also observed that Anhydroaureomycin-zirconium chelate (1:2) produces the 1:1 chelate by further warming with *N* HCl. Therefore, the above reversible equilibrium seems possible.

The remaining problem is to examine the chelating ability of the tricarbonylmethane system (I, 1).

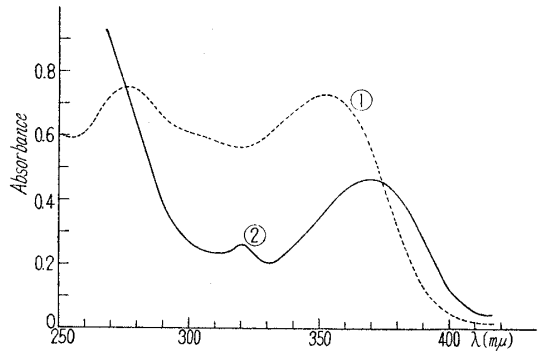


Fig. 9.
Apoterramycin in HCl Solution

① Terramycin 20 γ/cc.
② Apo-TM

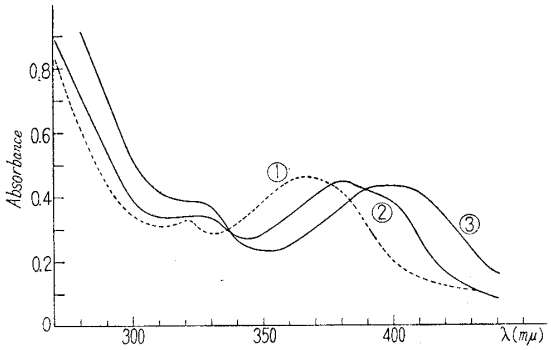


Fig. 10.
① Apo-TM (in HCl)
② Apo-TM (pH 3.9)
③ Apo-TM-Th (pH 4.0)

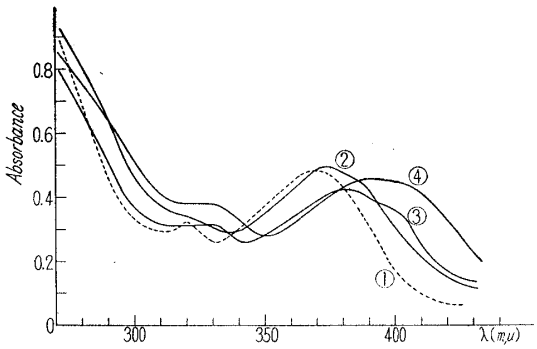
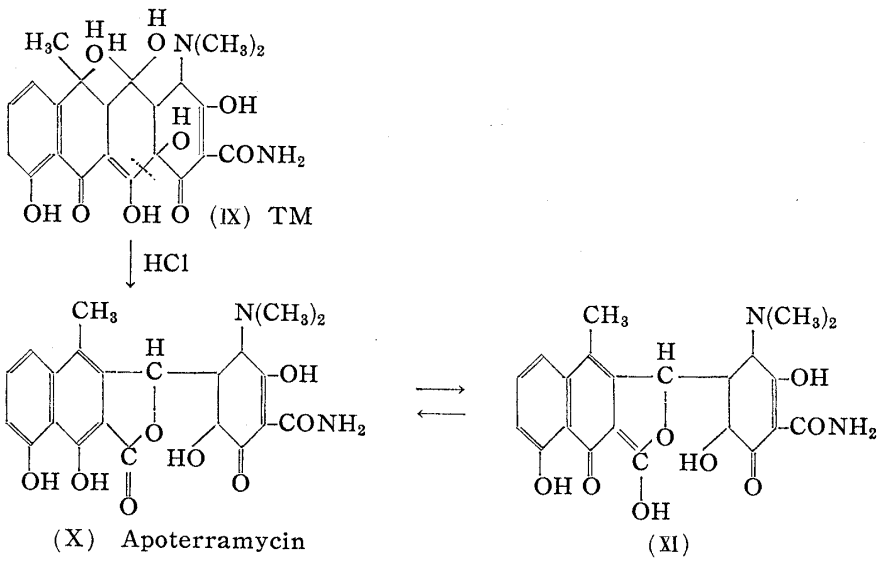


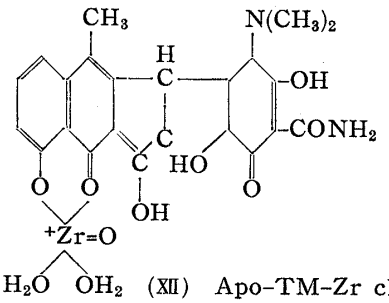
Fig. 11.
① Apo-TM (in HCl)
② Apo-TM-Zr (in HCl)
③ Apo-TM (pH 4.0)
④ Apo-TM-Zr (pH 4.0)



(IX) TM

(X) Apoterramycin

(XI)



(XII) Apo-TM-Zr chelate

Hydrolysis of Terramycin with *N* HCl gives a stable Apoterramycin[5] spectrophotometrically, though Aureomycin and Achromycin give only the anhydro derivatives under similar conditions (Figs. 9 and 10).

Apoterramycin proved to be a very useful derivative in this study which contains the system (1) intact and little changed in other places. We noted, however, that while Apoterramycin underwent chelation with some metals (Th and Zr) it failed to chelate with magnesium. Besides, it was seen that Apoterramycin-zirconium and -thorium chelates were also produced from Terramycin-zirconium and -thorium chelates on warming with *N* HCl (Figs. 10 and 11).

According to Stephens' experiment,[2] Isoaureomycin hydrochloride indicated no complex formation on adding calcium chloride.

The peak of β-diketone chelates of tetracycline is usually within 370~400 mμ as reported in our previous paper.[1] This suggests the importance of chelate formation.

Our experiment on Isoaureomycin showed no chelate formation in the range of 370~400 mμ by thorium, zirconium, and aluminum, so that we may conclude that the chelates* between the tricarbonylmethane group in Isoaureomycin, even if they were formed, are in different wave length from those of phenolic β-diketone group (Figs. 12~16).

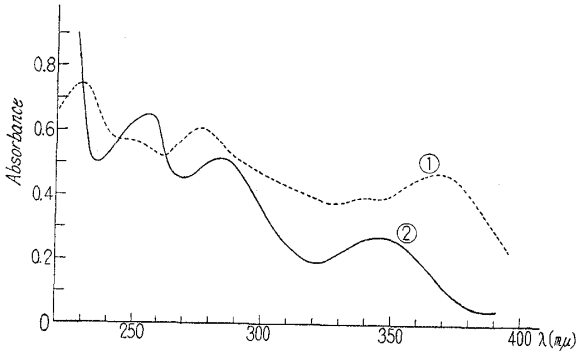


Fig. 12. Isoaureomycin
① Aureomycin 20 γ/cc.
② Isoaureomycin in KOH soln.

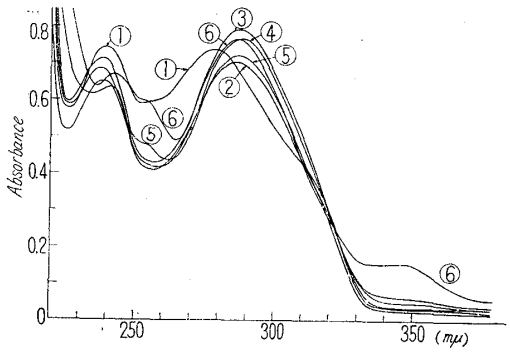


Fig. 13. Isoaureomycin at various pH (0.00005 *M* soln.)
① pH=3.30 ④ pH=5.82
② pH=4.18 ⑤ pH=6.52
③ pH=4.90 ⑥ pH=7.08

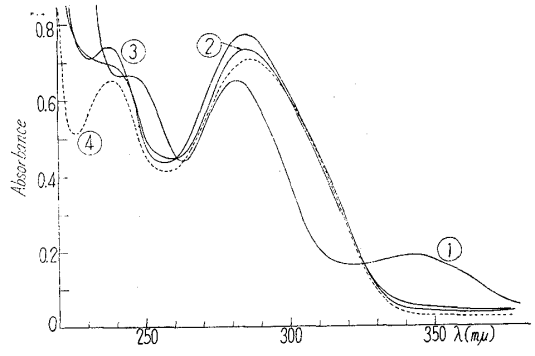


Fig. 14. Isoaureomycin-Metal (1)
① Iso-AM-Th pH=4.20
② Iso-AM-Zr pH=4.36
③ Iso-AM-Cu pH=4.60
④ Iso-AM pH=4.18

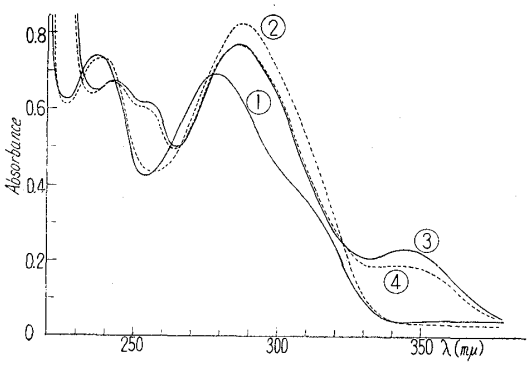


Fig. 15. Isoaureomycin-Metal (2)
① Iso-AM-Al pH=4.90
② Iso-AM pH=4.90
③ Iso-AM-Mg pH=7.12
④ Iso-AM pH=7.08

*) The pH drop was seen in a solution containing Isoaureomycin and Th, Zr, or other metal ions, but most likely it is based on other chelates between the tricarbonylmethane groups (cf. Table VI).

5) F. A. Hochstein, *et al.*: J. Am. Chem. Soc., **74**, 3707(1952).

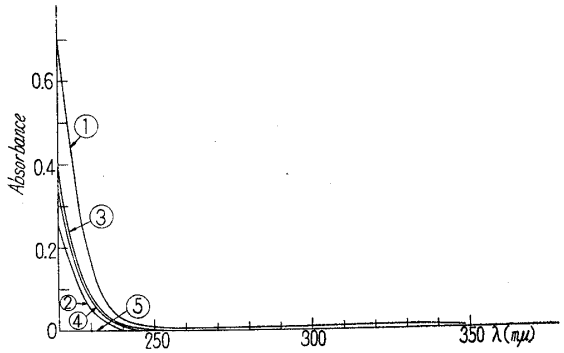
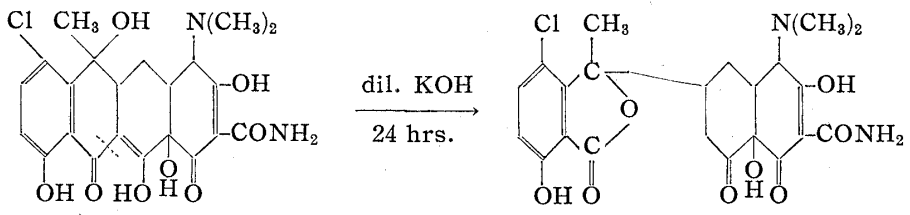


Fig. 16. Absorption Curve of 0.00005*M* Metal Solution

① Th pH=4.08
② Zr pH=4.92
③ Cu pH=5.28
④ Mg pH=7.22
⑤ Al pH=5.20



(VI) Aureomycin (XIII) Isoaureomycin

Thus the chelation of tetracycline involves the phenolic β-diketone group as well as the 12–1 system. Therefore, it may be safe to conclude that the Apoterramycin chelates have the structure shown by (XII).

The authors are greatly indebted to Prof. M. Ishidate of the University of Tokyo for valuable suggestion and encouragements. This study was supported by a Grant in Aid for Scientific Research from the Ministry of Education.

Experimental

The absorption curves of colored chelates of Terramycin and Achromycin with Zr(IV), Th(IV), Al, and other metals are shown in Figs. 1~4, and in Table IV.

Table IV. Absorption Spectra of Terramycin and Achromycin Chelates

1. Terramycin (Fig. 1)				2. Achromycin (Fig. 4)			
Metal	pH	λmax	ε	Metal	pH	λmax	ε
Th	4.6	395	23,300	Th	4.4	395	23,500
Zr	5.0	392	16,750	Zr	4.4	395	18,850
UO_2	5.6	405	10,000	UO_2	4.4	—	—
Al	5.0	372	17,500	Al	4.4	380	15,580
Mg*	no buffer	370	18,750	Mg	4.4	360	12,310
Cu*	5.0	370	17,500 (Fig. 3)				
Co*	no buffer	370	15,000 (Fig. 2)				

* In the case of Mg, Cu, and Co, the final concentration of 10 γ/cc. Terramycin and 0.02 *M* metal solution was used. Because of the ill effect upon chelate formation of those metals, the buffer solution was not added.

The chelate solution was prepared by adding 0.5 cc. of $10^{-2}M$ $Th(NO_3)_4$ to 1 cc. of $10^{-3}M$(500 γ/cc.) Terramycin and this solution was diluted to 25 cc. The final concentration of Terramycin in this chelate solution was $4\times10^{-5}M$ or 20 γ/cc., and the one of $Th(NO_3)_4$ $2\times10^{-4}M$.

The chelate solution of Achromycin was prepared by adding 2 cc. of 1% $Th(NO_3)_4$ to 0.5 cc. of 500 γ/cc. Achromycin-HCl with 1 cc. of acetate buffer, and diluting to 10 cc. with water. The final concentration of Achromycin was 25 γ/cc.

Molar Ratio of Thorium and Terramycin (Fig. 5)—The method of continuous variations proposed by Job was tried at 395 mμ and at pH 4.58. The results are summarized in Table V. Each solution was finally made up to 25 cc. with water after adding 5 cc. of acetate buffer. The blank is a solution containing 1 cc. of $10^{-3}M$ TM-HCl and 5 cc. of acetate buffer and made up to 25 cc. with water.

Molar Ratio Method for Thorium and Terramycin (Fig. 6)—One cc. of $10^{-3}M$ $Th(NO_3)_4$ in Fig. 6 was added to 1 cc. of $10^{-3}M$ Terramycin. This solution was made up to 25 cc. with water after adding 5 cc. of acetate buffer. The pH of this final solution was 4.58. The molar ratio of Ter-

Table V.

No. of Tubes	1	2	3	4	5	6	7	8	9
$10^{-3}M$ TM·HCl	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.225
$10^{-3}M$ Th	2.25	2.00	1.75	1.50	1.25	1.00	0.75	0.50	0.25
Absorbance of solution	0.196	0.393	0.522	0.667	0.755	0.740	0.635	0.497	0.354
Absorbance of blank	0.031	0.051	0.067	0.084	0.100	0.115	0.110	0.144	0.159
Absorbance 395*	0.165	0.342	0.455	0.583	0.655	0.625	0.505	0.353	0.195

* Corrected absorbance at 395 mμ.

ramycin to Th is 1:1.

Molar Ratio of Zirconium and Terramycin (Continuous Variation Method) at pH 5.0 (Fig. 7)—The solution was prepared by adding 5 cc. of acetate buffer and dil NaOH, and making up to 25 cc. with water. The molar ratio of Terramycin to Zr is 1 : 2.*

Molar ratio of Zr to Anhydroaureomycin (Fig. 8)—$10^{-3}M$ Anhydroaureomycin and $10^{-3}M$ Zr were used. Anhydroaureomycin was prepared as previously reported. Its hydrochloride has m.p. 223° (decomp.) and showed absorption maximum at 450 mμ.

Each solution was finally made up to 20 cc. with water after adding 10 cc. of $4N$ HCl. In Fig. 8, 490 mμ $2N$ HCl-curve is the one of this solution after warming and 490 mμ, pH 4.2-curve is the curve from a solution with acetate buffer instead of $4N$ HCl.

Therefore, the molar ratio of Anhydroaureomycin to Zr is 1:2 by optimal pH but 1:1 after warming with dil HCl.

Apoterramycin—Apoterramycin was prepared as follows : To 1 cc. of the solution of 500 γ/cc. Terramycin 1 cc. of $2N$ HCl (the total concentration of HCl is $1N$) was added and the resultant solution was warmed in water bath for 10 mins. at 80°. After warming, it was cooled and diluted to 25 cc. with water (it contained 20 γ/cc. as Terramycin) (Fig. 9). Apoterramycin showed maximum absorbancy at 380 mμ at pH 4.0 (Fig. 10).

To 25 cc. of Apoterramycin solution mentioned above 0.5 cc. of $0.01M$ metal solution was added in excess and pH was adjusted to about 4.0. The absorption curve was measured. It showed maximum absorbancy at 395 mμ, indicating formation of Apoterramycin-Th chelate (Fig. 10). In the same way Apoterramycin-Zr chelate showed maximum absorbancy at 395 mμ at pH 4.0 (Fig. 11).

Isoaureomycin—Isoaureomycin was prepared from Aureomycin with dil. KOH solution as follows : To 10 cc. of $0.02M$ Aureomycin solution was added 10 cc. of $0.4N$ KOH (total concentration of KOH is about $0.2N$). This solution was maintained for 24 hrs. when it showed fluorescence (Fig. 12). After careful buffering of this solution with dil. HCl, it was diluted to 40 cc. Thus, $0.005M$ Isoaureomycin solution was obtained in the proper pH. Figs. 12 and 13 show the curves of Isoaureomycin in KOH solution at various pH.

The pH drop was measured in a solution containing $0.005M$ Isoaureomycin and $0.005M$ metal ion (Table VI). The curve of Isoaureomycin and metal was measured with a solution containing 0.2 cc. each of $0.005M$ Isoaureomycin and $0.005M$ metal ion which was finally diluted to 20 cc., adjusting its pH (Fig. 14~15). Fig. 16 shows the blank of metal ions alone. These curves showed no maximum absorption in the range of 370 and 400 mμ. Table VI, however, showed that some metal ions such as Th, Cu, and Al can form chelates far different from those of phenolic β-diketone group.

Table VI. pH-Drop of Isoaureomycin Chelates

	pH before mix.	pH after mix.	Drop of pH	Ratio of two components
Iso-AM	2.95	2.50	0.42	2.5 : 1.5
Th	2.92			
Iso-AM	2.95	2.54	no	
Zr	2.45			
Iso-AM	2.95	2.60	0.18	1 : 1
Cu	2.78			
Iso-AM	3.98	3.45	0.33	2.5 : 1.5
Al	3.78			
Iso-AM	3.98	6.02	no	
Mg	5.98			
Iso-AM	3.98	6.15	no	
Ca	5.84			

* According to our previous report the molar ratio of Aureomycin to Zr was also 1 : 2 (This Bulletin, **3**, 147(1955)).

Summary

Terramycin and Achromycin form chelate compounds with various metals, especially stable compounds with zirconium and thorium, as previously reported with Aureomycin. Their molar ratio is 1:1 in thorium chelates and 1:2 in zirconium chelates. Further investigations on the chelate formation of Apoterramycin and Isoaureomycin showed that phenolic β-diketone in tetracycline structure was very important as the chelating group, as was indicated in the previous paper. It is also suggested that the chelation of tetracylines must further involve the 12-1 bond.

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2. Koiti Kimura and Akira Tanaka : Anodic Synthesis of Fatty Acids. II.[1] The Syntheses of 3,3-Dimethylated Branched Acids.

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There have been numerous data concerning the properties of pure synthetic branched acids, but those of 3,3-dimethylated branched acids have not been reported as a series.

Table I.

	$RCH_2C(CH_3)_2CH_2COOH$					m.p. (°C)	
Compd. No.	R	Formula	b.p. or m.p. (°C)	d_{25}	n_D^{25}	S-Benzylthiuronium salt	*p*-Bromophenacyl ester
(I)	CH_3	$C_7H_{14}O_2$	b.p. 203~204[a]	0.9348	1.4280	162.0	54.8
(II)	C_2H_5	$C_8H_{16}O_2$	b.p. 208~210	0.9009	1.4278	144.0	66.2
(III)	C_3H_7	$C_9H_{18}O_2$	b.p. 217~218[b]	0.9102[b]	1.4329[b]	136.0	
(IV)	C_4H_9	$C_{10}H_{20}O_2$	b.p. 242~244	0.9129	1.4346	123.0	
(V)	C_5H_{11}	$C_{11}H_{22}O_2$	$b.p_5$ 130~133	0.8995	1.4370	131.0	
(VI)	C_6H_{13}	$C_{12}H_{24}O_2$	$b.b_3$ 124~125	0.8954	1.4398	124.5	
(VII)	C_7H_{15}	$C_{13}H_{26}O_2$	$b.p_7$ 154~157	0.8827	1.4415	125.5	42.0
(VIII)	C_8H_{17}	$C_{14}H_{28}O_2$	$b.p_3$ 141~145	0.8841	1.4420	118.0	
(IX)	C_9H_{19}	$C_{15}H_{30}O_2$	$b.p_3$ 157~158	0.8892	1.4450	126.5	41.5
(X)	$C_{10}H_{21}$	$C_{16}H_{32}O_2$	$b.p_4$ 162~164[c]		n_D^{30} 1.4469	119.0	
(XI)	$C_{11}H_{23}$	$C_{17}H_{34}O_2$	$b.p_4$ 186~189 (m.p. 29.0)			126.0	50.5
(XII)	$C_{12}H_{25}$	$C_{18}H_{36}O_2$	$b.p_2$ 164~166 (m.p. 33.0)			123.5	47.5
(XIII)	$C_{13}H_{27}$	$C_{19}H_{38}O_2$	m.p. 40.5~41.0			123.5	54.0
(XIV)	$C_{14}H_{29}$	$C_{20}H_{40}O_2$	m.p. 44.0~44.8[d]			121.2	58.5
(XV)	$C_{15}H_{31}$	$C_{21}H_{42}O_2$	m.p. 47.5~48.0			121.3	62.0
(XVI)	$C_{16}H_{33}$	$C_{22}H_{44}O_2$	m.p. 53.5~54.0			121.5	60.5
(XVII)	$C_{17}H_{35}$	$C_{23}H_{46}O_2$	m.p. 57.0~57.3			120.0	66.0

All melting points are not corrected.

a) b.p. 209~210° by A. W. Crossley, W. H. Perkin, Jr. (J. Chem. Soc., **73**, 18, 35(1898)); $b.p_{778}$ 213°, anilide, m.p. 105.5~106.0° by N. L. Drake, G. W. Kline, W. G. Rose (J. Am. Chem. Soc., **56**, 2078(1934)); b.p. 201~202° by L. Schmerling (*Ibid.*, **67**, 1154(1945)).

b) $b.p_{18}$ 133~134°, d_{25} 0.9059, n_D^{25} 1.4319, by F. S. Prout (J. Am. Chem. Soc., **76**, 1913 (1954)).

c) $b.p_{10}$ 195°, m.p. 15°, by A. G. Birch, R. Robinson (J. Chem. Soc., **1942**, 494); m.p. 21 ~22.5° by G. Gustbée, E. Stenhagen (Chem. Zentr., **1943**, 1769).

d) m.p. 44.0~44.8° by J. Cason, *et al.* (J. Org. Chem., **15**, 855(1950)).

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1) Part I : Yakugaku Zasshi, **76**, 960(1956).