22. **Akira Miyake**: Studies on Antibiotics. II†. Acidomycin. (2). Antitubercular Activity of Compounds Related to Acidomycin.

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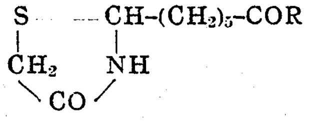
In the preceding paper1), the isolation and chemical structure of acidomycin were presented. Although this antibiotic exhibits specific high activity against human and avian types of *Mycobacterium tuberculosis*, *in vitro*, it shows no activity against the same bacilli *in vivo*. The antitubercular activity of its related compounds has never been reported**. In order to establish the relationship between the chemical structure and antibiotic activity of acidomycin and to obtain *in vivo* antitubercularly active compounds, some of its related compounds were synthesized. Although this work is not yet finished, some data which have so far been obtained are reported herein.

First of all, derivatives of the carboxyl group in acidomycin, (−)2-(5-carboxypentyl)-4-thiazolidone, were taken up as the subject of the study. They are listed in Table I. Some of them show neutral or basic characters and different solubilities to various solvents. Most of them have antitubercular activity *in vitro*. The activity *in vivo* is now under examination and the results of only two derivatives, both negative data, are in.

In order to test whether the antitubercular activity has a connection with the carbonyl group in acidomycin, it was reduced to a primary alcohol group (XII) and a methyl group

TABLE I



	R	m.p.	[α]	Toxity LD$_{50}$	Antitubercular activity *in vitro*b) (dil. unit)	Antitubercular activity *in vivo*c)
(I)	ONa	195~196°	$[\alpha]_D^{19}$ −31.9° ±1.4° (H_2O)	22 mg/. 10g.a)	1000~3000 u./mg.	not active
(II)	OCH_3	54.5~55.5°	$[\alpha]_D^{19}$ −42.68° ±1.5° (MeOH)		1000~2000 u./mg.	
(III)	OC_2H_5	49°	$[\alpha]_D^{23}$ −35.0° (MeOH)	11.2mg./ 10g.	1000~2000 u./mg.	
(IV)	$NHNH_2$	122~123°	$[\alpha]_D^{13}$ −43.4° (MeOH)	6 mg./ 10g.	800~2000 u/mg.	not active
(V)	$NHN{=}C(CH_3)_2$	152~153°	$[\alpha]_D^{19}$ −20° (MeOH)		800~1500 u./mg.	"
(VI)	$NHN{=}C_6H_{12}O_5$ (glucosyl)	120~123°	$[\alpha]_D^{18}$ +13.5° ±3.4° (MeOH)		500~1000 u./mg.	
(VII)	$NHN{=}C_6H_{12}O_5$ (fructosyl)	136~137°	$[\alpha]_D^{18}$ −27.5° ±2.0° (MeOH)		500~1000 u./mg.	

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† Sueo Tatsuoka: Studies on Antibiotics. II.

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** Recently it has been learned that some of compounds presented in this paper had been reported by R. K. Clark, Jr. and J. R. Schenck: Arch. Biochem. Biophys., **40**, 270 (1952).

1) Part I: This Bulletin, 1, 84 (1953).

(VIII)	NH_2	154.5~155.5°	$[\alpha]_D^{31}$ +10.1° (EtOH)	1500u./mg. (1 : 250, 000)[c]
(IX)	$NHCH_2COOC_2H_5$	108~111°	$[\alpha]_D^{19}$ −18.7° ±3.1° (MeOH)	±0.u/mg.
(X)	$NHNHSO_2C_6H_4NH(COCH_3)$	219~221°	$[\alpha]_D^{19}$ +15.6° ±7.8° (MeOH)	200~500 u./mg.
(XI)	$NH-C(NH_2)=NH$	130~131°	$[\alpha]_D^{19}$ +8.8° ±1.5° (MeOH)	1500u./mg. (1 : 250, 000)[c]

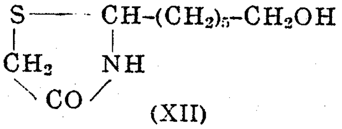
[a] When injected intravenously to mice. When it is injected subcutaneously, LD_{50}=200 mg./10 g.

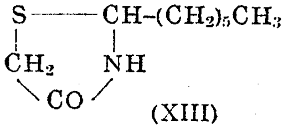
[b] Tested against avian type.

[c] Tested against human type.

TABLE II

Substance	m. p.	$[\alpha]_D$	Antitubercular activity *in vitro* Avian type	Antitubercular activity *in vitro* Human type (H 37 Rv)	*in vivo*
(XII)	78~79°	0	700~1500u./mg.		
(XIII)	43~44°	0	0		
(XIV)	b.p.$_{0.6}$175~177°	0	0		
(XV)	>200°		0		
(XVI)	87~ 88°	0	0		
(XVII)	131~132°	0	0		
(XVIII)	129~130°	0	0		
(XIX)	152~153°	0	0	1 : 50,000	
(XX)	149~150°	0	20u./mg.		
(XXI)	110~112°	0	0		
(XXII)	102°		0		
(XXIII)	181.5°		80u./mg.		
(XXIV)	208~209°	0	0		
(XXV)	143°(decomp.)		0		


(XII)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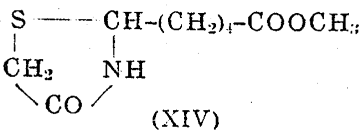
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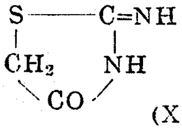
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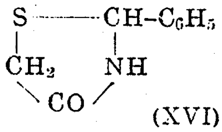
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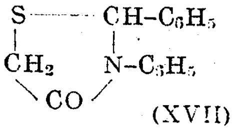
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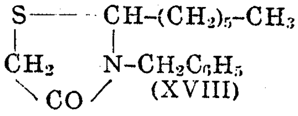
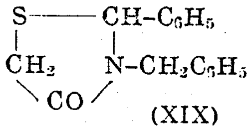

(XVIII)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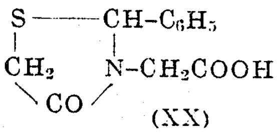
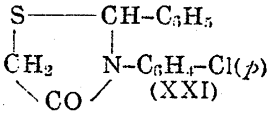

(XX)

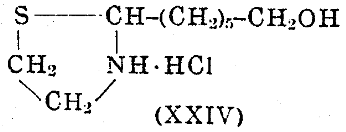

(XXI)

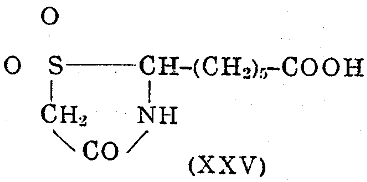
$HOOC-(CH_2)_5-COOH$

(XXII)

$H_2NNHCO-(CH_2)_5-CONHNH_2$

(XXIII)


(XXIV)


(XXV)

(XIII). In (XII) it is reasonable to suppose that the primary alcohol group is easily oxidized to a carbonyl group. (XII) has *in vitro* activity but not (XIII).

Recently, it was found in a test for several bacilli that biotin was antagonistic to acidomycin. ω-Carboxypentyl group is one of the common structures in both compounds. To remove the antagonistic effect of biotin, variations were made at the ω-carboxypentyl group of acidomycin (XIV～XVI in Table II), but none of the resulting products showed activity *in vitro*.

To test whether the activity is attributable to the imido group in acidomycin, the hydrogen atom in the imido group was exchanged for other radicals. In this instance, the side-chain in 2-position of the thiazolidone ring was also replaced with other groups (XVII～XXI in Table II). These derivatives showed no activity *in vitro*.

Pimelic acid and its dihydrazide, which was assumed to have a connection with the side-chain in 2-position of the thiazolidone ring, were also tested, but they showed no activity *in vitro*.

Finally, to find out the contribution of the carbonyl group in the thiazolidone ring to the activity of acidomycin, the biologically active thiazolidone (XII) was reduced to thiazolidine (XXIV). (XXIV) has no activity *in vitro*. The activity of acidomycin sulfone, which was recently reported by Solomons[2], was reëxamined, but it was found that this derivative had no activity *in vitro*.

Recently, it has been reported by Kawashima[3] that acidomycin antagonizes biotin *in vitro*. It is, therefore, interesting to know whether (XII), which is active against both human and avian types *in vitro*, also antagonizes biotin *in vitro*. Kawashima carried out the experiment concerning this point and confirmed the antagonism between (XII) and biotin *in vitro*. The details of these experiments are published elsewhere.

Discussion

The antitubercular activity of 25 compounds related to acidomycin are tested *in vitro*, 2 of them, *in vivo*. As far as *in vitro* activity is concerned, it is found that: 1) The derivatives of the carboxyl group in the side-chain attached to the 2-position of the thiazolidone ring are active; 2) the alcohol derivative, which is obtained by the reduction of the carboxyl group in the side-chain, has still some antitubercular activity, but when $-CH_2OH$ group is further reduced to a methyl group the activity disappeares; 3) in the same side-chain the straight, 5 methylenic linkage is essential to the activity; 4) but this side-chain itself has no activity; 5) the related compounds in which modifications were made at 2- and 3-positions of the thiazolidone ring have no activity; and 6) the carbonyl group at 4-position and the bivalent sulfur atom at 1-position of the thiazolidone ring are both essential to the activity.

The author wishes to express his thanks to Dr. S. Kuwada for his continuous advice throughout the course of this work, to Dr. M. Hori for taking charge of microbiological experiments, to Messrs. M. Honjo, H. Hitomi, J. Ueyanagi, M. Miyamoto, T. Kinoshita, T. Takewaka, and S. Kimata for their helpful suggestions and assistances, to Mr. K. Ogawa for the biological assay, to Mr. I. Ishikawa for examination of toxicity, to Mr. T. Ito for the physical data recorded herein, to Misses F. Suzuki and Y. Kobayashi, and to Messrs. M. Kan, H. Kashiwagi, and T. Nakata for the microanalyses.

Experimental

Acidomycin sodium salt (**I**)—Ten g. of acidomycin is suspended in 50 cc. of water, and under stirring, 20% NaOH solution is added dropwise to adjust the solution to pH 6.6～6.8 (excess NaOH tends to induce racemization). After filtering the insoluble material, the filtrate is allowed to cool. To the cold solution, 1000 cc. of dry acetone is added under stirring and kept standing for 2 hours in a refrigerator. The amorphous precipitate thus formed is filtered and dried at a room tem-

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[2] I. A. Solomons, *et al.*: J. Am. Chem. Soc., 74, 2946 (1952).

[3] Minoru Kawashima: Studies on Acidomycin. VI.: This Bulletin, 1, 93 (1953).

perature, under a reduced pressure to m.p. 195∼196°; yield, 90∼95%. *Anal.* Calcd. for $C_9H_{14}O_3NSNa \cdot 2\frac{1}{2}H_2O$: C, 38.02; H, 6.74; N, 4.93; S, 11.28; Na, 8.09; H_2O, 15.84. Found: C, 37.64; H, 7.12; N, 4.81; S, 11.14; Na, 8.27; H_2O, 16.22.

Acidomycin methyl ester (II)— The method of preparation is described in the preceding paper1).

Acidomycin ethyl ester (III)— Prepared by the same method as for the methyl ester. Colorless needles, m.p. 49°. Yield, 60∼70%. *Anal.* Calcd. for $C_{11}H_{19}O_3NS$: $-OC_2H_5$, 18.36. Found: $-OC_2H_5$, 18.21.

Acidomycin Hydrazide (IV) —A mixture of acidomycin methyl ester and a slight excess of hydrazine hydrate is warmed at 60° for 4 hours and allowed to stand over H_2SO_4 in a desiccator. Light yellow crystalline mass is obtained. Recrystallization from 100% methanol gives colorless plates, m.p. 122∼123°; yield, 50%. It is easily soluble in water and alcohols, and sparingly soluble in ether and benzene. *Anal.* Calcd. for $C_9H_{17}O_2N_3S$: C, 47.74; H, 7.41; N, 18.17. Found: C, 46.75; H, 6.70; N, 18.09.

Isopropylidene Derivative (V) of Acidomycin Hydrazide—Acidomycin hydrazide is dissolved in an excess of acetone, whereupon fine crystals soon begin to deposit. Recrystallization from methanol gives colorless fine crystals, m.p. 152∼153°; yield, 90∼95%. *Anal.* Calcd. for $C_{12}H_{21}O_2N_3S$: C, 53.11; H, 7.80; N, 15.94. Found: C, 53.11; H, 7.49; N, 15.13.

***d*-Glucosyl Derivative (VI) of Acidomycin Hydrazide**—One and one-fifth g. of acidomycin hydrazide and 0.9 g. *d*-glucose are heated at 65° in 30 cc. of 100% methanol. The solid soon dissolves. After heating for 2 hours, the methanol is evaporated. Addition of dry ether gives gummy substance. Recrystallization from ether-methanol (99.5 : 0.5) gives colorless hygroscopic crystalline mass, m.p. 120∼123°; yield, 90%. *Anal.* Calcd. for $C_{15}H_{27}O_7N_3S$: C, 43.57; H, 6.58. Found: C, 43.19; H, 6.35.

Fructosyl Derivative (VII) of Acidomycin Hydrazide— Prepared by the method similar to that used for glucosyl derivative of acidomycin hydrazide. Colorless and hygroscopic crystalline mass, m.p. 136∼137°; yield, 90%. *Anal.* Calcd. for $C_{15}H_{27}O_7N_3S$: C, 43.57; H, 6.58. Found: C, 42.92; H, 6.56.

Acidomycin Amide (VIII)—To a suspension of acidomycin ethyl ester in 10 times its volume of 28% ammonia, methanol saturated with ammonia is added dropwise till all the ester has dissolved and the solution is allowed to stand over night in a corked vessel. Evaporation of the solvent gives colorless mass. Recrystallization from water or dilute methanol gives colorless needles, m.p. 154.5∼155.5°; yield, 60∼70%. *Anal.* Calcd. for $C_9H_{16}O_2N_2S$: C, 49.97; H, 7.46; N, 12.95. Found: C, 50.11; H, 4.43; N, 13.18.

Glycyl Derivative (IX) of Acidomycin Hydrazide— Acidomycin azide is prepared from acidomycin hydrazide by the usual method. To an ether solution of this azide, equimolar glycine ethyl ester in ether is added and kept standing for 24 hours at a room temperature. Evaporation of the ether gives colorless needles. Recrystallization from ether gives needles, m.p. 109∼111°. *Anal.* Calcd. for $C_{13}H_{22}O_4N_2S$: C, 51.64; H, 7.34; N, 9.21. Found: C, 51.51; H, 7.46; N, 9.24.

***p*-Acetaminobenzenesulfonyl Derivative (X) of Acidomycin Hydrazide**—Seven-tenths g. of acidomycin hydrazide is dissolved in 3.2 cc. of pyridine, and to the solution 0.7 g. of *p*-acetoaminobenzenesulfochloride is added, little by little. After the completion of addition the mixture is kept to stand for 1.5 hours and poured into ice water. The gummy reaction product is recrystallized from methanol to m.p. 219∼221°; yield, 90%. *Anal.* Calcd. for $C_{17}H_{24}O_5N_4S_2$: C, 47.64; H, 5.65; N, 13.08; S, 14.97. Found: C, 47.64; H, 5.84; N, 13.57; S, 15.04.

Guanidine Derivative (XI) of Acidomycin—To 9 cc. of absolute ethanol, 0.36 g. of Na, and 1.5 g. of guanidine carbonate are added. The solution is stirred for 2 hours and filtered. To the filtrate 9 cc. of water and 3 g. of acidomycin methyl ester are added and warmed at 80° for 10 minutes. After leaving over night at a room temperature, the solvent is evaporated to dryness. Recrystallization from ethanol gives 3.2 g. of colorless plates, m.p. 130∼132°. It is soluble in ethanol and benzene, and insoluble in ether. *Anal.* Calcd. for $C_{10}H_{18}O_2N_4S \cdot 1\frac{1}{2}H_2O$: C, 42.09; H, 7.42; N, 19.63. Found: C, 42.44; H, 7.71; N, 19.79.

2-(6-Hydroxyhexyl)-4-thiazolidone (XII) — The method of preparation is descrided in the preceding paper1).

2-Hexyl-4-thiazolidone (XIII) — The method of preparation is described in the preceding paper1).

2-(4-Carbomethoxybutyl)-4-thiazolidone (XIV)—This compound is prepared according to the method of Solomons2).

2-Imido-4-thiazolidone (XV) — Prepared according to Mulder4), from monochloroacetic acid and thiourea.

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4) Mulder: Ber., 8, 1264.

2-Phenyl-4-thiazolidone (XVI)—Prepared according to Davis, *et al.*[5] from thioglycollic acid amide and benzaldehyde. m.p. 87~88°.

2, 3-Diphenyl-4-thiazolidone (XVII)—Prepared according to Surrey[6], from benzaldehyde, aniline, and thioglycollic acid. m.p. 131~132°.

2-Hexyl-3-benzyl-4-thiazolidone (XVIII)—A mixture of 1.7 g. of oenanthaldehyde, 1.6 g. of benzylamine, and 1.5 g. of thioglycollic acid is condensed by refluxing in 20 cc. of dry benzene for 2 hours, and the water is removed continuously by a separator. Evaporation of benzene gives colorless needles, m.p. 129~130°; yield, 0.7 g.

2-Phenyl-3-benzyl-4-thiazolidone (XIX)—Prepared according to Troutman, *et al.*[7] from N-benzylidenebenzylamine and ethyl thioglycollate. m.p. 152~153°.

2-Phenyl-3-carboxymethyl-4-thiazolidone (XX)—A mixture of 0.7 g. of sodium salt of N-benzylideneglycine and 0.45 g. of ethyl thioglycollate in ethanol is refluxed for 4 hours. After evaporating the solvent, a small amount of water is added and from the aqueous solution the unreacted ethyl thioglycollate is extracted with ether. The aqueous solution is then acidified and extracted with ether. Evaporation of the extract gives 0.4 g. of crystals of m.p. 149~150°; yield, 46%.

2-Phenyl-3-*p*-chlorophenyl-4-thiazolidone (XXI)—Prepared according to Surrey[3] from benzaldehyde, *p*-chloroaniline, and thioglycollic acid.

2-(6-Hydroxyhexyl)-thiazolidine Hydrochloride (XXIV)—The method of preparation is described in the preceding paper[1].

Acidomycin sulfone (XXV)—One-half g. of acidomycin is dissolved in 5 cc. of acetic acid, 1 cc. of 30% H_2O_2 is added, and the solution is allowed to stand over night. Evaporation of the acetic acid followed by recrystallization of the residue from hot water gives colorless needles, m.p. 143° (decomp.).

Summary

Twenty-five compounds related to acidomycin were tested *in vitro*, two of them *in vivo*, against human and avian type tubercle bacilli. As a result, some of the relationships between the chemical structure and antibiotic activity of acidomycin are discussed.

(Received January 9, 1953)

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5) W. Davis, *et al.*: J. Chem. Soc., 1949, 2636.
6) A. R. Surrey: J. Am. Chem. Soc., 69, 2911 (1947).
7) H. D. Troutman, *et al.*: J. Am. Chem. Soc., 70, 3436 (1948).