Summary

The coloring matter of naphthoresorcinol reaction with glucuronic acid was shown to consist of two main dyes, one of which was isolated by the present authors. The new dye was supposed to change to the Ogata and Nozaki's dye at a higher temperature or by a longer reaction time by hydrochloric acid. The newly presented structure of the dyes may explain all the analytical data and the reaction mechanism.

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13. Minoru Kawashima, Akira Miyake, Teruji Hemmi, and Shizuko Fujii : Biochemical Studies on Acidomycin. III. Antibiotin Activity of Acidomycin and Its Related Compounds.*

(*Research Laboratory, Takeda Pharmaceutical Industries, Ltd.***)

Recently, it was reported that acidomycin, 2-(5-carboxypentyl)-4-thiazolidone, was antagonistic to biotin on cultures of *Mycobacterium tuberculosis typus avium* and several lactic acid bacteria.[1] Miyake previously announced the antitubercular activity of various compounds related to acidomycin and discussed the relationship between their antitubercular activity and chemical structures.[2]

As necessary prerequisites for the compounds to show antitubercular activity he revealed that; (1) the side-chain attached to the 2-position of the thiazolidone ring must contain a carboxyl or a group derivable from carbonyl, (2) the alcohol derivatives obtained by the reduction of the carboxyl still have some antitubercular activity, but when the $-CH_2OH$ group is further reduced to a methyl group the activity disappears, (3) the side-chain must be a straight pentamethylene chain, (4) the side-chain is not a sole factor of the activity, (5) modifications at 2- and 3-positions of the thiazolidone ring cause disappearance of the activity, and (6) the carbonyl group at 4-position as well as the bivalent sulfur at 1-position is essential.

This report describes experiments designed to obtain some insight into the relationship between the chemical structures, antitubercular activity, and properties antagonistic to biotin of these compounds.

Material and Methods

The microörganism employed in these experiments was *Mycobacterium tuberculosis typus avium*. This was regularly maintained in the form of slant cultures containing 1% meat extract, 1%

Table I. Composition of the Basal Medium

Na_2HPO_4	3.0 g.
KH_2PO_4	4.0 〃
$MgSO_4$	0.6 〃
Sodium citrate	2.5 〃
Asparagine	5.0 〃
Glycerol	20.0 〃
Tween 80 (in 25% solution)	20.0 cc.
Distilled water	1,000.0 〃 (pH 7.0)

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** Juso-Nishinocho, Higashiyodogawa-ku, Osaka (川島 実, 三宅 彰, 逸見昭二, 藤井シヅ子).

1) M. Kawashima, Y. Hamada, S. Fujii : This Bulletin, **1**, 94 (1953).
2) A. Miyake : *Ibid.*, **1**, 89 (1953).

peptone, 0.5% NaCl, 10% glycerol, and 1.5% agar. Inoculum for studies was prepared by transferring the culture from the agar slant directly into 5 cc. of sterilized basal medium. The culture was incubated at 37° for 48 hours.

The basal medium employed is given in Table I.

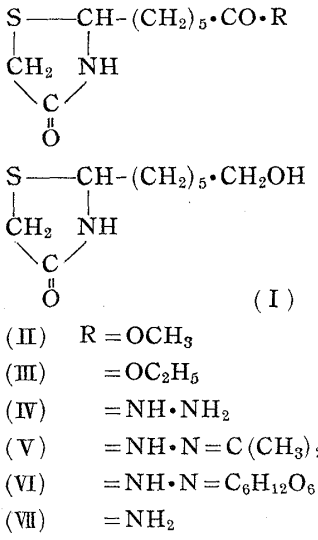
Procedures :

i) Determination of Antitubercular activity : Five milligrams of acidomycin sodium or its related compounds were added to 5 cc. of the basal medium in the initial tube, and then diluted by consecutive 5-fold dilution. The dilution series were sterilized by autoclaving at 15 lbs. for 10 mins. and, after inoculation with *M. tuberculosis typus avium* (48-hr. culture), incubated at 37° for 48 hrs. and the resulting turbidity was measured electrophotometrically with Coleman-Junior spectrophotometer at 650 mμ.

ii) Determination of Antibiotin activity : Bacterial growth in the basal medium was inhibited by the addition of 10 γ/cc. of acidomycin sodium or its related compounds having antitubercular activity. One cc. of a basal medium containing 10 γ/cc. of biotin was added to 4 cc. of the above medium in the initial tube and consecutively diluted by 5-fold dilution as described above. The resulting turbidity was measured as in i).

Samples tested are given in Chart 1, and it was previously reported by Miyake[2] that compounds (I) to (VIII) had antitubercular activity whereas compounds (IX), (X), and (XI) were not effective toward tubercle bacilli.

CHART 1.



(I)

(II) R = OCH_3

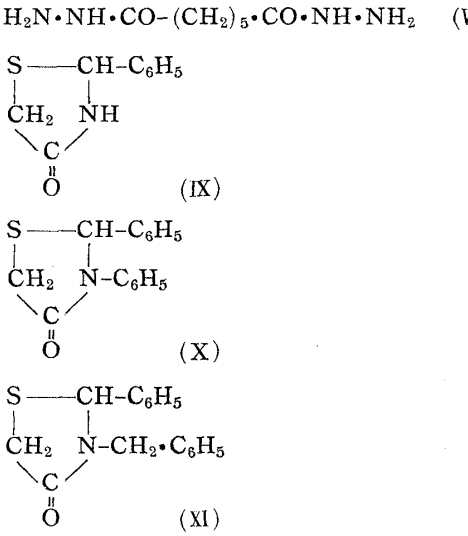
(III) = OC_2H_5

(IV) = $NH \cdot NH_2$

(V) = $NH \cdot N = C(CH_3)_2$

(VI) = $NH \cdot N = C_6H_{12}O_6$

(VII) = NH_2



(VIII)

(IX)

(X)

(XI)

Results and Discussion

Antitubercular activities of various compounds related to acidomycin are given in Table II. It was observed that compounds (I) to (VIII) also showed antitubercular activity under the present experimental conditions. They inhibited the growth of avian type tubercle bacilli at a dilution of 0.32~1.6 γ/cc., while compounds (IX) to (XI) affeccted the growth slightly.

TABLE II. Antitubercular Activity of Acidomycin or its Related Compounds

Compd. / γ/cc.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	INAH
1,000	−	−	−	−	−	−	−	−	−	−	−	−
200	−	−	−	−	−	−	−	−	−	−	−	−
40	−	−	−	−	−	−	−	−	+	+	+	−
8	−	−	−	−	−	−	−	−	+	+	+	−
1.6	−	−	−	−	−	−	−	−	+	+	+	−
0.32	−	−	−	−	±	±	−	±	+	+	+	−
0.064	±	±	−	+	+	±	+	+	+	+	+	+
0.013	+	+	±	+	+	+	+	+	+	+	+	+
0.0036	+	+	+	+	+	+	+	+	+	+	+	+
0.0007	+	+	+	+	+	+	+	+	+	+	+	+

* 37°, 48-hr. incubation.

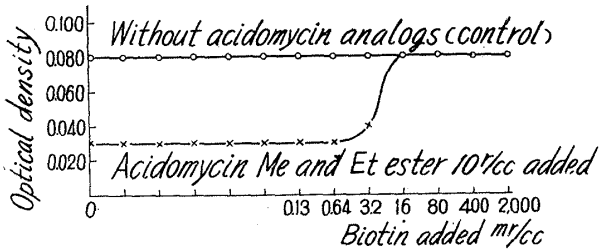


Fig. 1. *M. tuberculosis typus avium*, 37°, 48 hrs.

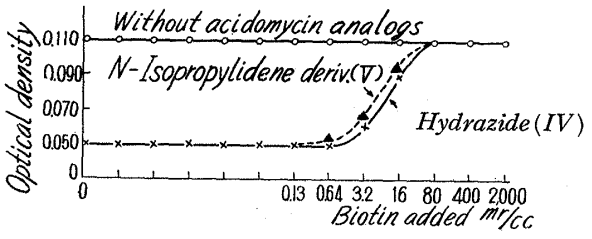


Fig. 2.

TABLE III. Effect of Added Biotin in the Culture Media containing 10 γ/cc. of Acidomycin or its Related Compounds

Biotin mγ/cc. \ Compd.	I	II	III	IV	V	VI	VII	VIII	INAH
2×10^{3}	+	+	+	+	+	+	+	+	−
4×10^{2}	+	+	+	+	+	+	+	+	−
8×10	+	+	+	+	+	+	+	+	−
1.6×10	+	+	+	+	+	+	+	+	−
3.2	+	±	±	±	±	±	±	±	−
6×10^{-1}	−	−	−	−	−	−	−	−	−
12×10^{-2}	−	−	−	−	−	−	−	−	−
25×10^{-3}	−	−	−	−	−	−	−	−	−
5×10^{-3}	−	−	−	−	−	−	−	−	−
1×10^{-3}	−	−	−	−	−	−	−	−	−
2×10^{-4}	−	−	−	−	−	−	−	−	−
4×10^{-5}	−	−	−	−	−	−	−	−	−
0	−	−	−	−	−	−	−	−	−

Antagonistic activity of biotin to the antitubercular activity of these compounds is shown in Figs. 1~2 and in Table III. The growth inhibition of tubercule bacilli caused by 10 γ/cc. of these compounds was reversed by the addition of 3.2 mγ/cc. of biotin in all cases.

The compounds (I) to (VIII) are all endowed with factors necessary for the compounds of these series to exhibit antitubercular activity, and the fact that all of them are antagonistic to biotin in activity seems to show a part, if not all, of the mechanism of their antitubercular activity.

Although pimelic acid was already reported to have no antagonistic activity to acidomycin, it is interesting to note that pimelic acid dihydrazide is antagonistic to biotin as shown in Table III and has itself a strong antitubercular activity.

Since this seemed to be due to the introduction of the hydrazide moiety, experiment was made with isonicotinic acid hydrazide (INAH) under the same conditions, but the growth inhibition caused by 10 γ/cc. of this hydrazide was not reversed even with 2 γ/cc. of biotin.

Recently, Pittilo *et al.*[3] reported that the growth inhibition of *A. aerogenes* and *Mycobacterium* strain AF2 caused by isonicotinic acid hydrazide was reversed by biotin, but in the present experiments with *M. tuberculosis typus avium* the growth inhibition was not reversed by biotin.

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Summary

The relation between the chemical structure, antitubercular activity, and anti-biotin activity of various compounds related to acidomycin was examined with *Mycobacterium tuberculosis typus avium*.

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3) R. F. Pittilo, J. W. Foster : J. Bacteriol., **67**, 53(1954).