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ANTIBACTRIAL ACTIVITY OF MYRMICACIN AND RELATED COMPOUNDS ON PATHOGENIC BACTERIA IN SILKWORM LARVAE, *STREPTOCOCCUS FAECALIS* AD-4

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Myrmicacin, a compound in the secretion of South American leaf-cutting ant (*Atta sexdens*), was identified as 1-β-hydroxydecanoic acid by SCHILDKNECHT and KOOB (1971). It is assumed that the substance prevents germination of collected seeds and spores of fungi such as *Altenaria* and *Botrytis* in the ant's nest. With respect to its physiological activity, IWANAMI and IWADARE (1978) reported that 50 ppm of synthesized myrmicacin inhibited pollen growth and pollen tube mitosis of several higher plants.

In view of that myrmicacin inhibits fungal spore germination, it seems reasonable to assume that the compound has antibacterial activity as well. This paper describes antibacterial activities of synthesized myrmicacin (dl-β-hydroxydecanoic acid) and some related compounds on several bacterial speices isolated from silkworm gut by TAKIZAWA and IIZUKA (1968) and on pathogenic bacteria, *Streptococcus faecalis* AD-4, isolated by IIZUKA and TAKIZAWA (1969) from diseased silkworm larvae reared on the artificial diet.

Materials and Methods

The test bacterial species are listed in Table 1.

The submitted dl-myrmicacin (mp. 58.5°−59°C) was synthesized, from which β-ketodecanoic acid (mp. 85°−86.5°C), dl-methyl β-hydroxydecanoate (bp. 94°−95°C/0.5 mm), dl-acetoxydecanoic acid (bp. 132°−135°C/0.1 mm) and dl-decan-1, 3-diol (bp. 167°−168°C/14mm) were derived by the conven-

tional procedures. The other compounds were purchased from Tokyo Kasei Co., and their purity (>98%) was guaranteed by the supplier.

All the submitted compounds were assayed paper disc method, for which each solution was prepared by dissolving 20 mg of compound in 1 ml of methanol. In addition, activity of myrmicacin and five related compounds, with which strong antibacterial activity was recognized in the paper disc assay, was tested by variation of living cells in the synthetic medium developed by IIZUKA *et al.* (1978). One (1000 ppm) of the substances was added to the medium followed by pH adjustment to 7.0. Thus six kind media were prepared. In another examination, pH was adjusted to 10.0. To each medium 10^6/ml of living cells were inoculated and incubated at 30°C for 24 hrs., and number of the living cells was counted.

Results and Discussion

Result of the assay of myrmicacin on 11 bacterial species is shown in Table 1, which reveals that multiplication of the Gram positive species such as *Staphylococcus* and *Streptococcus* are more inhibited than that of the others.

TABLE 1. Antibacterial activity of myrmicacin on some bacterial species

Species	Antibacterial activity
Staphylococcus aureus	++
S. epidermidis	+++
Streptococcus faecalis	++
Bacillus cereus	+
B. subtilis	−
B. thuringiensis	−
Escherichia coli	−
Pseudomonas riboflavina	−
P. aeruginosa	−
P. fluorescens	−
Alcaligenes metalcaligenes	++

−: No inhibitory zone was formed, +: 1−2 mm, ++: 2−3 mm, +++: 3−4 mm.

Table 2 presents the antibacterial activity of 27 compounds related to myrmicacin on *Streptococcus faecalis* AD-4. Strong activity was recognized with (8) caprylic acid, (9) pelargonic acid, (10) capric acid and (12) Δ^2-decenoic acid. The structural relations of myrmicacin and main related compounds

TABLE 2. Antibacterial activity of myrmicacin and related compounds

Number	Compounds	Molecular formula	Antibacterial activity
(1)	Formic acid	CH_2O_2	+
(2)	Acetic acid	$C_2H_4O_2$	±
(3)	Propionic acid	$C_3H_6O_2$	+
(4)	Butyric acid	$C_4H_8O_2$	−
(5)	Valeric acid	$C_5H_{10}O_2$	+
(6)	Caproic acid	$C_6H_{12}O_2$	+
(7)	Enanthic acid	$C_7H_{14}O_2$	±
(8)	Caprylic acid	$C_8H_{16}O_2$	++
(9)	Pelargonic acid	$C_9H_{18}O_2$	++
(10)	Capric acid	$C_{10}H_{20}O_2$	++
(11)	Lauric acid	$C_{12}H_{24}O_2$	±
(12)	Δ^2-Decenoic acid	$C_{10}H_{18}O_2$	++
(13)	Octanal	$C_8H_{16}O$	−
(14)	Decan-3-ol	$C_{10}H_{22}O$	−
(15)	Decan-1-ol	$C_{10}H_{22}O$	+
(16)	γ-Decalactone	$C_{10}H_{18}O_2$	−
(17)	δ-Decalactone	$C_{10}H_{18}O_2$	−
(18)	Sodium γ-hydroxydecanoate	$C_{10}H_{19}O_3Na$	−
(19)	Sodium δ-hydroxydecanoate	$C_{10}H_{19}O_3Na$	−
(20)	4-Cyclohexyl butyric acid	$C_{10}H_{18}O_2$	+
(21)	4-Phenyl butyric acid	$C_{10}H_{12}O_2$	−
(22)	Sebacic acid	$C_{10}H_{16}O_2$	+
(23)	*l*-D-β-Hydroxydecanoic acid*	$C_{10}H_{20}O_3$	not tested
(24)	β-Ketodecanoic acid	$C_{10}H_{18}O_3$	−
(25)	β-Acetoxydecanoic acid	$C_{12}H_{22}O_4$	+
(26)	Methyl β-hydroxydecanoate	$C_{11}H_{22}O_3$	+
(27)	Decan-1.3-diol	$C_{10}H_{22}O_3$	++

* not tested because of small amount available.

is described in Fig. 1. From the consideration of chemical structures of the active compounds, it seems reasonable to assume that the terminal carboxyl group and normal $C_{8\sim10}$ hydrocarbon chain are essential for the activity. The assumption is in good agreement with a previous report (IWANAMI and IWADARE, 1979).

Comparing the activity of myrmicacin and the five related compounds demonstrated by paper disc, much weaker inhibitory activity was observed in the synthetic medium at pH 7.0 with the same compounds. However, Fig. 2 shows that they apparently have inhibitory activity at pH 10.0. Among

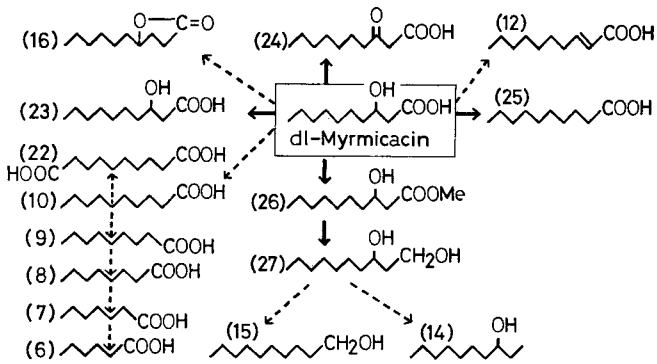

Fig. 1. Structural relationship of myrmicacin and related compounds.
⟶ : directly derived
⇢ : closely related
( ) : the number indicates that cited in the Table 2

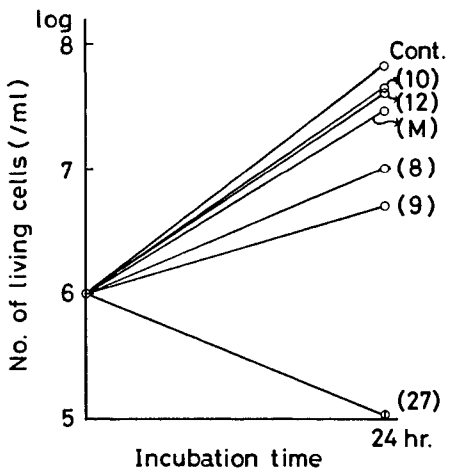

Fig. 2. Antibacterial effect of some compounds in the medium at pH 10.0.

them, decan-1.3-diol was the strongest in antibacterial effect. It is assumed that the strong activity of decan-1.3-diol in the synthetic broth medium is attributed to combination of the terminal alcohol and secondary alcohol, since both of decan-1-ol and decan-3-ol are negative in the test.

Study on the effect of the compounds (myrmicacin, 8, 9, 10, 12 and 27) added to the artificial diet, on which silkworm larvae are reared, is in progress.

Summary

Antibacterial activity of myrmicacin (dl-β-hydroxydecanoic acid), a compound in the secretion of the South American leaf-cutting ant and the related compounds was tested. The test revealed myrmicacin had the antibacterial activity on Gram positive cocci such as *Staphylococcus* and *Streptococcus*. Myrmicacin, caprylic acid, pelargonic acid, capric acid and Δ^2-decenoic acid and decan-1.3-diol exibit strong activity in the assay by paper disc method. Inhibitory activity of the six compounds was also tested in the synthetic medium at pH 7.0 and 10.0. While they were inactive at pH 7.0 in the medium, activity was observed at pH 10.0. Decan-1.3-diol had a paticular strong activity under the latter condition.

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