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Pharmacology of Iridoid: Antimicrobial Activities of Aucubin

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Abstract—Antimicrobial activities of aucubin, an iridoid glycoside, were investigated. Gram-positive bacterium, *S. aureus* appeared to be more sensitive to aucubin's aglucone, aucubigenin than Gram-negative, *E. coli* did. Antimicrobial activities produced by aucubigenin may result in part from the inhibition of RNA and protein biosyntheses in bacterial cells. The conversion of aucubin iridoid glycoside into aucubigenin, an aglucone, appears to be a prerequisite step to exhibit the antimicrobial activities.

Keywords—Antimicrobial activities of aucubin • inhibition of RNA and protein biosyntheses • biologically active form of iridoid glycoside

Iridoids represent a group of natural constituents that belongs to monoterpenoid with cyclopenta(c)pyran ring system and are found usually as glycosides. To present, more than 250 compounds have been isolated from plants and insects and their chemical structures were reported.[1] They are also widely distributed in many Chinese herbal drugs, but relatively few biological activities have been reported, summarizing as follows: 1) liver-protective activities against hepatic damages induced by toxic chemicals such as CCl_4 and galactosamine 2) antidotal activities against alpha-amanitin poisoning 3) antimicrobial activities 4) inhibitions of liver RNA and protein biosyntheses 5) cholerectic action 6) hypotensive activities 7) enhancement of sexual activities, etc.[2~14]

Iridoid glycoside can be hydrolyzed by β-glucosidase (E.C. 3.2.1.21.) enzymatically or by acid into its aglycone and sugar(Fig. 1). Then the cyclopentapyran ring is easily opened to form the dialdehyde structure. This dialdehyde structure is once formed, it can be very rapidly proceeded to polymerization. In this regard it is interesting to note that which chemical structure would be responsible for exhibitions of the biological activities.

Present study aims to investigate antimicrobial activities of iridoid compounds against Gram-positive and -negative bacteria. In relation to antimicrobial activities, we examined a possible mechanism of antimicrobial actions and biologically active form of drug.

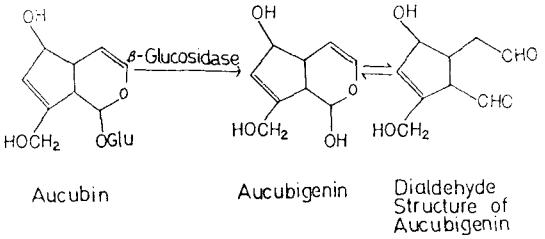

Fig. 1. Hydrolysis of aucubin to its aglucone.

Study on Antimicrobial Activities against Gram-positive and Negative Bacteria

An iridoid glycoside, aucubin was isolated

from fresh leaves of *Aucuba japonica* (Cornaceae) and examined potential antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli*, Gram-positive and -negative bacteria, respectively. All bacteria were grown in the MS-2 media and the growth rates were measured by the MS-2 microbial research system in which degree of turbidity was employed as a measure of growth rates of bacteria.

Firstly, aucubin, final concentrations of 0.3 mM and 3.0 mM were employed (2 and 1). Penicillin G (500 IU) was also treated as a positive control (5). Two negative control (3 and 4) were made. As the data shown in Fig. 2, 2,500 IU of penicillin G blocked completely the growth of *S. aureus* during approximately 2 hr incubation period. In case of aucubin at 0.3 mM and 3.0 mM, no inhibition of bacterial growth was observed. Therefore, aucubin as a glycoside form did not apparently exhibit antimicrobial activites against *S. aureus*.

Next we tested aucubin to Gram-negative, *E. coli* under the same condition employed as the preceding experiment. Aucubin at two concentrations of 0.3 mM and 3.0 mM (2 and 1) did not inhibit the growth of *E. Coli*, whereas chloramphenicol (9 μg/ml) employed as a positive control blocked completely bacterial growth. These results indicate that aucubin as a glycoside form of iridoid did not possess any antimicrobial activities in Gram-positive and -negative bacteria.

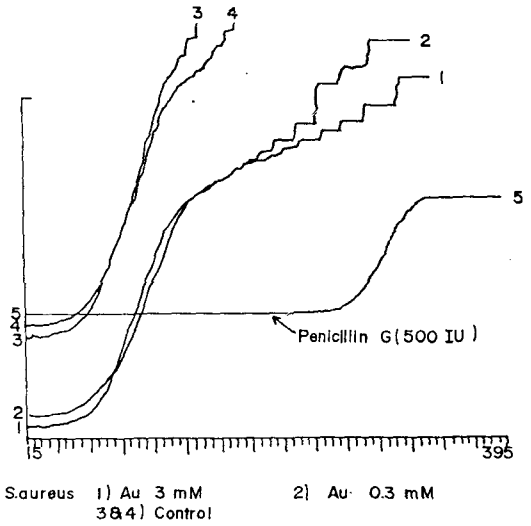

Fig. 2. Effect of aucubin on the growth of *S. aureus*. 1, aucubin 3 mM, 2, aucubin 0.3 mM, 3 & 4, negative control, 5, positive control, penicillin G(500 IU).
Bacteria were pretreated with drugs for 15 min, then incubation was carried out further to 395 min.

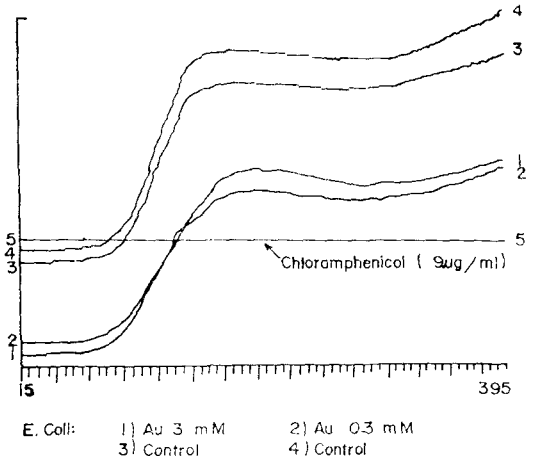

Fig. 3. Effect of aucubin on the growth of *E. coli*. 1, aucubin 3 mM, 2, aucubin 0.3 mM, 3 & 4, negative control, 5, positive control, chloramphenicol(9 μg/ml).
Other experimental conditions were same as those in Fig. 2.

When aucubin is treated with β-glucosidase or acid, it is converted to aglucone (aucubigenin) and glucose. Then the aucubigenin is easily converted to its aldehyde structure. In order to investigate whether the hydrolytic product, aucubigenin, can exhibit antimicrobial activities or not, aucubin was pretreated with β-glucosidase (at pH 5.5), before added to bacterial incubation media.[15] We could obtain aglucone (>90%) during 2 hr enzyme treatment, then total hydrolytic products were added to the incubation chamber of *S. aureus*. As the data shown in Fig. 4, aucubigenin (3.0 mM treated - 1) showed marked inhibition of the growth of *S. aureus*.

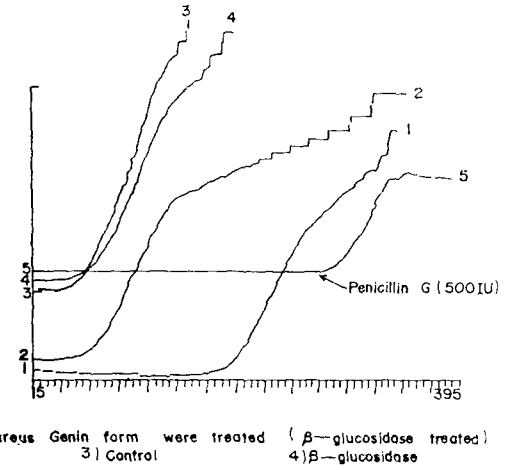

Fig. 4. Effect of aglucone, aucubigenin on the growth of *S. aureus*.
1, aucubigenin 3 mM, 2, aucubigenin 0.3 mM, 3, control 4, β-glucosidase only treated, 5, penicillin G(500 IU).
To hydrolyze the aucubin, β-glucosidase was treated at pH 5.5 for 2 hr. Total hydrolyzed products represent as aglucon, aucubigenin. Other experimental conditions are same as those in Fig. 2.

Also we could observed similar inhibition, but relatively weak inhibition at 0.3 mM of aucubigenin. It is interesting to note that when aucubin is hydrolyzed into its aglycone, it shows

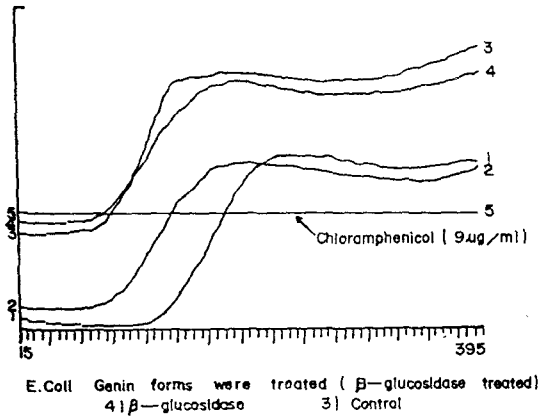

Fig. 5. Effect of aglucone, aucubigenin on the growth of *E. coli*.
1, aucubigenin 3 mM, 2, aucubigenin 0.3 mM, 3, control, 4, β-glucosidase only treated, 5, chloramphenicol (9 μg/ml).
Other experimental conditions are same as those in Fig. 4.

antimicrobial activities.

Therefore, we further tested aucubigenin to *E. coli*. Under the same experimental conditions employed as the preceding, aucubigenin at 3.0 mM concentration exhibited very slight inhibition and at 0.3 mM concentration no inhibition was observed (Fig. 5). These results indicate that Gram-positive bactera, *S. aureus* appears to be more sensitive to aucubigenin (aglucone) than that of Gram-negative, *E. coli*.

Mechanism of Antimicrobial Activities

It was reported previously that aucubin exhibits the inhibitory effects on liver RNA and protein biosyntheses. In relation to this, we examined that whether such inhibition of RNA and protein biosyntheses in bacteria can be produced by aucubin glycoside/aucubigenin or not. After *S. aureus* was inoculated into medium from the stock culture, aucubin (0.1 mg/ml and 1 mg/ml) or aucubigenin (0.1 mg/ml and 1 mg/ml) was added into culture flask and the preincubation was carried out for 45 min at 37°C. Then ^{3}H-uridine (10 μc/ml) was added into culture flask. Incubation was further continued for 75 min. During incubation, aliquot (1 ml) was taken from culture flask at every 15 min time-interval and placed in 5% ice-cold TCA solution. Then the bacterial cells were denatured and disrupted, whole homogenate was centrifuged in cold at 4°C. The precipitated pellet was washed three times more with 5% ice-cold TCA solution. Finally the pellet was washed once with 95% ethyl alcohol. After the washed pellet was dissolved in the solubilizing agent, Soluene, toluen-based scintillation fluid was added and the radioactivities were measured in the liquid scintillation spectrometer. Radioactivities incorporated into acid-insoluble portion were considered as a measure of RNA biosyn-

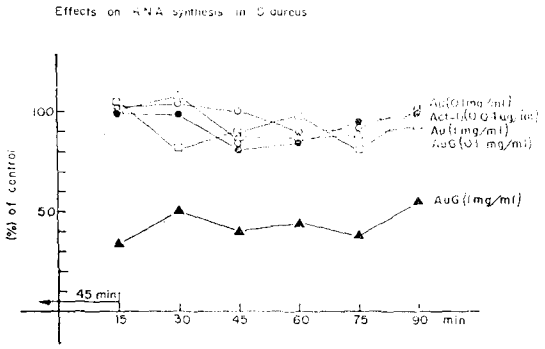

Fig. 6. Effects of aucubin and its aglucone on RNA biosynthesis in *S. aureus.*
^{3}H-uridine was pulse labeled. Radioactivities incorporated into TCA-insoluble nucleotides were considered as a measure of RNA biosynthesis in bacteria. Drugs and cells were preincubated for 45 min, then radioactive uridine was added. Aliquots were removed from culture flask every 15 min. Bacteria cells were washed with 5% ice-cold TCA four times and once with 95% ethyl alcohol. Au;aucubin, AUG; aucubigenin, Act-D; actinomycin D.

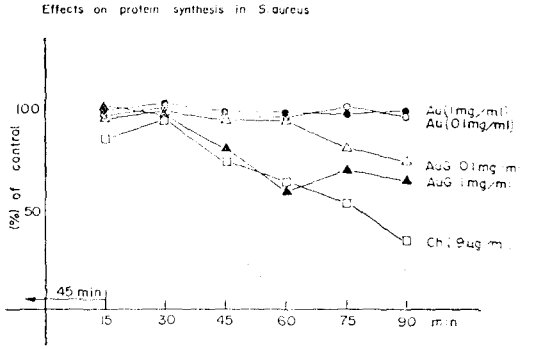

Fig. 7. Effects of aucubin and its aglucon on protein biosynthesis in *S. aureus.*
^{3}H-Leucine was pulse-labeled. Radioactivities incorporated into TCA-insoluble protein were considered as a measure of protein biosynthesis in bacteria. Other experimental conditions are same as those in Fig. 6.
Au;aucubin, AUG;aucubigenin, Ch;chloramphenicol.

thesis in bacterial cells.

As the results shown in Fig. 6, aucubin glycoside did not inhibit appreciably the RNA synthesis in *S. aureus.* However, when aucubigenin(1 mg/ml) was treated, the RNA synthesis was inhibited approximately 60% in comparison with that of control group throughout the 75 min incubation period. When 0.1 mg/ml concentration of aucubigenin was treated, no significant inhibition occurred.

We also examined a possible influence of aucubin and aucubigenin to protein synthesis in *S. aureus* cells. Same experimental conditions were employed as the preceding one except the radioactive precussor. Instead of ^{3}H-uridine, ^{3}H-leucin(4, 5-H) was used. As the data shown in Fig. 7, treatments of aucubin(0.1 mg/ml and 1 mg/ml) did not inhibit appreciably the protein synthesis in *S. aureus* cells while aucubigenin (0.1 mg/ml and 1 mg/ml) inhibited significantly the protein synthesis. At the concentration of 1 mg/ml of aucubigenin, the protein synthesis was inhibited almost as much as the chloramphenicol, a positive control, did inhibit.

It is interesting to us that the degree of inhibition of RNA synthesis produced by aucubigenin treatment (1 mg/ml) appeared to be more profound than that of protein synthesis inhibition in *S. aureus* cells. This results suggest that the inhibition of RNA synthesis by aucubigenin is more sensitive than the inhibition of protein. It is also noted that similar results were obtained when liver cells were treated with aucubigenin.

Present study demonstrated that aucubin, an iridoid glycoside, exhibits antimicrobial activities against Gram-positive bacteria, *S. aureus* and Gram-negative bacteria, *E. coli.* However, Gram-positive bacteria, *S. aureus* appears to be more sensitive to aucubigenin than *E. coli* does. Such antimicrobial activites may result in part from the inhibition of RNA and protein biosyntheses caused by aucubigenin. In addition, aucubin should be converted to its aglycone, aucubigenin in order to exhibit antimicrobial activities. Therefore, hydrolysis of iridoid glycoside appears

to be a prerequisit step to from an active form of drug.

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