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Antibacterial Activity of Venom Alkaloids from the Imported Fire Ant, *Solenopsis invicta* Buren[1]

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The antibacterial properties of synthetic fire ant venom alkaloids were tested by disc-diffusion procedures against a variety of bacteria and by doubling concentrations in broth cultures of *Staphylococcus aureus* and *Escherichia coli*. Gram-positive bacteria were inhibited by lower concentrations of *trans*-2-methyl-6-*n*-undecylpiperidine, *trans*-2-methyl-6-*n*-tridecylpiperidine, and *trans*-2-methyl-6-*n*-pentadecylpiperidine than were gram-negative bacteria. A fourth alkaloid, *trans*-2-methyl-6-(*cis*-6-pentadecenyl)piperidine, which was available in minute quantity and was tested by disc-diffusion only, was ineffective against all organisms.

The antibiotic properties of the venom of the imported fire ant (IFA), *Solenopsis invicta* Buren (Hymenoptera:Formicidae), were reported by Blum et al. (1), who found that paper discs impregnated with a 1:50 dilution of raw venom produced zones of inhibition on agar plates inoculated with *Micrococcus pyogenes*, *Streptococcus pyogenes*, *Escherichia coli*, *Lactobacillus casei*, and a variety of molds. Caro et al. (2) reported that the pustules arising from experimentally inflicted stings of IFA are apparently free from bacteria.

MacConnell et al. (4) identified and synthesized an alkaloid component of IFA venom, *trans*-2-methyl-6-*n*-undecylpiperidine, for which they proposed the name solenopsin A. MacConnell et al. (5) have since identified and synthesized four additional alkaloids which, together with solenopsin A, constitute more than 99% of the detectable substances in the poison gland secretion. These compounds are *trans*-2-methyl-6-*n*-tridecylpiperidine (solenopsin B), *trans*-2-methyl-6-(*cis*-4-tridecenyl)-piperidine (dehydrosolenopsin B), *trans*-2-methyl-6-*n*-pentadecylpiperidine (solenopsin C), and *trans*-2-methyl-6-(*cis*-6-pentadecyl)-piperidine (dehydrosolenopsin C).

The present paper presents the results of susceptibility tests conducted with four of the synthetic IFA venom alkaloids.

MATERIALS AND METHODS

Test organisms. Cultures of *Bacillus pulvifaciens* and *B. thuringiensis* were obtained from the American Type Culture Collection, Rockville, Md. The remaining cultures were obtained from Edward M. Hoffman, Department of Microbiology, University of Florida.

Alkaloids. The alkaloids (as hydrochlorides) were synthesized by the method of MacConnell et al. (5). Dehydrosolenopsin C was not available.

Procedures for the bactericidal tests. The inhibition of various bacteria by solenopsins was tested by the paper disc-diffusion method. Sterile white blotter-paper discs, 6.0 mm in diameter, were saturated with a 1:1,000 aqueous solution of solenopsin HCl and air-dried at 37 C. Petri dishes containing 20.0 ml of brain heart infusion (BHI) agar (Difco) were inoculated by flooding the surface with 1.0 ml of a 24-hr BHI broth culture of the test organism. Excess culture was removed by pipette. The solenopsin-impregnated discs were placed on the inoculated agar surfaces, and the resulting zones of inhibition were measured after incubation at 37 C for 24 hr. Each test was conducted in duplicate, and plain sterile discs were used as controls.

Dehydrosolenopsin B, which was ineffective against all organisms tested by disc diffusion, was available in minute quantity and was not tested further. Additional bactericidal tests were conducted with solenopsins A, B, and C against *Staphylococcus aureus* (inhibited in the disc-diffusion tests described) and *E. coli* (not inhibited in the disc-diffusion tests). The latter organism was tested because solenopsins are not highly soluble in water, and concentrations higher than those utilized in the disc-diffusion tests might be inhibitory to gram-negative bacteria.

The test method was essentially method 2-A of Grundy et al. (3). A series of screw-cap culture tubes (25 by 150 mm) containing 9.0 ml of BHI broth and successively doubled concentrations of solenopsin HCl were inoculated with 1.0 ml of a 1:1,000 dilution of a 24-hr broth culture which had

been subcultured daily in broth for 3 days. This procedure resulted in an initial concentration per tube of approximately 10^5 cells/ml. The tubes were agitated and were then incubated at 37 C. At intervals of 3, 6, 24, and 48 hr, 1.0 ml was withdrawn from each tube, and the number of surviving cells was estimated by standard dilution-plating procedures. Reduction in volume of the test cultures was minimized by removing 0.5 ml (instead of 1.0 ml) when undiluted samples were plated.

RESULTS

The results of the disc-diffusion inhibition tests are shown in Table 1. Solenopsins A, B, and C inhibited all of the gram-positive bacteria to some degree, and the order of inhibition of these organisms was always the same (A > B > C). Solenopsin A slightly inhibited 4 of the 12 gram-negative bacteria, solenopsin B slightly inhibited 1, and solenopsin C had no observable effect on any of the gram-negative bacteria. None of the bacteria tested was inhibited by dehydrosolenopsin B.

The results of the doubling concentration tests with *S. aureus* are shown in Table 2. The minimal bactericidal concentration (MBC) of a compound has been defined by Otto et al. (6) as the concentration killing more than 99.9% of the cells within 48 hr as determined by plate count. Under the conditions of this assay, the MBC was 8.0 μg/ml for solenopsin A, 2.0 μg/ml for solenopsin B, and 4.0 μg/ml for solenopsin C.

The results of the doubling concentration tests with *E. coli* are shown in Table 3. The MBC was 40.0 μg/ml for solenopsin A and 20.0 μg/ml for

TABLE 1. *Antibacterial disc-diffusion tests with imported fire ant venom alkaloids against various bacteria*

Organism	Diam (mm)[a] of zone of inhibition		
	Solenopsin A	Solenopsin B	Solenopsin C
Streptococcus salivarius	18.5	18.0	8.0
S. pyogenes	16.5	13.0	8.0
S. equisimilis	14.0	10.0	8.5
S. faecalis	10.5	9.0	7.0
Staphylococcus epidermidis	11.5	9.0	8.0
S. aureus	11.0	9.0	8.0
Bacillus pulvifaciens	17.5	11.0	9.0
B. thuringiensis	9.0	9.0	+
Shigella flexneri	8.0	+	−
S. boydii	+	−	−
S. sonnei	−	−	−
Salmonella typhimurium	8.0	−	−
S. paratyphi	+	−	−
S. schottmuelleri	−	−	−
S. enteritidis	−	−	−
Escherichia coli	−	−	−
Proteus spp.	−	−	−
Klebsiella pneumoniae	−	−	−
Alcaligenes faecalis	−	−	−
Pseudomonas aeruginosa	−	−	−

[a] Mean of two replicates (includes diameter of paper disc); − = no inhibition; + = zone of inhibition around disc less than 1.0 mm wide. Dehydrosolenopsin B was also tested but did not inhibit any of the organisms.

TABLE 2. *Antibacterial action of imported fire ant venom alkaloids on Staphylococcus aureus*

Solenopsin[a]	Time (hr)	Viable cells/ml[b] in indicated venom alkaloid concn						
		None	0.5 μg/ml	1.0 μg/ml	2.0 μg/ml	4.0 μg/ml	8.0 μg/ml	12.0 μg/ml
A	3	1.1×10^6	—	6.5×10^5	3.4×10^5	9.0×10^4	3.2×10^2	1.8×10^2
	6	4.0×10^7	—	2.3×10^7	4.4×10^6	4.0×10^4	1.2×10^2	33
	24	1.0×10^9	—	1.0×10^9	9.5×10^8	6.8×10^3	0	0
	48	—	—	—	1.3×10^9	2.3×10^6	0	0
B	3	4.4×10^5	1.0×10^5	3.9×10^4	8.8×10^2	5	0	—
	6	4.9×10^7	5.4×10^6	8.7×10^3	1.6×10^2	0	0	—
	24	5.2×10^8	3.7×10^8	7.7×10^5	7	0	0	—
	48	—	—	2.2×10^9	0	0	0	—
C	3	1.5×10^6	9.2×10^5	3.2×10^4	3.5×10^2	0	0	—
	6	5.5×10^7	3.4×10^6	1.5×10^4	1.7×10^2	0	0	—
	24	6.5×10^8	7.4×10^8	5.7×10^6	6.0×10^4	0	0	—
	48	—	—	8.4×10^8	1.2×10^5	0	0	—

[a] Inocula (cells/ml): solenopsin A, 1.0×10^5; solenopsin B, 9.0×10^4; solenopsin C, 1.2×10^5.
[b] Mean of two replicates.

TABLE 3. *Antibacterial action of imported fire ant venom alkaloids on Escherichia coli*

Solenopsin[a]	Time (hr)	Viable cells/ml[b] in indicated venom alkaloid concn				
		None	5.0 μg/ml	10.0 μg/ml	20.0 μg/ml	40.0 μg/m
A	3	6.4×10^6	4.8×10^6	1.7×10^6	9.2×10^4	0
	6	1.4×10^8	7.5×10^6	7.0×10^6	6.0×10^5	0
	24	1.3×10^9	1.0×10^9	5.5×10^8	3.7×10^7	0
	48	1.5×10^9	7.2×10^8	3.7×10^8	8.8×10^8	0
B	3	7.9×10^6	1.2×10^6	5.1×10^1	0	0
	6	3.3×10^8	1.8×10^7	8.0×10^2	0	0
	24	1.6×10^9	8.0×10^8	1.2×10^7	0	0
	48	1.0×10^9	6.4×10^8	1.1×10^9	0	0

[a] Inocula (cells/ml); solenopsin A; 1.6×10^5; solenopsin B, 1.9×10^5.
[b] Mean of two replicates.

solenopsin B. Satisfactory tests could not be conducted with solenopsin C because this compound tended to precipitate at the concentrations of 20.0 μg/ml. *E. coli* increased in number from 1.6×10^5 to 8.0×10^8 cells/ml when exposed to theoretical concentrations of solenopsin C of 20.0 and 40.0 μg/ml over a 24-hr period.

DISCUSSION

Solenopsin A was slightly less effective as a bactericidal agent than solenopsins B and C against *S. aureus* and was less effective than solenopsin B against *E. coli*. The larger zones of inhibition produced by solenopsin A compared with solenopsins B and C in the disc-diffusion tests probably resulted because of the greater solubility of this compound. Solenopsin A HCl precipitated in BHI broth (37 C) at concentrations between 250 and 500 μg/ml, solenopsin B HCl precipitated at concentrations between 125 and 250 μg/ml, and solenopsin C HCl precipitated at concentrations between 10 and 20 μg/ml. Relatively high bacteriostatic activity by solenopsin A could also produce wider zones of inhibition in disc-diffusion tests; however, this is not indicated by the data presented in Tables 2 and 3 for 24-hr incubation. Incubation of disc-diffusion plates for 72 hr did not result in detectable changes in the zones of inhibition.

The gram-positive bacteria tested were less resistant to solenopsins than were the gram-negative bacteria. The relative resistance of *B. thuringiensis* compared to *B. pulvifaciens* and the other gram-positive organisms (Table 1) probably reflects a tendency of this species to gram-variability (*Bergey's Manual*, 7th ed.).

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LITERATURE CITED

1. Blum, M. S., J. R. Walker, P. S. Callahan, and A. K. Novak. 1958. Chemical, insecticidal, and antibiotic properties of fire ant venom. Science 128:306–307.
2. Caro, R., V. J. Derbes, and R. Jung. 1957. Skin responses to the sting of the imported fire ant (*Solenopsis saevissima*). A.M.A. Arch. Dermatol. 75:475–488.
3. Grundy, W. E., E. F. Alford, and J. C. Sylvester. 1959. Antibiotic bactericidal studies. I. Effect of methods on apparent bactericidal concentration. Antibiot. Annu. 1958–1959, p. 847–854.
4. MacConnell, J. G., M. S. Blum, and H. M. Fales. 1970. Alkaloid from fire ant venom: identification and synthesis. Science 168:840–841.
5. MacConnell, J. G., M. S. Blum, and H. M. Fales. 1971. The chemistry of fire ant venom. Tetrahedron 26:1129–1138.
6. Otto, R. H., E. F. Alford, W. E. Grundy, and J. C. Sylvester. 1961. Bactericidal and bacteriostatic tests with various antibiotics, p. 104–122. P. E. Gray, B. E. Tabenkin, and S. G. Bradley (ed.), *In* Proc. Conf. on Antimicrobial Agents, 1960. Antibiotics Annual.