IIT RESEARCH INSTITUTE

Report No. IITRI C222-7
(Quarterly Progress Report)

DEVELOPMENT OF AN ORALLY EFFECTIVE INSECT REPELLENT

Headquarters
U.S. Army Medical Research and
Development Command
Office of the Surgeon General
Washington 25, D.C.

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Attention: Major Lee Roy J. Jones

DEVELOPMENT OF AN ORALLY EFFECTIVE INSECT REPELLENT

IITRI Project No. C222
Contract No. DA-49-193-MD-2281

November 1, 1963, to January 31, 1964

I. INTRODUCTION

The object of this program is the development of an insect repellent which will be effective when given internally. In order to be effective after internal administration, a compound must possess repellent properties at a very low concentration in the body fluid. Information on minimum effective concentration of repellent compounds is not available for most compounds. Therefore, during the period covered in this report, the repellent properties of several compounds were determined at low concentrations.

II. PROCEDURES

The testing procedures employed in this investigation have been described in detail in our previous reports (No. C222-2, -3, -6). In essence, the compounds are applied to the skin of mice

for in vivo testing and are homogenized in blood for in vitro testing. A mixture of a fluorescent dye and radioiodinated serum albumin is used as an indicator. It is injected in the mice intravenously or is homogenized in the blood. In the in vivo tests the mosquitoes are allowed to bite the mice, and in the in vitro tests they ingest blood through a specially prepared animal membrane. The number of mosquitoes that bite are counted, and the amount of blood ingested is determined quantitatively.

The compounds tested were selected from the USDA Agriculture Handbook No. 69 and other reports on insect repellents. They were initially tested in vitro at two concentrations, one at which diethyltoluamide was effective (1.0 mg/ml) and the other at which it was not effective (0.1 mg/ml). The compounds which were effective at a concentration of 0.1 mg/ml were tested further in vivo.

III. RESULTS

Table 1 shows the effectiveness of several compounds as repellents when homogenized in blood at concentrations of 1.0 and 0.1 mg/ml. Of the 28 compounds tested, only N-amyl succinamide reduced mosquito biting to less than 50% at a concentration of 0.1 mg/ml. This compound will be studied further.

Table 1

EFFECT OF CHEMICAL COMPOUNDS EMULSIFIED IN BLOOD ON MOSQUITO FEEDING

Compound	Conc, mg/ml	Feeding, % of Controls: Number Engorged	Feeding, % of Controls: Blood Ingested
Benzoic acid, benzyl ester	1.0	0	0
	0.1	101	110
Anthranilic acid, methyl ester	1.0	0	0
	0.1	69	71
Cyclohexanol, 2-cyclohexyl	1.0	0	0
	0.1	92	85
α,α-Dimethyl-α-carbobutoxydihydro-γ-pyrone (Indalone)	1.0	11	9
	0.1	95	84
Acetanilide, N-n-propyl	1.0	4	3
	0.1	58	63
O,O-dimethyl-S-p-chlorophenyl thiomethyl phosphorodithioate (methyl Trithion)	1.0	62	23
	0.1	92	81
Decanoic acid	1.0	63	23
	0.1	73	64
Phthalic acid, dimethyl ester	1.0	51	19
	0.1	75	61
Hexadecanoic acid	1.0	92	58
	0.1	108	121
Acetamide, N-amyl-α-butoxy	1.0	0	0
	0.1	108	96
Ethanol, 2- [2-(3-methyl-2-norcamphanyl methoxy) ethoxy]	1.0	25	6
	0.1	110	118
Bicyclo [2.2.1] -5-heptene-2,3-dicarboximide, N-(mixed)amyl	1.0	0	0
	0.1	64	40
Succinamide, N-amyl	1.0	0	0
	0.1	34	31

Table 1 (cont.)

Compound	Conc, mg/ml	Feeding, % of Controls: Number Engorged	Feeding, % of Controls: Blood Ingested
5-m-Dioxanol, 2-hexyl	1.0	15	11
	0.1	78	67
S-bis(4-chlorophenyl)methyl,O,O-diethyl phosphorodithioate (Stauffe.'s R-2371)	1.0	108	92
	0.1	99	103
O-4-tert-Butyl-2-chlorophenyl, O-methyl, methylphosphoramidate (Ruelene)	1.0	95	81
	0.1	93	81
1,3-Hexanediol, 2-ethyl	1.0	51	29
	0.1	95	106
Thiamine hydrochloride	1.0	82	76
	0.1	103	111
Anisyl alcohol	1.0	86	44
	0.1	105	90
Citronellic acid	1.0	49	22
	0.1	123	111
10-Undecenoic acid	1.0	31	15
	0.1	95	92
O,O-dimethyl-O-2,4,5-trichlorophenylphosphorothioate (Ronnel)	1.0	89	41
	0.1	87	74
O-O-dimethyl-O,p-(dimethyl sulfamoyl) phenylphosphorothioate (Famphos)	1.0	32	21
	0.1	60	52
Acetic acid, chloro-, 2-nitroisobutyl ester	1.0	113	91
	0.1	113	122

Table 1 (cont.)

Compound	Conc, mg/ml	Feeding, % of Controls: Number Engorged	Feeding, % of Controls: Blood Ingested
Bicyclo [2.2.1] -5-heptene-2,3-dicarboximide, N-amyl	1.0	6	5
	0.1	86	128
1,2-Propanediol, 3-isoamoxy	1.0	110	82
	0.1	113	117
Benzoic acid, m-amino, ethyl ester	1.0	57	33
	0.1	108	102
Glycine, N-butyl, isobornyl ester	1.0	18	11
	0.1	103	93

Each experimental value represents an average of 2 replicates; each control value represents an average of 4 to 6 replicates. Each replicate consisted of 50 mosquitoes exposed to one blood meal.

Allethrin was also effective at a concentration of 0.1 mg/ml. The effect of allethrin on in vitro mosquito biting is shown in Table 2. This table also includes data on other allethrine-like compounds and on the acid moiety of allethrin, chrysanthemumic acid. Of the four allethrin-like compounds tested, only allethrine was more active than diethyltoluamide; preliminary results showed allethrin to be more than 100 times more active than diethyltoluamide.

Allethrin and allethrine-like compounds were also tested in vivo. It can be seen from Table 3 that allethrin lost its effectiveness only at a concentration of 0.0001 mg/ml. All the other allethrin-like compounds tested were ineffective at a concentration of 0.1 mg/ml.

The present investigation shows allethrin to be the most effective drug in preventing mosquito biting. Further investigation on allethrin will be reported in future reports.

IV. CONCLUSIONS AND FUTURE WORK

Several compounds were tested for their effectiveness in preventing mosquitoes from biting mice and from ingesting blood through an animal membrane. Of the compounds tested, only N-amyl succinamide and allethrin were effective at a concentration of 0.1 mg/ml. Allethrin was effective both in vitro and in vivo at a concentration of 0.001 mg/ml.

Table 2

EFFECT OF CHRYSANTHEMUMIC ACID ESTERS EMULSIFIED IN BLOOD ON MOSQUITO FEEDING

Ester of Chrysanthemum, monocarboxylic acid	Conc., mg/ml	Feeding, % of Controls	
		Number Engorged	Blood Ingested
Ethyl	10.00	0	0
	1.00	33	28
	0.01	81	78
2,4-Dimethylbenzyl (Dimet[illegible]rin)	1.0	52	22
	0.1	76	49
6-Chloropiperonyl (Barthrin)	1.0	30	13
	0.1	74	48
dl-2-Allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one (allethrin)	0.010	3	-
	0.001	52	-

Each experimental value represents 2 replicates; each control value represents 4 to 6 replicates.
Each replicate consisted of 50 mosquitoes.

Table 3

EFFECT OF CHRYSANTHEMUMIC ACID ESTERS APPLIED ON MOUSE SKIN ON MOSQUITO FEEDING

Ester of Chrysanthemum, monocarboxylic acid	Number of Replicates	Conc., mg/ml	Feeding, % of Controls	
			Number Engorged	Blood Ingested
Ethyl	2	10.00	22	21
	2	1.00	100	96
	2	0.01	98	96
2,4-Dimethylbenzyl (Dimethrin)	2	1.00	23	12
	1	0.10	87	73
	1	0.01	85	77
6-Chloropiperonyl (Barthrin)	2	1.00	20	15
	1	0.10	81	63
	1	0.01	96	100
dl-2-Allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one (allethrin)	2	1.00	3	4
	2	0.10	5	8
	2	0.01	7	32
	2	0.001	10	18
	2	0.0001	70	90
	1	0.00001	97	95

Each control value represents 4 to 6 replicates.
Each replicate consisted of 50 mosquitoes.

Work on succinamides and allethrin will be continued. Evaluation of other compounds in vitro and in vivo will also be continued. In addition, tests are being designed to detect mosquito biting in the absence of blood sucking.

S. Ginocchio contributed to the progress of this investigation. The data are recorded in Logbooks C13244 and C14373.

Respectfully submitted,

IIT RESEARCH INSTITUTE

Harbans Lal
Research Pharmacologist
Life Sciences Research

Approved by:

E. J. Hawrylewicz
Assistant Director
of Life Sciences Research

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