Onderstepoort J. vet. Res. 45, 39–42 (1978)

THE ACTIVITY OF INSECT JUVENILE HORMONE MIMICS IN LARVAL *AMBLYOMMA HEBRAEUM* KOCH (ACARINA : METASTRIATA : IXODIDAE)

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ABSTRACT

SOLOMON, K. R. & EVANS AILEEN A., 1978. The activity of insect juvenile hormone mimics in larval *Amblyomma hebraeum* Koch (Acarina : Metastriata : Ixodidae). *Onderstepoort Journal of Veterinary Research* 45, 39–42 (1978).

A total of 14 insect juvenile hormone mimics was tested for activity in *Amblyomma hebraeum* by exposing newly engorged larvae to filter paper impregnated with hormone mimics. The most active compounds used in this assay were HS 103 (6-ethyl-3-pyridyl geranyl ether; EC_{50}=0,0018 mg/cm²), ZR 512 (Ethyl 3,7,11-trimethyldodeca-2,4-dienoate; EC_{50}=0,0022 mg/cm²), HS 2 (6-methyl-3-pyridyl geranyl ether; EC_{50}=0,0035 mg/cm²), ZR 615 (N-ethyl 3,7,11-trimethyldodeca-2,4-dieneamide; EC_{50}=0,0035 mg/cm²), ZR 777 (Prop-2-ynyl 3,7,11-trimethyldodeca-2,4-dienoate; EC_{50}=0,0039 mg/cm²) and ZR 515 (Isopropyl-11-methoxy 3,7,11-trimethyldodeca-2,4-dienoate; EC_{50}=0,0094 mg/cm²). Activity in this assay was similar to that reported in insects and was consistent with the susceptibility of these compounds to metabolic inactivation. The results suggest that ZR 615 may be of possible use in tick control.

Résumé

*ACTIVITÉ DE MIMÉTIQUES D'HORMONE JUVÉNILE D'INSECTES SUR LA LARVE D'*AMBLYOMMA HEBRAEUM *KOCH (ACARINA: METASTRIATA: IXODIDAE)*

On a vérifié l'activité sur Amblyomma hebraeum *de 14 mimétiques d'hormone juvénile d'insectes, en exposant des larves fraîchement repues à du papier-filtre imprégné de ces mimétiques. Les plus actifs des composés utilisés dans ce test ont été HS 103 (6-ethyl-3-pyridyl geranyl ether; EC_{50}=0,0018 mg/cm²), ZR 512 (Ethyl 3,7,11-trimethyldodeca-2,4-dienoate; EC_{50}=0,0022 mg/cm²), HS 2 (6-methyl-3-pyridyl geranyl ether; EC_{50}=0,0035 mg/cm²), ZR 615 (N-ethyl 3,7,11-trimethyldodeca-2,4-dieneamide; EC_{50}=0,0035 mg/cm²), ZR 777 (Prop-2-ynyl 3,7,11-trimethyldodeca-2,4-dienoate; EC_{50}=0,0039 mg/cm²) et ZR 515 (Isopropyl-11-methoxy 3,7,11-trimethyldodeca-2,4-dienoate; EC_{50}=0,0094 mg/cm²). Dans ce test l'àctivité a été semblable à celle que l'on a mentionnée chez les insectes et s'est montrée en accord avec la sensibilité de ces composés à l'inactivation métabolique. Les résultats suggèrent que le ZR 615 pourrait être utilisé dans la lutte contre les tiques.*

INTRODUCTION

While much information on the activity of insect juvenile hormone mimics in a number of insect species has been given in the literature (Henrick, Willy & Staal, 1976; Staal, 1975), few reports on the activity of these compounds in other arthropods have appeared. Sannasi & Subramonian (1972) showed that juvenile hormone mimics do not affect rupture of larval diapause in *Rhipicephalus sanguineus* but according to the findings of Bassal (1974), working with *Hyalomma dromedarii*, and of Solomon & Evans (1977), working the *Boophilus decoloratus, B. microplus* and *Amblyomma hebraeum*, they prevent egg hatch when applied to adult engorged females. Mansingh & Rawlins (1977) report that high doses of insect juvenile hormone mimics applied to adult *B. microplus* cause mortality, a reduction in the number of eggs produced and an inhibition of egg hatch in those eggs that were laid. Koch (1975) reported that insect juvenile hormone mimics have also been shown to have toxic and ovicidal effects on the mites *Tetranychus urticae* and *Panoychus ulmi*.

In developing insects, juvenile hormone mimics act at a "sensitive stage" of the life cycle during which the animal is undergoing a metamorphosis (Wyatt, 1972). It thus seems likely that the effects of juvenile hormone mimics will be most noticeable at similar sensitive stages in the life cycle of non-insect arthropods. After engorgement, larval ticks undergo a metamorphosis to the nymphal stage, involving, amongst other changes, the development of an extra pair of legs. As this metamorphosis could possibly be a sensitive stage, we decided to investigate the effects of these compounds in larval ticks. This paper describes a bio-assay for juvenile hormone mimics in newly engorged *A. hebraeum* larvae and summarizes the results of the screening of 14 of these compounds.

MATERIALS AND METHODS

The juvenile hormone mimics ZR 512 (Ethyl 3,7,11-trimethyldodeca-2,4-dienoate), ZR 515 (Isopropyl-11-methoxy 3,7,11-trimethyldodeca-2,4-dienoate), ZR 725 (11-methoxy 3,7,11-trimethyldodeca-2,4-dienoic acid), ZR 614 (N,N-diethyl-11-methoxy 3,7,11-trimethyldoceca-2,4-dienamide), ZR 615 (N-ethyl 3,7,11-trimethyldodeca-2,4-dienamide), ZR 618 (N-ethyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienamide), ZR 619 (Ethyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienethiolate), ZR 777 (Prop-2-ynyl 3,7,11-trimethyldodeca-2,4-dienoate) and ZR 856 (Hexadecyl cyclopropanecarboxylate) were kindly supplied by Zoecon Corporation of California. HS 2 (6-methyl-3-pyridyl-geranyl ether), HS 103 (6-ethyl-3-pyridyl-geranyl ether), HS 104 (6-ethyl-3-pyridyl-6,7-epoxygeranyl ether), R 20458 (1,4′-ethylphenyl-6,7-epoxygeranyl ether) and R31026 (N-ethyl-1,2-bis (isobutylthiocarbamoyl) ethane were kindly supplied by Stauffer Chemical Corporation, also of California.

Since preliminary trials showed that the topical application of acetone or ethanol in volumes as low as 0,1 µl is toxic to engorged *A. hebraeum* larvae, exposure to impregnated filter paper discs had to be used as an application method. Stock solutions of test substances were made up in AR cyclohexane and stored at —20°C. Known amounts of each substance in 0,5 ml cyclohexane were applied to Whatman No. 1 filter paper discs (with an area of 3,8 cm²), supported on stainless steel pins. The solvent was evaporated by placing the discs in a current of air at room temperature (22°C) for 30 min, after which the discs were placed at the bottom of 16 ml pill vials.

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Received 24 November 1977—Editor

Tick larvae were fed on the ears of albino rabbits and the engorged larvae collected between 08h00 and 09h00 within 24 h of drop. They were washed with distilled water, dried in a current of air and immobilized on a cold plate at 4°C. Groups of about 50 larvae each were transferred to the pill vials which were then stoppered with an open-cell polyurethane plastic bung and maintained in an incubator at 25°C, 80% RH, 14 h light:10 h dark until nymphal emergence was complete. At least 6 replicates were set up for each concentration of hormone mimic used.

Abbott's formula (Abbott, 1925) was used to correct for control response and the dose effect lines were plotted on log-probit paper. Fit of the dose-response line was optimized and the EC_{50} calculated by the method of Litchfield & Wilcoxon (1949). Controls, exposed to paper impregnated with cyclohexane only, were set up for each batch of ticks used.

Result and Discussion

The only obvious effect of juvenile hormone mimic treatment was that fever nymphs emerged. Unemerged nymphs were surrounded by a loose larval cuticle and appeared to have died just prior to emergence. Despite careful dissection and inspection of emerged and pharate nymphs, no larval-nymphal intermediates such as those seen in insects (Wyatt, 1972) were found. Apparently, therefore, juvenile hormone mimics do not block nymphal emergence by interfering with the easily observable changes that occur during metamorphosis. The nymphs may die because they have been affected at a cellular or biochemical level. Where larvae were treated with HS 104, nymphs died shortly after emergence at all concentrations of the compound used. However, since nymphal emergence was used as the activity criterion, these deaths were not taken into account in calculating EC_{50} values.

While the application of hormone mimic by the exposure of tick larvae to impregnated paper may be affected by various factors, such as the volatility, physical state and viscosity of the substance under test as well as the degree of activity of the test organisms, this method was the only practicable one available. It is similar to the type of exposure one would expect in the field if these compounds were used on the host animals. The differences in the physical state, viscosity and vapour pressure of the juvenile hormone mimics were minimal (Staal, personal communication). This, plus the uniform lack of activity and the large number of larvae used per concentration, suggests that variations of effectiveness due to physical factors are not great and that the results are comparable. EC_{50} values for the compounds are shown in Fig. 1.

Those compounds that had already been tested in insects (Henrick, *et al.*, 1976) were shown to have similar effects on ticks. ZR 512, 615, 777 and 515 were all quite active in insects and in the ticks in this assay. ZR 614 and 618, however, are less active in ticks than in insects and are also relatively less active than the structurally similar ZR 615 in ticks. Unlike ZR 615, both ZR 614 and 618 contain a methoxy group which may act as a site for inactivation and result in a more rapid breakdown and reduced activity. The same argument can be used for ZR 515 which is less active than ZR 512. ZR 725 has been reported to be a metabolite of ZR 515 and has lower activity than the parent compound in insects (Solomon & Metcalf, 1974). Its lack of activity in this assay is not surprising as it contains a carboxylic acid group which would be expected to reduce its lipid solubility and hence its penetration through the cuticle as well as allow for more rapid conjugation and excretion. ZR 856, although highly active against mites (Staal, Ludvik, Nassar, Henrick & Willy, 1975), has low activity in insects, and its lack of activity in ticks points to the similarity in response of ticks and insects to juvenile hormone mimics.

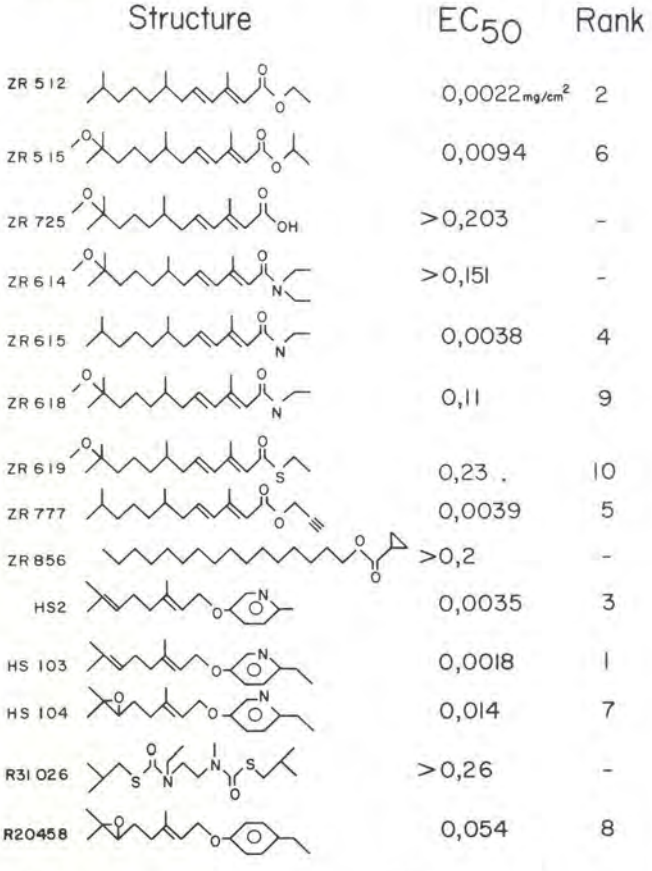

FIG. 1 Structure of juvenile hormone mimics aud EC_{50} for prevention of nymphal emergence in engorged *Ambiyomma hebraeum* larvae

The highest activity was shown by HS 103, followed quite closely by HS 2, while HS 104 and R 20458 were much less active than HS 103. Both the former compounds contain an epoxide functional group which is hydrolyzed to the diol by epoxide hydrase enzymes in insects (Hammock, Gill & Casida, 1974) and this may account for the lower activity of these two compounds. The low activity of R 31 026 is difficult to explain as no isosteres of this compound were tested.

There is little correlation between the activity of the juvenile hormone mimics in this assay and in the egg desiccation assay carried out by Solomon & Evans (1977). These mimics apparently cause egg desiccation by changing the properties of the egg waxes, and this suggests that the modes of action in the two assays are different. There is a close similarity between tick larvae and insects in their response to insect juvenile hormone mimics. Whether ticks possess a hormone similar to insect juvenile hormone is debatable, however, because, unlike that of the insects, the life cycle of ticks (at least in the ixodid ticks) is short and metamorphosis takes place at each moult. The major function of juvenile hormone in the developing insect is the prevention of premature metamorphosis into the nymph, pupa or adult (Wyatt, 1972) and it would seem unnecessary in ixodid ticks unless it is involved

in the control of the development of adult characters such as the reproductive system. In this regard, the effect of juvenile hormone mimics on the nymphal-adult metamorphosis will be interesting.

It is likely that these compounds will have similar effects on ticks other then those tested in this assay. The compounds could possibly be used for tick control, particularly of single-host species such as *B. decoloratus* and *B. microplus* which would be accessible during the sensitive period. *A. hebraeum* larvae do not feed exclusively on cattle in the field and the control of these and other multihost ticks by these compounds would be less successful. The relatively high activity of ZR 615 in preventing nymphal emergence and in causing egg desiccation (Solomon & Evans, 1977) suggests that his compound should be considered as a candidate for further *in vivo* trials.

Acknowledgements

We would like to thank Dr H. M. Schwartz and Miss J. B. Walker for their constructive comments on this manuscript. We are indebted to Dr G. B. Staal of Zoecon Corporation and Dr A. H. Frieberg of Stauffer Chemical Corporation for the supply of juvenile hormone mimics and to Messrs J. Putterill, P. Mokoena and F. Lebelo for their able assistance in this work.

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