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Uptake, Retention, Metabolism and Excretion of [22,23-$^3H_2$]Dihydroazadirachtin in *Schistocerca gregaria*

P. A. PARANAGAMA,* H. LOVELL,† A. A. DENHOLM,† S. V. LEY,† J. D. CONNOLLY,‡ R. H. C. STRANG*,§

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[22,23-$^3H_2$]dihydroazadirachtin was used as a tracer of azadirachtin to follow the distribution and metabolism of the tertranortripenoid in the locust *Schistocerca gregaria*. Injected tracer was rapidly removed from the haemolymph into many of the locust tissues. This uptake was inhibited by a large excess of injected dihydroazadirachtin, suggesting that the uptake was by a specific mechanism.

High concentrations of injected unlabelled derivatives of azadirachtin inhibited to different extents the clearance of the tracer from the haemolymph. The results suggest that azadirachtin and its derivatives have different affinities for the uptake mechanism. Radiolabelled dihydroazadirachtin applied topically was shown to penetrate the locust cuticle quite slowly. A large fraction of the tracer was present in the fat body as well as gut and nervous tissue. Binding was persistent and not easily displaced. There was no evidence that dihydroazadirachtin was secreted into the primary urine by an active pumping mechanism. Metabolism of the dihydroazadirachtin was largely restricted to fat body and crop.

[22,23-$^3H_2$]Dihydroazadirachtin *Schistocerca gregaria* Tissue uptake Metabolism Excretion

INTRODUCTION

Azadirachtin (Fig. 1), one of many complex terpenoids present in the seeds of the neem tree (*Azadirachta indica*), was originally identified as the major component in the antifeedant activity of the plant, to which the locust *Schistocerca gregaria* is particularly sensitive (Butterworth and Morgan, 1971).

Since then, the compound has been shown to have a range of biological activities in many insects, mainly concerned with metamorphosis and development. Although these effects have been well characterised in whole insects, the mode of action at the physiological and biochemical level of specific tissues still remains obscure, although much evidence points to effects on neurohormonal metabolism and release (Subrahmanyam and Rembold, 1989).

In view of its biological effects, it has been proposed that some insect tissues possess specific receptors for azadirachtin (Käuser and Koolman, 1984). Evidence that close analogues of the compound have different biological potencies support this contention (Rembold, 1988).

To investigate this hypothesis more exactly, a radiolabelled tracer is required and Rembold *et al.* (1983) introduced the use of [22,23-$^3H_2$]dihydroazadirachtin. Although the dihydro derivative is structurally different from azadirachtin (Fig. 1), current evidence suggests that its biological activity is the same (Rembold *et al.*, 1983). Rembold and his co-workers (Rembold *et al.* 1983, 1988; Subrahmanyam and Rembold, 1989; Sieber and Rembold, 1983) have done preliminary work with both locusts and other insects which has indicated that specific tissues, e.g. Malpighian tubules, do have differential affinities for tritiated dihydroazadirachtin.

The aim of the present work was to confirm and extend the previously reported results, and in particular to try to establish, by the use of unlabelled analogues, the nature of the uptake of dihydroazadirachtin into locust tissues, as a preliminary to attempting more exact binding studies on identified tissue fractions.

A secondary objective was to examine uptake after topical application of the radiolabelled compound, and so determine if the route of application determined tissue distribution.

Finally, as the effects of azadirachtin and its analogues are often apparent over periods of days rather than minutes, the rate and extent of metabolism is obviously

*Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, U.K.
†University Chemical Laboratory, Cambridge CB2 1EW, U.K.
‡Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.
§To whom all correspondence should be addressed.