Acta Tropica 160 (2016) 53–57

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Responses of *Glossina pallidipes* and *Glossina morsitans morsitans* tsetse flies to analogues of δ-octalactone and selected blends

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ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 20 April 2016
Accepted 23 April 2016
Available online 30 April 2016

Keywords:
Lactones
Structure-activity
Glossina pallidipes
Glossina morsitans morsitans
Repellency
Attraction

ABSTRACT

Previous studies have shown that δ-octalactone is an important component of the tsetse-refractory waterbuck (*Kobus defassa*) repellent odour blend. In the present study, structure-activity comparison was undertaken to determine the effects of the length of the side chain and ring size of the lactone on adult *Glossina pallidipes* and *Glossina morsitans morsitans*. The responses of the flies to each compound were studied in a two-choice wind tunnel. Increasing the chain length from C3 (δ-octalactone) to C4 (δ-nonalactone) enhanced repellency to both species (*G. pallidipes* from 60.0 to 72.0%, and *G. m. morsitans* from 61.3 to 72.6%), while increasing the ring size from six (δ-octalactone) to seven members (ε-nonalactone) changed the activity from repellency to attraction that was comparable to that of the phenolic blend associated with fermented cow urine ($p > 0.05$). Blending δ-nonalactone with 4-methylguaiacol (known tsetse repellent) significantly ($p < 0.05$) raised repellency to 86.7 and 91.7% against *G. pallidipes* and *G. m. morsitans* respectively. Follow-up Latin Square Designed field studies (Shimba hills in coastal areas in Kenya) with *G. pallidipes* populations confirmed the higher repellence of δ-nonalactone (with/without 4-methylguaiacol) compared to δ-octalactone (also, with/without 4-methylguaiacol). The results show that subtle structural changes of olfactory signals can significantly change their interactions with olfactory receptor neurons, and either shift their potency, or change their activity from repellence to attraction. Our results also lay down useful groundwork in the development of more effective control of tsetse by 'push', 'pull' and 'push-pull' tsetse control tactics.

1. Introduction

Previous surveys of different tsetse species (*Glossina* spp.) in varying habitats have shown differential attraction to and feeding on available vertebrates irrespective of their relative abundance (Weitz, 1963; Vale, 1974; Grootenhuis, 1986; Turner, 1987; Moloo, 1993; Grootenhuis and Olubayo, 1993; Clausen et al., 1998). In a follow up set of studies, comparison of the behaviour of teneral *Glossina morsitans morsitans* on waterbuck (*Kobus defassa*), a tsetse refractory bovid, and on two preferred hosts, buffalo (*Syncerus caffer*) and ox (*Bos indicus*), suggested the presence of volatile and short-range allomones on the waterbuck odour (Gikonyo et al., 2000). Examination of odour profiles of the three bovids by Gas chromatography–linked Mass spectrometry and electroantennography (GC–MS and GC-EAD, respectively), showed some constituents (phenols and aldehydes) that are common to the three bovids, but there were also a series of others (15 compounds) that were specific to waterbuck (Gikonyo et al., 2002). These included straight chain carboxylic acid (C5–C10), 2-alkanones (C8–C12 homologues and geranylacetone), phenols (guaiacol and carvacrol), and δ-octalactone (Fig. 1). In a laboratory 2-choice wind tunnel, *G. m. morsitans* showed a pattern of responses to synthetic blends of these compounds that suggested avoidance behaviour, significantly different from their responses to attractive blends associated with odours of preferred hosts (Gikonyo et al., 2003).

In a recent study, the effects of different blends of these compounds on catches of *Glossina pallidipes* in attractant-baited NG2 G traps were evaluated in the field (Bett et al., 2015). Each class of constituents (acids, ketones, phenols and δ-octalactone) was found to significantly reduce catches, indicating that each contributes incrementally to the repellency of the waterbuck odour. However, within each multi-component class of compounds (carboxylic acids, ketones, and phenols), large variations in intrinsic individual repellency to *G. pallidipes* were found. Among the

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http://dx.doi.org/10.1016/j.actatropica.2016.04.011
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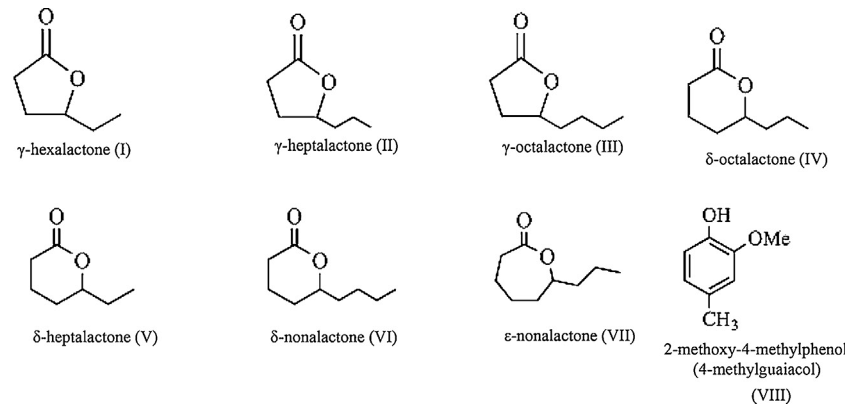

Fig. 1. Schematic diagrams of δ-octalactone and its selected analogues and 4-methylguaiacol evaluated.

carboxylic acids, the lower (C5–C7) were found to be repellent unlike the higher (C8–C10) homologues, which did not show any significant repellency. On the other hand, of the ketones, the higher molecular weight compounds (C11, C12 homologues and geranylacetone) were significantly more repellent than the lower (C8–C10) homologues. Of the two phenols, guaiacol was found to be more repellent than carvacrol. Guaiacol was previously shown to be a mild tsetse repellent (Torr et al., 1996). In a structure-activity study with different analogues of this phenol, replacement of H with a CH_3 group (4-methylguiacol) was found to significantly increase repellency to tsetse (Saini and Hassanali, 2007). The *G. m. morsitans* and *G. pallidipes*, restricted to the savannah, are most widespread and common vectors of livestock and human trypanosomiasis in sub – Sahara Africa (Jordan, 1986; Onyango et al., 1966; Willett, 1965).

The objective of the present study was to extend the study on structural variants of δ-octalactone, and to see how these modifications affect the olfactory responses of *G. pallidipes* and *G. m. morsitans* flies to the different analogues. Specifically, the effects of two types of structural variants were studied: increased or decreased size of the lactone ring and that of the hydrocarbon side chain. In addition, the effect of blending more repellent lactones with 4-methylguiacol was also evaluated.

2. Materials and methods

2.1. Laboratory test insects

Glossina pallidipes and *G. m. morsitans* were obtained from colonies maintained at Biotechnology Research Institute, Kenya Agricultural and Livestock Research Organization, Muguga, Kenya. Booth colonies were established from seed puparia material received from the large colonies maintained at the International Atomic Energy Agency (IAEA) labs, Seibersdorf, Austria. The IAEA *G. pallidipes* colony originated from wild pupae collected from Lugala, Uganda in 1975, and northern Zimbabwe in 1987 (Ciosi et al., 2014). The *G. m. morsitans* colony originated from wild pupae collected from Zimbabwe in 1983. The flies were reared in an insectary under controlled environmental conditions (25 ± 2 °C, 75 ± 2% RH and LD 12:12 h photoperiod), and received defibrinated bovine blood through an artificial feeding system three times per week (Moloo, 1971).

2.2. Test compounds

Racemic blends (98–99% pure) of γ-hexalactone, γ-heptalactone, γ-octalactone, δ-octalactone, δ-heptalactone, δ-nonalactone and 4-methylguaiacol (Fig. 1) were sourced from Sigma-Aldrich, Taufkirchen, Germany. Racemic ε-nonalactone (Fig. 1) was synthesized in the laboratory using the method of Gikonyo et al. (2002) and its structure confirmed by HR-MS, ^{13}C NMR, ^{1}H NMR and IR spectrophotometry. In addition, two blends (A, δ-octalactone + 4-methylguaiacol) and B (δ-nonalactone + 4-methylguaiacol) were prepared in 1:1 ratio.

2.3. Responses of G. pallidipes and G. m. morsitans to δ-octalactone, analogues and selected blends in a wind tunnel

The study was undertaken in a two-choice cuboidal plexi-glass wind tunnel (195 cm × 20 cm × 20 cm) following the procedure outlined by Gikonyo et al. (2003). Briefly, the effect of each test compound and selected blends at 3 doses (0.05, 0.25 and 0.5 g/ml) in 1000 μl dichloromethane (CH_2Cl_2) was compared with that of the pure solvent (CH_2Cl_2) in three sets of replicates, each with ten individual flies of each species. The experiment was conducted in the mornings (0800–1200 h) and afternoons (1500–1700 h) coincident with the natural tsetse feeding hours. Purified air from an air cylinder flowed from both sides of the tunnel at 12.63 l/min. The following observations were recorded three minutes post-exposure: (i) number of flies departing from the midsection, (ii) initial direction of flights upwind, and (iii) final landing and resting position (control or treated arms of the tunnel). After each replicate cycle, the tunnel, metallic racks and release cages were cleaned with water and then with 70% ethanol. Blank tests were conducted to confirm no residual effects of previous test materials.

2.4. Responses of G. pallidipes to δ-octalactone and δ-nonalactone with/without 4-methylguaiacol in the field

Studies were conducted at Shimba Hill National Reserve (004° 15′ 26″S, 039° 23′16″E) altitude 403 m) in Kwale County, Kenya where wild populations of *G. pallidipes* occur. Large mammal populations in the reserve include sable antelopes, elephants, buffalos, bushbucks, warthogs, bush pigs, giraffes, leopards, duikers, hartebeest and monkeys. The vegetation consists mostly of coastal rainforest and semi-evergreen woodland and grassland. The area experiences long and short rainy seasons from April to June, and October to November, respectively. The mean annual rainfall level is between 855 and 1682 mm. Maximum daily temperatures are highest in March and November, often reaching 31 °C. June to July are the coolest months, with daily maximum temperatures of about 27 °C.

The effects of δ-octalactone and δ-nonalactone with/without 4-methylguaiacol on *G. pallidipes* catches in NG2 G traps baited with acetone (∼500 mg/h) and fermented cow urine (1000 mg/h) (Brightwell et al., 1991) were evaluated in the field in