EFFECTS OF STRUCTURAL MODIFICATIONS OF TSETSE-REPELLANT δ-OCTALACTONE ON THE RESPONSES OF *Glossina pallidipes* and *G. morsitans morsitans*

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of any degree in any other university.

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Signature……..Date…….

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We confirm that the work reported in this thesis was carried out by the candidate under our supervision as university supervisors.

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DEDICATION

I dedicate this work to my late aunt Trusillah Nyaburi, my grandparents Charles Makang'a and Grace Nyambori. My mother Loice Kerubo, my wife Everlyne Mogoi and my daughter Adrianna Nyaburi for their love, encouragement and immeasurable support that enabled me to complete my work.

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ABBREVIATIONS AND ACRONYMS

AAT	Animal African Trypanosomiasis
ANOVA	Analysis of Variance
BHC	Benzene hexachloride
DCM	Dichloromethane
DDT	Dichloro Diphenyl Trichloroethane
EAG	Electroantennography
GC-EAD	The gas chromatography–electroantennographic detector
GC-MS	Gas chromatography linked to mass spectrometer
GC-MSD	Gas Chromatography linked to Mass Spectrophotometer detector
HAT	Human African Trypanosomiasis
IAEA	International Atomic Energy Agency
IR	Infra red Spectroscopy
NG2B	Type of tsetse fly trap
NGU	Type of tsetse fly trap used in Nguruman
NMR	Nuclear Magnetic Resonance Spectroscopy
PVC	Polyvinyl chloride
R_t	Retention time
SAT	Sequential aerosol technique
SIT	Sterile-insect technique
SNK	Student-Newman-Keul test
TLC	Thin layer chromatography
WHO	World health organization

ABSTRACT

Tsetse flies (*Glossina spp*.) are vectors of Animal African Trypanosomiasis and Human African Trypanosomiasis. Two approaches have been used to combat the diseases: parasite and vector control. Parasitic control by trypanocidal drugs has so far failed due to problems of availability, toxicity and resistance development. Vector control by use of insecticides is ineffective. Trapping using baits have been relatively successful, except among pastoralists. Integration of repellants and attractants to create push-pull strategy may provide an effective control tactic at individual farmer and pastoralist level. A previous study on waterbuck, led to the identification of a blend of 15 electrophysiologically active constituents: six C_8-C_{13} methylketones, two phenolic compounds, six C_5-C_{10} fatty acids and δ-octalactone that is repellant to savanna tsetse flies. δ-Octalactone has been shown to singly elicit allomonal responses, and a follow-up study has shown that its structural modification can bear both repellants and attractants. In the present study, the effects of some δ-octalactone analogues on two *Glossina spp.* were carried out to elucidate their activities. (RS)-3-propylcyclohexanone (**15**) and (RS)-δ-valerolactone (**16**) and were synthesized and their structures confirmed using spectroscopic techniques, while 2-propyloxane (**14**) and (RS)-3-propylcyclohex-2-enone (**17**) were acquired commercially. The responses of the two *Glossina spp.* to each of the four analogues were determined in a two-choice wind tunnel. Data collected were subjected to Analysis of Variance and means ranked using Student-Newman-Keuls test and the tsetse preferences were compared using t-Test. In the bioassays, it was noted that (RS)-δ-valerolactone (**16**) and (RS)-2-propyloxane (**14**) elicited avoidance; whereas (RS)-3-propylcyclohex-2-enone (**17**) and (RS)-3-propylcyclohexanone (**15**) elicited attraction. There was no significant difference between the repellency of (RS)-δ-valerolactone (**16**) and 2-propyloxane (**14**) ($p > 0.05$) to both *Glossina spp.* However, the repellency of both, (RS)-2-propyloxane and (RS)-δ-valerolactone, were significantly lower than that of δ-octalactone (**5**) ($p < 0.05$). When (RS)-δ-valerolactone (**16**) and (RS)-2-propyloxane (**14**) were blended it was noted that the repellency was comparable to that of δ-octalactone (**5**) ($p > 0.05$) when *G. pallidipes* were deployed, but there was no significant improvement in repellency against *G. m. morsitans.* The attractancy of (RS)-3-propylcyclohex-2-enone (**17**) and (RS)-3-propylcyclohexanone (**15**) to the two tsetse species were comparable ($p > 0.05$). Blending the two attractants did not lead to any change to both tsetse species ($p > 0.05$). The study identifies potent attractants and repellants on the two *Glossina spp.* and therefore lays a useful basis for the development of more effective behavioural control of the tsetse species.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Tsetse flies are hematophagous insects that feed solely on vertebrate blood and transmit protozoan parasites of the *Trypanosoma* genus, which cause two types of Trypanosomiasis: Human African Trypanosomiasis (HAT) – Sleeping sickness and Animal African Trypanosomiasis (AAT) – Nagana (Maniania *et al*., 2003). They are classified as insects of medical and agricultural importance (Dr, 2005). Regardless of their low fertility, tsetse flies show great resilience which makes population control costly and untenable to the capacities of private and public sectors (Gooding, 2005).

Tsetse flies infest an area of 9 million km^2 in Sub-Saharan Africa, making 60 million people in 37 countries susceptible causing a human disease prevalence of about 500,000 (Cattand *et al*., 2001). HAT, is fatal if not treated, affect a wide range of people in Sub-Saharan Africa (Symeonakis *et al.*, 2007). HAT coupled with AAT have been a critical hindrance to Sub-Saharan African rural development, and a serious problem to agricultural production (Simarro *et al*., 2008). Human infections shrink labour and disrupt human settlement patterns throughout much of Sub-Saharan Africa. On the other hand, animal infections reduce availability of meat, milk, and deprive African farmers off draught animal power which significantly minimizes crop production (Vreysen, 2001). HAT and AAT, are therefore responsible for the underdevelopment of the African continent, and are considered a major obstacle in the establishment of a prosperous agriculture to provide food security and create sustainable economic growth and healthy

populations (Koné *et al*., 2011). The estimated livestock production losses due to tsetse flies infestation is estimated to be US$ 5 billion annually (Symeonakis *et al*., 2007; Vreysen, 2011; WHO, 2012).

In Kenya, tsetse flies infest different places equivalent to an area covering 138,000 km^2 (Muriuki *et al*., 2005). They not only impoverish livestock farmers and threaten food security and livelihoods, but also have negative impact on tourism sector. Concerted control measures are key to avert tsetse fly effects on humanity and livestock where in addition to negative health effects, leads to annual economic losses estimated at Kshs. 20 billion (MoLD, 2011).

1.2 African Trypanosomiasis

African Trypanosomiasis is a contagious disease which infects both humans and animals (Barrett *et al*., 2003). The causative agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of the mammalian hosts, and are transmitted by the sting of infected tsetse flies (Krafsur, 2009). The distribution of Trypanosomiasis in Africa corresponds to the range of tsetse flies between 14° N and 20° S (Molyneux *et al.,* 1996).

1.2.1 The mammalian host

Wildlife such as warthog, bush pig, duiker, bush buck, kudu, buffalo and monitor lizard are the natural hosts of tsetse flies and may acquire lingering and symptomless trypanosome infections. Livestock exhibit a range of vulnerabilities to AAT infection

from refractory to highly susceptible (Bourn, 2001). The wildlife in Africa generally tolerates infection and often serves as a reservoir for human and livestock infective trypanosomes (Taylor, 2004). Monkeys, rats, mice, guinea pigs and rabbits can also be infected by trypanosomes. Wild ruminants, wild equidae, lions, leopards and wild pigs can serve as carriers. Susceptibility of cattle to Trypanosomiasis depends on breed, age, behaviour, previous exposure and health (Taylor, 2004).

1.2.2 Animal African Trypanosomiasis

AAT is caused by Protozoan parasites of *Trypanosoma* genus which are transmitted from one animal to another through the sting of an infected tsetse fly after biting either an infected human or animal (Desquesnes and Dia, 2004; Menu *et al*., 2005). These trypanosomes are: *Trypanosoma congolense, T. vivax* and *T. brucei brucei.* The latter species can be classified into three types namely: savannah, forest and kilifi types. Other species such as *T. simiae* and *T. godfreyi* have also been implicated to cause AAT. Multiple infections arise with more than one species of trypanosome (Fiennes *et al.,* 1970).

The host preferences of each trypanosome species may vary but *T. congolense, T. vivax* and *T. brucei brucei* have a wide host range among domesticated animals such as cattle, sheep, goats, horses, donkeys, pigs, camel, dogs and cats and wild animals such as buffalo. *T. godfreyi* and *T. suis* are responsible for infections in pigs. *T. simiae* appears to be most important in pigs but have also been reported in camels, horses and cattle (Taylor and Authié, 2004).

For most parts of Sub-Saharan Africa, cattle are the mainly affected because of the feeding preferences of tsetse flies hence in effect they can protect goats and pigs from Trypanosomiasis (Simo *et al*., 2006). Over 30 species in the wild or zoos and non-human primates are also known to be prone to infection and many of them remain active carriers and reservoirs of the trypanosomes (Grootenhuis, 2000). In domestic animals, trypanosomes cause a severe and often deadly disease whereas in wild animals they cause relatively mild infections (Mulligan *et al* 1970; Steverding, 2008).

The primary manifestation of AAT is a nodule at the site of the fly bite, which is not clearly manifested and is followed by other clinical signs such as intermittent fever, signs of anaemia, lymph-swelling, inactivity, eye discharge, emaciation and weight loss (Steverding, 2008). As the sickness progresses the animals continually weaken and ultimately become unproductive (Garcia *et al*., 2006). Consequently, AAT stifles and debilitates all aspects of animal production: fertility loss, milk yields get depressed, growth and work output are suppressed and the mortality rate reduces herd size (Barrett *et al*., 2003; Hotez *et al*., 2007; Miles, 2003).

AAT is often destructive at herd level and its effect on human populations is extensive and far-reaching. In most tsetse infested areas, not only does AAT cause a drastic decrease in meat production but it also causes a reduction in the amount of milk for consumption by African citizens (Simarro *et al*., 2008). The decrease in these products contributes to shortage of protein as well as risk of food insecurity for the tsetse infested countries (Shaw, 2004). Furthermore, animal draught is adversely affected, which limits

farm ploughing and transportation. In addition, AAT causes poor calving rates in cattle including sheep and goats, abortions in expectant animals, reduced herd size and high treatment costs. These factors overstretch and lower household incomes and as a result impede socio-economic development (Kabayo, 2002).

The death of animals due AAT causes direct monetary losses estimated at more than $1.2 billion every year. In addition, its wider costs in terms of reduced milk yields and dairy products, abortion and lost fertility results in reduced agricultural productivity estimated at some $4.5 billion every year (Fevre *et al*., 2008).

1.2.2.1 Epidemiology of African Animal Trypanosomiasis

AAT is a prevalent protozoan disease affecting wildlife and livestock in Sub-Saharan Africa with a scale of pathologies ranging from chronic and terminal to acute and rapidly fatal depending on the prevailing conditions (Bourn *et al*., 2001). The epidemiology of AAT is determined by four biological factors namely: trypanosomes, tsetse flies, reservoir hosts and livestock. Cattle are the domestic animals in which the disease is most frequently diagnosed and treated. When dealing with AAT, much depends on the distribution and the vectorial capacity of tsetse flies. Of the three groups of tsetse flies, the savannah and riverine species are the most important since they populate areas suitable for grazing and watering (Simukoko e*t al*., 2007).

1.2.2.2 Control of African animal Trypanosomiasis

1. Drug treatment

In most of Sub-Saharan Africa, AAT is curtailed solely by trypanocidal drugs (Steverding, 2008). Three products namely: Isometamidium chloride, homidium and diminazene aceturate are used and have been on the market more than 50 years. Isometamidium chloride is mainly used as a prophylactic drug and can provide up to six months protection against AAT. Homidium salts have both curative and prophylactic properties. Although it has been shown that diminazene provides a short term protection of two to three weeks, it is basically used for therapeutic purposes (Jamison *et al*., 2006).

Administration of these drugs is largely done by the individual farmers devoid of supervision by veterinary personnel. Consequently, this has lead to underdosing and overdosing which is a critical risk factor for side effects and drug resistance (Steverding, 2008). Drug resistance is on an upward surge and is now reported in 21 Sub-Saharan countries (Holmes *et al*., 2004). However, used properly, veterinary drugs permit higher levels of production, improve animal welfare and secure the livelihood assets which serve as economic mainstay for 700 million poor farmers in developing countries (Anene *et al*., 2001).

Due to the rampant use of trypanocidal drugs to tackle AAT in Sub-Saharan Africa, it is approximated that about 35 million doses of trypanocidal drugs are administered each year to an estimated 60 million cattle at risk of AAT (Holmes *et al*., 2004). Although there is a continuous and sustained demand for trypanocidal drugs by livestock keepers,

the African market of trypanocidal drugs estimated at about US$ 30 million, it is not viable to justify investment by pharmaceutical companies in the development of new animal trypanocidal drugs. (Jamison *et al*., 2006). Consequently, the control of AAT will continue to rely on the use of old drugs (Holmes *et al*., 2004).

2. Host management

The prevalence and presence of tsetse flies is popularly known in Sub-Saharan Africa. They therefore exploit this knowledge to cope the exposure of livestock to the flies. However, this approach is not viable especially in the dry season where livestock keepers are compelled to bring their herds to wetter places with more grass but often habouring tsetse flies (Kinung'Hi *et al*., 2006; Ohaga *et al.*, 2007).

Livestock keepers also exploit trypanotolerance characteristic of some livestock breeds to deal with AAT (d'Ieteren *et al*., 1998). In West Africa, the N'dama and in Kenya the Orma Boran which are trypanotolerant can be embraced to enhance livestock production in the tsetse infested areas. Rearing of trypanotolerant cattle breeds is among economically sound and environmentally friendly controlling methods of the effects of AAT. However, their small size and lack of strength to provide sustainable draught power, has made them very unpopular among farmers (Holmes, 2004; Geerts *et al*., 2009).

3. Tsetse Flies eradication

The elimination of tsetse flies is central to the curtailment of AAT. A variety of techniques for tsetse control exist. Most current methods depend on the severe vulnerability of tsetse flies to eco-friendly pyrethroid insecticides. These insecticides are applied by spraying the known fly resting sites or at minute volumes from the air. Spraying is carried out sequentially, consistently and sustainably to eradicate all tsetse flies (Allsopp, 2001).

They can also be decimated using traps and targets. Targets are an integration of cloth and netting baited with an odour attractant and impregnated with a pyrethroid insecticide. Traps work on the same mechanism but the fly is allured to go into a net chamber where it remains snared. Live bait techniques are also used. This method has been successfully used in Burkina Faso, Ethiopia, Kenya, Tanzania, Zambia, and Zimbabwe (Omolo *et al*., 2009).

Another approach is sterile insect technique which involves mass production of sterilized male flies, which compete with local males to mate with females. Because female tsetse flies generally mate only once, the result is unproductive offspring and a decrease of the wild tsetse population. This technique is costly, because it requires macro-rearing of flies, and it is only recommended for use once the wild tsetse population has been squeezed to low levels using other techniques (Vreysen, 2001). Despite the substantial progress made in eliminating the vector, an ideal method easily accessible to the population at risk still does not exist.

1.2.3 Human African Trypanosomiasis

HAT occurs in two forms: a chronic form caused by *T. b. gambiense*, which occurs in West and Central Africa, and the acute form, caused by *T. b. rhodesiense*, which occurs in Eastern and Southern Africa (Kabayo, 2002; Kennedy, 2008).). The chronic infection lasts for years while the acute disease may take only weeks before death occur (Simarro *et al*., 2008; Biryomumaisho *et al*., 2013). *T. b. gambiense* accounts for over 95% of reported cases while *T. b. rhodesiense* accounts for 5% of the reported cases (Kabayo, 2002 and Simarro *et al*., 2008). Several species of tsetse flies, *G. palpalis*, *G. morsitans*, *G.pallidepes* and *G. swynnertoni*, are the vectors of HAT (Smith *et al*., 1998). Tsetse flies come into contact with people, cattle and wild animals, all acting as reservoirs for the Trypanosoma parasites (Stich, 2002; Brun, 2010).

In the first stage of HAT, the trypanosomes multiply rapidly in the blood and the lymphatic system (Fevre *et al*., 2008). In HAT caused by *T .b. gambiense*, the first phase lasts for years without the infected person noticing other than occasional fever with headaches and rheumatic pains. Yet it is also during this phase that severely swollen lymph glands in the neck ultimately show the disease's presence. HAT becomes fatal in its second phase, when the trypanosomes cross the blood-brain barrier and invade the central nervous system (Simarro *et al*., 2011).

The initial symptoms of HAT include bouts of fever, headaches, joint pains and itching, anaemia and swollen cervical glands, which mimic malarial symptoms (Ruiz, 2002). If left untreated, the patient becomes confused and has sensory disturbances, poor coordination and a general disruption of the sleep-wake cycle (sleeping sickness). These

signs characterize the second phase of the disease, which is always lethal (Legros *et al*., 2002).

Both forms of HAT leads to a confused mental state, severe sensory disturbances and abrupt changes in the sleep cycle that causes victims to fall asleep instantly regardless of the activities (Dumas and Bisser, 1999). These symptoms are followed by increasingly severe convulsions and neurological changes which culminate death. If HAT is diagnosed and treated in time, it can always be cured (Kennedy *et al*., 2004).

HAT is an incapacitating disease which impacts man's welfare in many ways such as disruption of family life, reduction in household labour, induction of abortions, sterility and gynecological disorders (Brun, 2010). It also leads to loss of time and income when seeking treatment. Treatment is often prolonged, complicated and costly (Welburn, 2001). HAT remains a major obstacle to the development of the entire affected regions (Feasey, 2010). Although, the epidemics of HAT were more widespread in the past, the most recent WHO estimates put 60 million people at risk currently with about 500,000 people with infections (WHO, 2012).

Most measures to diminish spread of HAT to humans and other vertebrate reservoirs target the control of the vector. Clinical treatment of both early and late onset HAT is limited and outdated and thus ineffectual for arresting the spread of the disease during the periods of epidemics. Therefore, understanding the ecological aspects that shape patterns of transmission to people and those that play a role in the re-emergence of HAT is

fundamental to the devise of new effective programs to curtail the burden of HAT in human populations (Cattand *et al.*, 2001).

1.2.3.1 Epidemiology of Human African Trypanosomiasis

1. West African Human African Trypanosomiasis

West African HAT is typically a terminal illness making it complex to be readily and easily diagnosed. In comparison to the East African form, *T. b. gambiense* has a longer evolutionary history with humans, having successfully adapted to establishing infections in human hosts without showing severe symptoms (Pépin and Méda, 2001). Vectors of the West African HAT are *G. palpalis*, most of which are in the vicinity of humans owing to their habitat and distribution. Although it is extensively recognized that the human-fly contact is the main corridor of transmission, some studies show a minor cycle involving an animal reservoir which may help to elucidate the re-emergence and lingering of the disease in Western Africa (Kennedy, 2008).

The epidemiology of West Africa form of HAT is far from being entirely understood. Despite the low levels of parasitemia in humans, it has successfully stamped endemic levels in many regions of West Africa (Pépin and Méda, 2001). It has also long been noted that the incidences of HAT do not correspond to the density of the *Glossina spp* populations and that epidemics often occur in areas where the density of the vector is low (Garcia *et al.*, 2006).

2. East African Human African Trypanosomiasis

East African form of HAT differs from West African form in both its epidemiology as well as its clinical signs in mammalian hosts. The clinical symptoms of East African HAT are more severe and the onset of the disease is swift. In contrast to *T. b. gambiense*, *T. b. rhodensiense* occurs with higher levels of parasitaemia in ruminants and humans which are the adventitious hosts. The vectors of *T. b. rhodensiense* are the *G. morsitans* subspecies, *G. pallidipes* and *G. swinnertoni* and on minor cases the *G. fuscipes* and *G. tachinoides* (Wint, 2001). Although the vectors normally feed on wild animals, under extreme situations where personal contact is pronounced due to social and environmental factors, a human-fly-human transmission sequence may arise causing an outbreak (Ford *et al.,* 2007).

1.2.3.2 Control of Human African Trypanosomiasis

1. Tsetse fly population suppression

The elimination of tsetse flies remains central to the control of HAT since tsetse flies are the sole vectors of disease transmission. Tsetse population suppression seeks to curtail the interaction between the flies and the host to destroy the transmission cycle. A number of methods have been engaged to control tsetse flies such as trapping tsetse flies using traps and targets, insecticides and sterile insect technique. These methods have worked with varying degrees of success (Kabayo, 2002). Despite the considerable progress made in eliminating the vector, an ideal method easily accessible available to the population at risk still does not exist.

2. Drug treatment

The drugs used vary depending on the phase of the disease. The first stage of *T.b. gambiense* HAT is treated with pentamidine, while suramin is used for the treatment of the first stage of *T. b. rhodesiense*. The second stage disease for both *T. b. gambiense* and *T.b. rhodesiense* are treated with melarsoprol. Eflornithine is also used for the treatment of second stage *T.b. gambiense* patients (Docampo and Moreno, 2003).

This approach has been futile in eradicating HAT because of a number of reasons: The disease often occurs in localized remote areas where it is often difficult to detect or predict, low prioritization of the disease by governments in endemic countries as a result budgetary constraints, the existence of domestic and wild animal reservoirs and absence of commercial diagnostic tests (Bouteille *et al*., 2003).

1.3 Tsetse fly distribution

1.3.1 Tsetse fly distribution in Africa

There are 23 species of tsetse flies in addition to eight sub-species currently confined to Africa (Cecchi *et al*., 2008). Tsetse sub-genera correspond to their three preferred habitats: Morsitans flies occupying woodland savannah, palpalis flies in lowland rain forests along watercourses and Fusca flies inhabiting lowland rain forests and gallery forests. Their distribution is limited primarily by ecological factors: Temperatures (20-28^{o}C), relative humidity (50-80%) and rainfall (630-1520mm) (Symeonakis *et al*., 2007).

1.3.1.1 The distribution and habitat of Morsitans group

There are seven species in this group. All are potential vectors of both HAT and AAT. They occupy the savannah woodlands that enclose the two major belts of lowland rain forests in Africa (Krafsur *et al.*, 2000). Their distribution corresponds to that of wildlife and water sources. In the wetter areas they roam frequently over the woodland but in drier regions their activities are limited to mesophytic vegetation of the watercourses (Rogers *et al*., 2004).

In Eastern and Southern Africa where *G. m. morsitans* is the most significant vector of HAT and AAT, the Miombo woodlands that spread from Mozambique to Tanzania in addition to the Mopane woodlands in Zambia and Zimbabwe are the typical habitats. The other sub-species *G. m. centralis* populate northwards from Botswana and Angola into Southern Uganda, nearer inland towards the lowland forests but also occurring in Miombo vegetation (Krafsur *et al*., 2000). *Glossina morsitans submorsitans* have an East to West pattern from Ethiopia to Senegal in Doka woodlands and can be irregularly found to occur in the southern Guinea savannah vegetation zone as well in the drier Sudan zone (DeVisser *et al*., 2010).

G. swynnertoni is confined to a small belt between Serengeti in Northern Tanzania and Maasai Mara in Southern Kenya. Both *G. longipalpis* and *G. pallidipes* have a much wider scope of possible habitats showing adaptability to different vegetation types (Sciarretta *et al*., 2010). *G. longipalpis* occurs mainly in the constricted Savannah belt just North of the rain forest in West Africa, from Guinea to Cameroon (Van den Bossche

and De Deken, 2002). The highly mobile *G. pallidipes* occurs in East Africa from Mozambique to Ethiopia. Finally, *G. austeni* occupy secondary scrub, thicket and islands of forest along the East African coast from Mozambique to Somalia (Cecchi *et al*., 2008).

1.3.1.2 The distribution and habitat of Palpalis group

Out of the nine species in this group, five *palpalis* and *fuscipes* sub-species transmit both HAT and AAT (Krafsur *et al*., 2008). Though they are continuously found in the lowland rainforests, some extend out to the savannah places mostly along watercourses. Their habitat exist mostly in the water bodies leading to the Atlantic Ocean and stretches from the wet mangrove and rainforests along the Coastal regions of West Africa to the drier savannah areas just north of the rain forests (Bouyer *et al*., 2007). They are less tolerant to the wide range of climatic conditions of the savannah belt and are therefore restricted to the eco-climate of the watercourses. Many of them such as the *G. p. palpalis* in Ivory Coast, prefer peridomestic conditions (de La Rocque *et al*., 2001).

Generally, most them are less suited to dehydrating conditions and therefore continue to exist in thick riverine forests (Hendrickx *et al.*, 2001). This is especially the case for the three fuscipes subspecies which are confined to hygrophytic habitats, rarely far from open water lacustrine habitats (Omolo *et al*., 2009). *G. tachinoides*, a riverine species, are found in Northern Nigeria and extend into human-inhabited savannah woodlands during the wet season, also showing strong adaptability to peridomestic habitats (Rayaisse *et al*., 2010).

1.3.1.3 The distribution and habitat of the Fusca group

With the exception of *G. brevipalpis* and *G. longipennis*, they are found in West African forests. Fusca group flies are vectors of HAT, but both *G. fusca* and *G. medicorum* are efficient vectors of AAT (Rogers *et al*., 2004). Their distributions rely mainly on forest vegetation and climatic factors. With the exception of *G. longipennis*, most of them inhabit moist, evergreen habitats either in riverine forests within savannahs such as *G. medicorum* or in dense and wet rain forests such as *G. tabaniformis* and *G. nigrofusca* (Malele, 2011). In contrast to the rest, the *G. longipennis* species lives in one of the driest habitats. Due to its pupal adaptation to dry conditions, its primary habitat consists of dry deciduous acacia bush that are intermittently spread around East Africa (Hendrickx *et al.,* 2001).

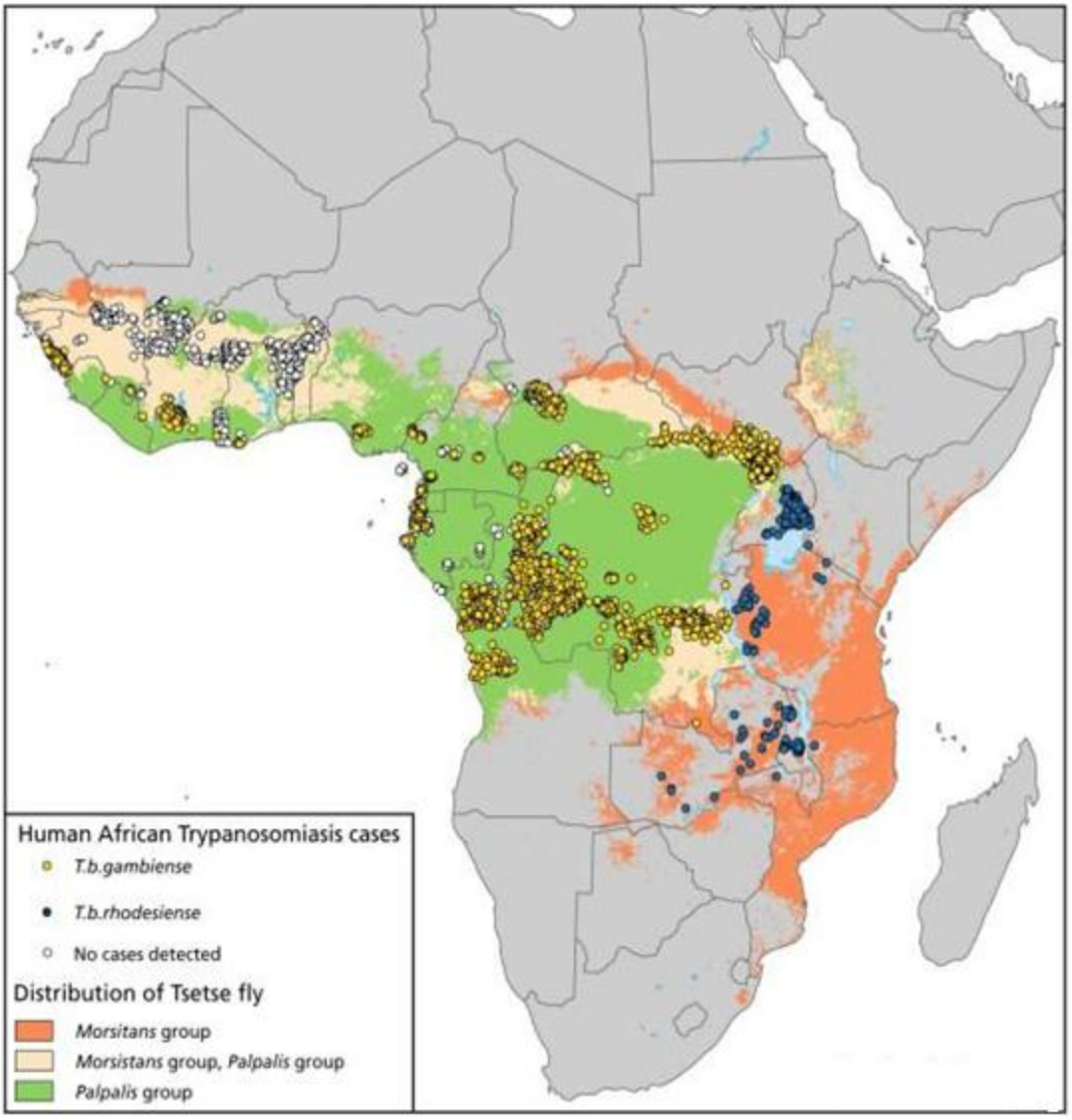


Figure 1.1: A map showing distribution of human African Trypanosomiasis (HAT) and tsetse flies in Africa (WHO, 2012).

1.3.2 Tsetse fly distribution in Kenya

Kenya has eight species of tsetse flies notably *G. pallidipes, G. austeni, G. swynnertoni* and *G. morsitans* in the Morsitans group; *G. longipennis, G. brevipalparis* and *G. fuscipleuris* in the Fusca group and *G. fuscipes* in the Palpalis group (Cecchi *et al*., 2008; DeVisser and Messina, 2009). Though the eight species of tsetse flies in Kenya exist and live in diverse habitats, their populations are concentrated in distinct zones: Arid and

semi-arid lands, western Kenya and Lake Victoria, North and South Rift Valley, Central Kenya, Transmara-Narok-Kajiado and Coastal belts (Muriuki *et al*., 2005). The tsetse fly belts are infested with one or more tsetse species with boundaries set by a variety of physical, biological and anthropogenic barriers. *G. pallidipes* and *G. fuscipes* are the two most significant tsetse species in Kenya because they are considered "efficient spreader" of Nagana and sleeping sickness respectively (DeVisser and Messina, 2009).

Belt 1: North and South Rift Valley belts

Belt 3 and 4 are clustered together since they are geographically connected. These zones comprise the South and North Rift Valley belts that lie just East of Uganda within the Rift Valley Province and are longitudinally split by the Great Rift Valley. The climate ranges from dry sub-humid in the southern part of the belt to semi-arid in the north with dramatic wet and dry seasons (Mattioli *et al*., 2004). Vegetation cover consists mainly of desert shrubs and grasses with savanna bordering the region to the south, east and west (Delgado *et al*., 1999). As of 1996, the most common species of tsetse confirmed in the belts was *G. pallidipes* (Swallow, 2000), although no HAT cases have been reported in this zone. *G. pallidipes* may also be a vector for *T. brucei, T. congolense* and *T. vivax* capable of infecting cattle. AAT infections among cattle in belt 3 and 4 have been reported (Courtin *et al*., 2008).

Belt 2: Arid and semi-arid lands

Found in north of Mt. Kenya, including parts of the Rift Valley and Eastern Provinces. The area lies north of high potential agricultural lands and has typically been used as a rangeland (Mattioli *et al*., 2004). Since 1967, an influx of people into traditional grazing

lands resulted in shrubland deforestation and the subsequent obliteration of tsetse fly habitat resulting in a redistribution (Oluwafemi, 2009). The presence of *G. longipennis* has been confirmed in the southwest area of the zone (Swallow, 2000) and may be a reservoir for *Trypanosome brucei spp*. AAT is not often found in areas infested by *G. longipennis* since this species thrives in tropical forests unfavourable to the tending of livestock (Cecchi *et al*., 2008).

Belt 3: The Central Kenya belt

It is found in the Central and Eastern Provinces. This area includes parts of Kenya's agricultural highlands which have a higher elevation and much moister climatic conditions. These areas have high agricultural potential and are intensely farmed (Mattioli *et al*., 2004). *Glossina pallidipes* are found in this zone but no HAT cases have been reported (Hursey and Slingerbergh, 1995).

Belt 4: The Coastal belt

It lies in the Coast Province along Kenya's eastern edge bordering the Indian Ocean whereas the southwestern portion of the zone includes the Coast and Tana River regions. Adjacent to the west of the zone are the Tsavo East and West National Parks and Chyulu Hills National Park (Delgado *et al*., 1999). Tsetse species found in this belt include *G. pallidipes, G. brevipalpis, G. austeni* and *G. longipennis* which are capable vectors of *T. rhodesiense* responsible for AAT infection (Oluwafemi, 2009). The AAT infection rate among cattle in this zone is the highest of all tsetse belts (Swallow, 2000).

Belt 5: Transmara-Narok-Kajiado belt

This belt lies within the Southern part of the Rift Valley Province along the Tanzanian border and includes the Masai Mara Game Reserve. The belt lies just south of the high potential agricultural lands and the climate is primarily semi-arid (Mattioli *et al*., 2004 and Delgado *et al*., 1999). The confirmed tsetse species are *G. swynnertoni, G. brevipalpis, G. pallidipes, G. fuscipleuris,* and *G. longipennis* which are capable vectors of *T. rhodensiense* that cause AAT (Swallow, 2000).

Belt 6: The Western Kenya and Lake Victoria belt

It encompasses the Western and Nyanza provinces along the Ugandan border and the banks of Lake Victoria (Delgado *et al*., 1999). The climate is humid and the region is part of the high potential agricultural zone and is therefore used for intensive crop farming (Mattioli *et al*., 2004). Tsetse species include widespread *G. f. fuscipes* and *G. pallidipes* (Hursey *et al*., 1995). *G. pallidipes* may host *T. b. rhodesiense* and HAT cases have been reported in this zone. Both tsetse species are vectors for AAT infection in this zone (Rutto and Karuga, 2001).

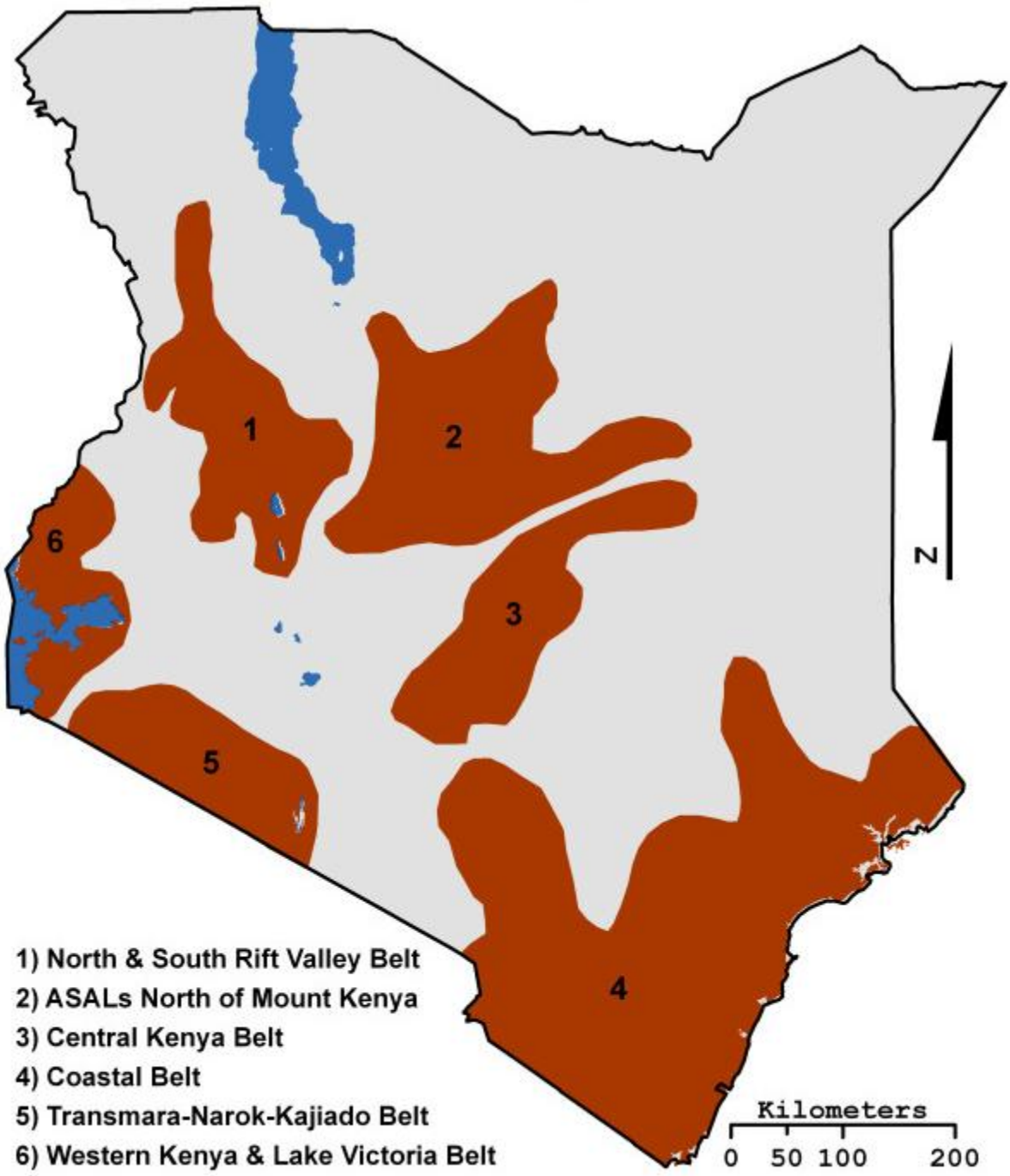


Figure 1.2: A map showing distribution of tsetse flies in Kenya (Muriuki *et al*., 2008).

1.4 Statement of the problem and justification

Tsetse flies (*Glossina spp*.) transmit different species of trypanosomes which cause Animal African Trypanosomiasis (AAT) in livestock and Human African Trypanosomiasis (HAT) in humans. In Sub-Saharan Africa, AAT threatens over 45 million cattle while HAT threatens over 60 million people. Two approaches have been used to combat the effects of AAT and HAT: parasitic and vector control. Parasitic control with trypanocidal drugs has not been successful due to problems related to their availability, toxicity and resistance development. Attempts to develop vaccines have been futile due to the trypanosomes' antigenic variations. This has led to search for effective vector control tactics to mitigate the impacts of the diseases. Some of the vector control measures used such as bush clearing, game destruction and use of insecticides are ecologically and environmentally harmful. Sterile insect technique (SIT) only works in ecological islands and, therefore, not effective in most of the African mainland. Community-based bait technologies have been relatively successful, except among pastoralists who move from one area to another in different seasons. Another approach to control the vectors is to develop potent repellants that can protect livestock.

A previous study on waterbuck, a tsetse refractory non-host, led to the identification of a blend of 15 electrophysiologically active constituents (six C_8-C_{13} methylketones, two phenols, six C_5-C_{10} straight chain fatty acids and δ-octalactone) that is repellant to savanna tsetse flies. Of these constituents, δ-octalactone has been shown to be a critical component. This study seeks to understand structural features associated with the repellency of δ-octalactone, which may lead to the identification of even more potent

analogues, and therefore expanding the arsenal of techniques to suppress tsetse flies' population and mitigate the effects of Trypanosomiasis. This will make livestock farming economically viable and also contribute towards enhanced food security.

1.5 Hypothesis

Analogues of δ-octalactone with different structural profiles may contribute toward better understanding of the feature(s) associated with their activity on *G. pallidipes* and *G. m. morsitans.*

1.6 Objectives

1.6.1 General objective

To carry out structure-activity studies so as to identify structural feature(s) in δ-octalactone analogues responsible for responses of *G. pallidipes* and *G. m. morsitans.*

1.6.2 Specific objectives

i. To synthesize 3-propylcyclohexanone and δ-valerolactone and confirm their structures using spectroscopic techniques.

ii. To determine responses of *G. pallidipes* and *G. m. morsitans* to each of the analogue.

iii. To determine the effect of structural modification δ-octalactone on the behaviour of *G. pallidipes* and *G. m. morsitans*.

1.7 Scope and limitation of the study

The study only focussed on four selected δ-octalactone analogues. The repellency of each synthesized, commercially available analogues and blends of the analogues were carried

out only in the wind tunnel assay. Only two species of tsetse flies were used in repellency studies: *G. pallidipes* and *G. m morsitans*.

1.8 Significance of the study

This research is of critical importance since it has laid a basis to the discover attractants that can be used individually or in blends in combination with already known repellants to control tsetse flies through 'push-pull' tactic. In this approach, the tsetse flies are repelled from the animal (push) and instantly get attracted to traps (pull). This will help in the mitigation of the effects associated with Animal Trypanosomiasis hence enabling pastoralists and livestock keepers to maximize profits from animal farming. On the other hand, the outcome of this research has laid some foundation for further studies on the repellency/attractancy of δ-octalactone analogues.

CHAPTER TWO

LITERATURE REVIEW

2.1. Tsetse fly vector

Tsetse flies (*Glossina spp*.) are arthropods which fall within the class insecta. There are thirty three species and sub-species of tsetse flies, and all except two are restricted to Sub-Saharan Africa (Jordan, 1993). They are highly mobile winged dipterans. The adults range in length from 6-14 mm and in all these known species are various shades of brown (ranging from light yellowish brown to dark blackish brown) (Leak, 1999; Barrett *et al*., 2003). They are the mechanical and biological vectors of trypanosomes which cause Trypanosomiasis and as a result present a potent and constant threat to humans and livestock in most of Sub-Saharan Africa (Gooding and Krafsur, 2005).

Only few species of tsetse flies are vectors of HAT but all are potential vectors of AAT (Dr, 2008). Tsetse flies feed exclusively on human and animal blood and are viviparous insects whereby females give birth to full-grown larvae which rapidly pupate in the soil (Krafsur, 2009). Development of trypanosomes in tsetse flies after sucking blood depends on the species of *Trypanosoma*. *T. vivax* only develops in and colonises the proboscis, *T. congolense* and *T. simiae* the midgut and the proboscis, whereas *T. b. gambiense, T. b. rhodesiense* and *T. b. brucei* develop in different regions of the intestine (Krafsur, 2009).

2.1.1 The Life Cycle of tsetse fly

The life cycle of tsetse fly begins with the female adult producing a single egg (Figure 2.1) which after fertilization hatches to a first instar larva in the uterus where it is

nurtured and nourished. Through a process of development and moulting, this progresses to the third stage larva that the female deposits on suitable sandy soil. The larva burrows down to its optimum depth into the soft soil or sand depending on the species hardens to form a puparium during which time it changes colour from white to brown and finally black. A teneral fly will appear after the puparial period which changes according to temperature, but on average is between thirty to forty days at 24°C. Tsetse fly females are capable of producing larvae (adenotrophic viviparity) every nine to ten days during a life span that can range up to four months depending on the climatic conditions. This form of reproduction ensures the higher level of survival of each offspring, but is also the basis why reproductive rates are significantly low in tsetse fly populations (Leak, 1999).

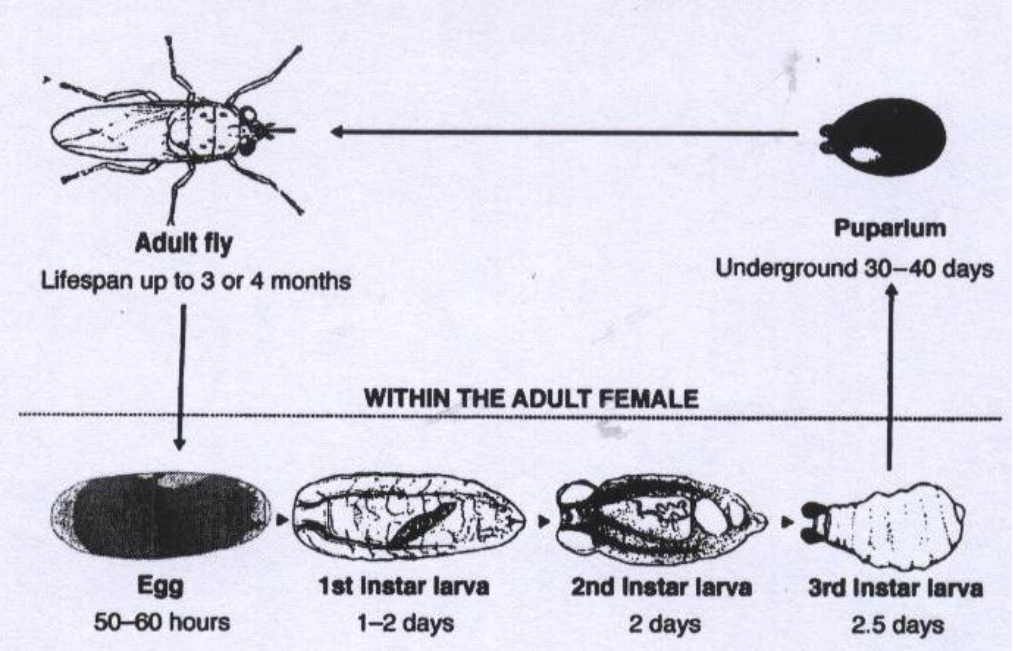


Figure 2.1: Life cycle of a tsetse fly (Leak, 1999).

Within the adult female the egg takes between fifty to sixty hours and first to third instar larvae takes about six to seven days. The puparium takes 30 to 40 days (in the soft soil)

and adult surviving for three to four months takes place in the environment. Sex determination is done by observing the tsetse fly genitalia where the presence of the hypopygium and hectors in males is used to differentiate males from females (Figure 2.2). For a quick identification the female abdomen is flat/smooth while the male has protruding structures (Leak, 1999).

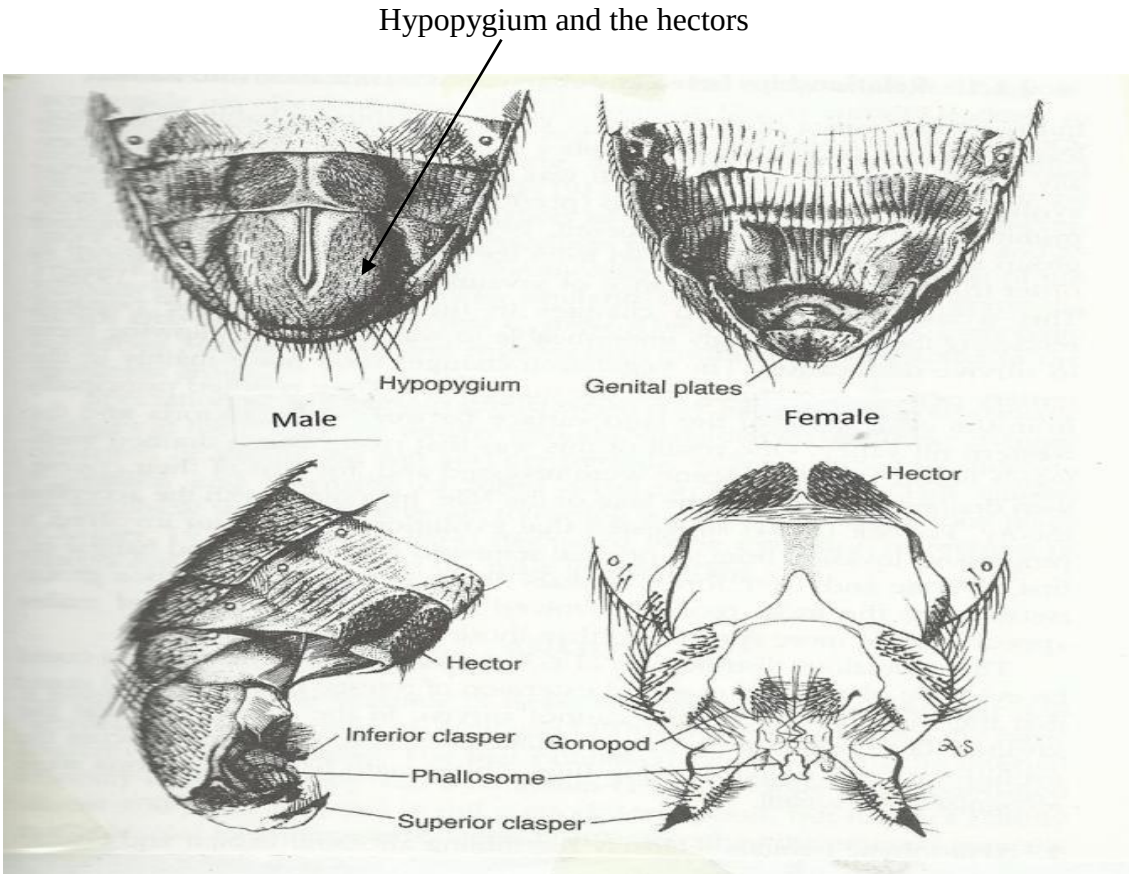


Figure 2.2: Differences between the posterior abdomens of male and female tsetse flies (Leak, 1999).

2.1.2 Methods of tsetse fly control

1. Bush clearing

In savannah areas, it involves large-scale destruction of bushes and vegetation cover where tsetse flies rest which destroys their shelter hence driving them away (Brightwell

et al., 2001; Kurugundla *et al*., 2012). Non-selective clearing of bushes was used in Uganda to control for *G. m. centralis* by cutting taller Acacia trees in the Ankole district (Kinung'Hi *et al*., 2006). In Tanzania, between 1923 and 1930, bush-clearing methods were also extensively employed to curtail the spread of HAT epidemic in Maswa district, where *G. swynnertoni* was prevalent (Leak, 1999). Similar tactics were used in Ghana to eliminate flies around villages were human-fly interactions were high (Courtin *et al*., 2008). This approach causes soil erosion, decreased soil fertility, its adverse effects on water supplies and loss of biodiversity. It is also labor intensive (Symeonakis *et al*., 2007).

2. Game destruction

This is modification of tsetse habitat, which entails killing of wild animals so as to remove reservoirs of infection in the wild animal populations. This method was used extensively in the past (Gooding and Krafsur, 2005). This measure is ecologically destructive since it leads to loss of biodiversity which consequently impacts negatively on the tourism sector hence making it unpopular (Peter *et al.*,, 2005).

3. Use of insecticides

Chemicals such as DDT, BHC, endosulfan and dieldrin are used for treatment of the tsetse habitats (Peter *et al*., 2005). Tsetse flies' biology and ecology render them susceptible to insecticides-based technique (Grant, 2001). Insecticides are applied with ground or aerial sprayers depending on the type, accessibility and size of the target area (Vreysen, 2001). Because of the harmful effects on the environment, they are being

replaced by the less toxic synthetic pyrethroids (Torr *et al*., 2005) which are also used for sequential aerosol technique (SAT).

SAT has been used successfully used to treat 10,000 km^2 in Botswana where the Okavango delta was cleared from tsetse flies without destructive effects on the environment and 48,000 km^2 in Zimbabwe (Vreysen and Marc, 2006). The method has also been applied in Kenya, Nigeria, Somalia, Uganda, and Zambia with varying successes. SAT is unsustainable due to high treatment frequency, the high cost of the insecticides, killing of non-target beneficial insects and the potential development of resistance to the insecticides (Vreysen and Marc, 2006).

Insecticides can also be applied through pour-ons. Currently most African farmers use pour-ons as they are simply and quickly applied without using complex equipment (Matthews *et al*., 2014). Furthermore, the insecticides kill also biting flies and ticks resulting in higher productivity. Applications can be made on the preferred biting sites of tsetse flies allowing a significant reduction of the amount of insecticide needed (Torr *et al.*, 2005). In case of zero grazing animals, insecticide impregnated mosquito nets installed around the zero grazing unit have been used effectively to defend the cattle against tsetse flies (Torr *et al*., 2007).

3. Use of sterile-insect technique (SIT)

SIT involves mass production of the target male insect species, sterilization and releasing them into the field on a sustained basis in sufficient numbers to achieve over-flooding

ratios (Feldman *et al.*, 2005). It is done by irradiation using gamma rays and beta rays, Chemo-sterilization and Physiological-sterilization (Dyck *et al*., 2005).

This technique relies on the mating of wild females with sterile male flies. Physiologically, female tsetse flies are only needed to mate once to store sperm in its spermathecae in adequate quantity such that fertilization can occur over its entire reproductive life. Mating with a sterile male would thus result in no offspring leading to suppression of the natural tsetse fly population (Hendrichs, 2000; Gooding and Krafsur, 2005). In 1994, an eradication program conducted in Zanzibar by the government and the IAEA used SIT to completely remove the entire tsetse population. This campaign may have been victorious in part as there is almost no immigration of tsetse flies into the island (Vreysen *et al*., 2001). Although Unguja is a small island infested with only one tsetse species *G. austeni*, it took several years and many millions of dollars to suppress the flies. Contrary to the theoretical 10:1 ratio of sterile males to wild males, a ratio of more than 100:1 was required on Unguja. This can be elucidated by the fact that contrary to the common belief some tsetse flies mate more than once (Lance and McInnis, 2005; Bonomi *et al*., 2011). This means that huge tsetse breeding units have to be built in order to produce the required large amounts of sterile flies (Rogers and Randolph, 2002).

SIT is a species-specific and environmentally non-polluting method of tsetse fly control (Thomas *et al*., 2000; Vreysen, 2001; 2005). However, this technique is costly as it requires macro-rearing of tsetse and it is only practicable for use once the wild tsetse flies population has been suppressed to low levels using other methods (Cattand *et al*., 2006).

4. Trapping of tsetse flies

Traps and targets are automatic structures used to eradicate tsetse flies through insecticides. The use of traps and targets to control tsetse populations have been successful mainly because they have a small rate of reproduction and require very little sustained mortality pressure to bring about a reduction in population (Lindh *et al*.,, 2009). The traps and targets attract tsetse flies by using their primary host-seeking behaviors, visual and olfactory stimulation (Esterhuizen *et al*., 2011).

Tsetse flies are lured by specific odors (ketones, phenols and carbon dioxide) identified in preferred hosts and by visual cues (blue colour) and shape (Nagel and Peveling, 2005). Targets are combinations of cloth and netting baited with an odor attractant and impregnated with an insecticide. Traps work on the same principle, but the fly is encouraged to enter a net where it remains trapped. Traps and targets are used to kill tsetse flies in a controlled manner (Mangan, 2005; Cattand *et al*., 2006). This method is only suitable to protect small areas, but the high target densities required against certain species and in certain dense habitats make the use of these devices over large areas uneconomical (Mihok, 2002).

There are many models of traps and targets designed to attract as many tsetse flies as possible in diverse environments, with a strong prominence on designs that are easy to replicate and maintain locally (Vale *et al*., 2004). Though most traps are robustly reliant on attractants and insecticides, some have recently been customised to attract tsetse flies based on visual stimulation alone and to kill tsetse flies through a trapping mechanism

(NGU and NG2B traps). While these traps may not be as efficient in attracting and killing tsetse flies, they are far more affordable and feasible to implement in resource poor settings (Omolo *et al*., 2009). Such traps were used to successfully suppress the tsetse fly populations in Nguruman, Kenya (Lindh *et al*., 2009). They are usually deployed in and around areas where human-fly contacts are greatest, such as streams frequented by villagers or in cultivated fields (Torr *et al.,* 2005).

2.2 Semiochemicals and blood-feeding insects

Semiochemicals are chemicals secreted by living organisms that stimulate a behavioural response in other individuals. They are classified into two groups: Pheromones (intraspecific) and allelochemicals (interspecific) (Heuskin *et al*., 2011). Pheromones convey information between members of the same species, usually with mutual effects for both the emitter and receiver. They include sex pheromones, aggregation pheromones and trail pheromones (Metcalf, 1994). Pheromones mediate interactions between members of the same species such as mating, aggregation, oviposition and invitation behavior (Rayaisse *et al*., 2010). Allelochemicals include allomones which conveys an adaptive advantage to the emitter (repellant), kairomones which provides an adaptive advantage to the receiver (attractant) and synomones which conveys a message of mutual advantage to both the emitter and receiver. However, at times a single chemical signal may act as both a pheromone and allelochemical (Heuskin *et al*., 2011).

Kairomones play a greater role in enabling and supporting hematophagus insects to track and locate their hosts (Torto, 2004). These insects possess highly developed olfactory systems and mainly use their antennae to detect kairomones (Birkett *et al*., 2004).

Volatile kairomones mediate interspecific communication, where the responding insects gain a behavioural or physiological advantage, while the kairomone-emitting host does not. Kairomones are secreted from various locations on the vertebrate including breath, skin (which may include gland secretions and breakdown products of microorganisms), urine and faeces (Birkett *et al*., 2004).

2.3 Host location by tsetse fly

The location of the preferred host is an integrated but flexible behavioural process that gathers momentum as the host is tracked down. The behaviour patterns involved are not organised in a particular order. They do not occur in a strict sequence with behaviour one always being followed by behaviour two, followed by three, and so on. This versatility allows a flexible response on the part of the insect to the differing situations in which it will encounter hosts (Torr and Solano, 2010). However, it is likely that insects mostly meet host-derived stimuli in a specific sequence. The insect often makes use of this predictability by permitting the current behavioural pattern in the host location sequence to lower the response threshold for subsequent host-related stimuli. For example, an insect that would not normally respond to a certain visual stimulus may respond strongly if it has just been exposed to an increase in carbon dioxide levels. In this way a behavioural momentum is built up during host finding. This behavioural momentum is further enhanced by the wide range of increasingly strong host stimuli that the insect encounters as host location proceeds (Sutcliffe, 1987).

Studies on blood-sucking insects both in the field and laboratory and from the clear evidence on the discrimination and selectivity they can show, it can be predicted that a

variety of host signals are used in host locating. In general, visual and olfactory stimuli, supported by anemotactic and optomotor responses, are the most critical signals when the insect is still at some distance from the host. Closer to the host, different stimuli become significant particularly humidity and heat.

The behaviour patterns involved in host location are divided into three phases (Sutcliffe, 1987):

(i) Appetitive searching

This stage is driven by hunger whereby the insect indulges in non-oriented behaviour likely to bring it into contact with stimuli derived from a potential host. This usually takes place at specific times of the day regulated by the insect's internally programmed activity cycle.

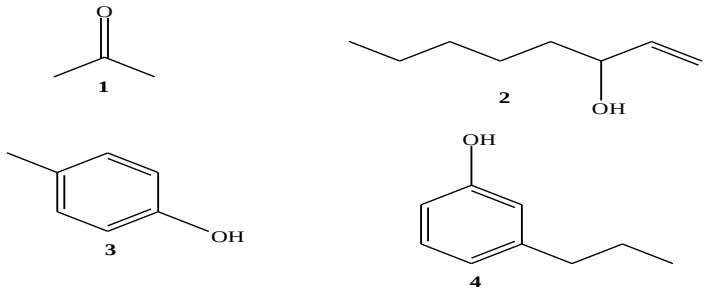
(ii) Activation and orientation

Upon getting stimulated by the host odour, the insect switches from behaviour patterns driven from within to oriented host location behaviour driven by host stimuli. The insect uses these host-derived stimuli to track down the host. These stimuli increases in variety and strength as the insect and host come closer together.

(iii) Attraction

This is the final phase, in which host stimuli are used to bring the insect into the host's immediate vicinity, and in which the decision of whether or not to contact the potential host is made.

Tsetse flies utilize the above systematic procedure to locate and recognize preferred hosts (hippopotamus, bushpig, cattle, elephant and bushbuck), beyond their visual range upwind (60–120 m) through odor-mediated anemotaxis, with visual cues supplementing host odors closer to the host (≈10 m) (Gibson and Torr, 1999). Thermal, tactile and contact chemical stimuli induce landing, probing and feeding which may contribute to host acceptability (Van der Goes van Naters *et al.*, 1998; Gikonyo *et al*., 2000). Tsetse flies are sensitive to spectral ranges from 300-700nm, the near UV and most of the range visible to the human eye that is why they prefer landing on black animals (Gooding, 1984). The long-range kairomone components that attract tsetse flies include breath constituents made up of carbon dioxide, acetone (**1**) and 1-octen-3-ol (**2**) (Hall *et al*., 1984) whereas the medium-range attractants include phenolic products, particularly 4-cresol (**3**) and 3-n-propylphenol (**4**), of host skin secretions and urine (Hassanali *et al*., 1986; Warnes, 1990; Saini *et al*., 1993). Different combinations of these attractants have been successfully deployed in the development of bait technologies for the control of *Glossina spp* in the field (Brightwell *et al*., 2001).



(Hassanali *et al*., 1986; Warnes, 1990; Saini *et al*., 1993)

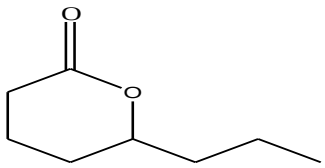
2.4 Non-host odor repellant to tsetse fly

The term non-host is used to describe organisms not apparently accepted as hosts by ectoparasites. Non-hosts may belong to a species that is regularly not accepted or one that includes both accepted and unaccepted individuals. Non-host odours may be induced, as

when the physiological state of chemical emitters affects their acceptability to ectoparasites (Weldon, 2010). Tsetse flies use chemoreception to avoid vertebrates that are known to be unprofitable for blood meal. Previous blood-meal analyses of tsetse flies (*Glossina spp*.) in Sub-Saharan Africa demonstrated the existence of differential susceptibilities of various vertebrates to attack (Gikonyo *et al*., 2002). This phenomenon led to the study of the semiochemical basis for the biased selection between hosts (ox and buffalo) and non-host (waterbuck). The waterbuck (*Kobus defassa*) was common in most study areas, but it was among the least fed by tsetse flies and this is attributed to its thick and shaggy coat which obstructs the tsetse flies from feeding (Weldon, 2010).

Previous studies focussed on feeding preferences, including the existence of non-host allomones produced by the non-preferred host waterbuck, *K. defassa* (Gikonyo *et al*., 2000). Analysis of the odour composition of preferred (buffalo, *Syncerus caffer*, and ox, *Bos indicus*) and non-preferred (*K. defassa*) hosts (Clausen *et al.*, 1998), and electrophysiological studies, has shown that the odours of the two preferred hosts are comparable. They comprise medium-chain, saturated or unsaturated aldehydes and phenolic compounds, with the non-host odour containing fewer aldehydes but more phenolic components and a series of 2-ketones (C_8–C_{13}), moderate amounts of C_5–C_9 straight chain fatty acids and δ-octalactone (**5**) (Gikonyo *et al*., 2002). The electro-physiological responses of *Glossina spp*. show that 2-ketones and the lactone from the non-host odour are physiologically active (Gikonyo *et al*., 2002). Follow up behavioural studies have shown that when presented with EAG-active components found specifically in the non-host odour, typical upwind flight behavior of flies is disrupted, with flies

avoiding the non-host blend (Gikonyo *et al*., 2003). Although, the 15 components in the repellant blend acted synergistically, δ-octalactone has been shown to singly elicit allomonal responses (Mwangi *et al*., 2008).



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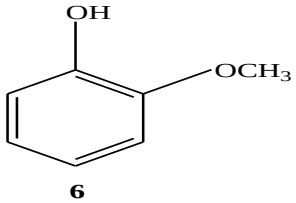
The behavioural responses of *Glossina spp*. to putative repellents based on guaiacol (**6**), an identified mild repellent, also have been investigated, with the aim of identifying analogues with more potent repellent activity (Saini and Hassanali, 2007). Of the compounds tested, 4-methylguaiacol (**7**), elicited stronger repellent effects, compared with guaiacol. Furthermore, the 4-methyl derivative reduced significantly trap catches of attractant-baited traps, and when applied to ox hosts, reduced the proportion of flies feeding on the host. Application of the repellent to approximately seventy five percent of cattle herds has been shown to protect entire cattle herds (Saini and Hassanali, 2007).



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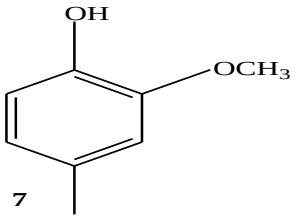

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2.5 Repellent properties of δ-octalactone

Lactones are cyclic esters of hydroxycarboxylic acids containing a 1-oxacycloalkan-2-one structure (Albertsson and Varma, 2003). A previous study on the feeding of *Glossina spp.* revealed a biased preference in host choices due to semiochemical differences between hosts (ox and buffalo) and non-host (waterbuck) (Gikonyo *et al*., 2000). The results from the study exposed the presence of volatile and short-range or contact

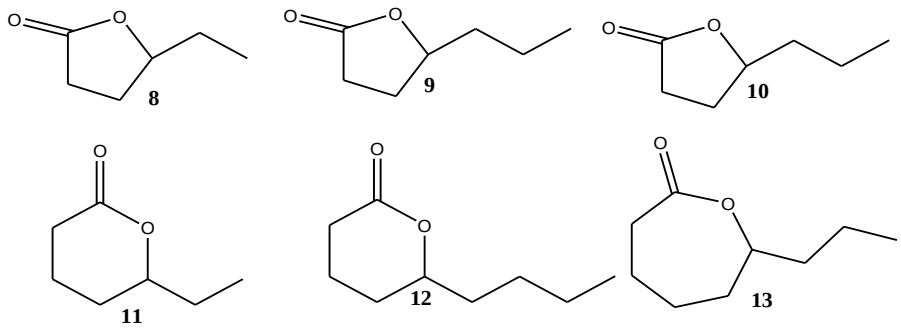
allomones from the waterbuck. The composition of the body volatiles of waterbuck and the ox and buffalo were compared. The GC-EAD profiles of the odour from the preferred hosts were comparable, comprising of phenols and medium-chain, saturated or unsaturated aldehydes. Water buck odour gave a richer profile, consisting of fewer aldehydes but more phenolic components, δ-octalactone and a series of methylketones (C_8-C_{13}), which were either not detected or found in trace amounts. Of these constituents, δ-octalactone has been shown to be a critical component since it elicited greater repellency (Gikonyo *et al*., 2002).

A follow-up study on δ-octalactone confirmed the repellency of δ-octalactone (Mwangi *et al*., 2008). Analysis of the data obtained demonstrated the potential repellent activity of the racemic δ-octalactone. The δ-octalactone was shown to be a tsetse fly repellent at ≥0.5 mg in 200 µl paraffin oil. However, significantly large amounts (1.0 or 2.5 mg per 200 µl) of δ-octalactone are required to make the flies stay away from the dispenser. This was consistent with earlier observations, when allomonal blends were dispensed in a choice wind tunnel bioassay and compared with kairomones and synthetic attractants (Gikonyo *et al*., 2002; 2003).

2.6 Structure-repellency studies of δ-octalactone

Structure-repellency studies help in elucidating the critical aspects in a compound that play the role of determining the behavioural responses of tsetse flies. This is done by obtaining homologues (compounds with similar functional groups but different structures) and carrying out behavioural responses tests so as to understand the link between the activity of the structure and the responses of the flies. A study carried out by

(Wachira *et al*., 2016) using selected δ-octalactone analogues [γ-hexalactone **(8)**, γ-heptalactone (**9**), γ-octalactone (**10**), δ-heptalactone (**11**), δ- nonalactone (**12**) and ε-nonalactone (**13**)]. These compounds had a similar functional group (lactone aspect). The outcome of this research manifested that reducing the ring size of δ-octalactone lead to a decrease in repellency while an increase gives an attraction effect, and an increase in the chain length of δ-octalactone lead to an increase in repellency while the reduction lead to reduced repellency against *Glossina spp.*



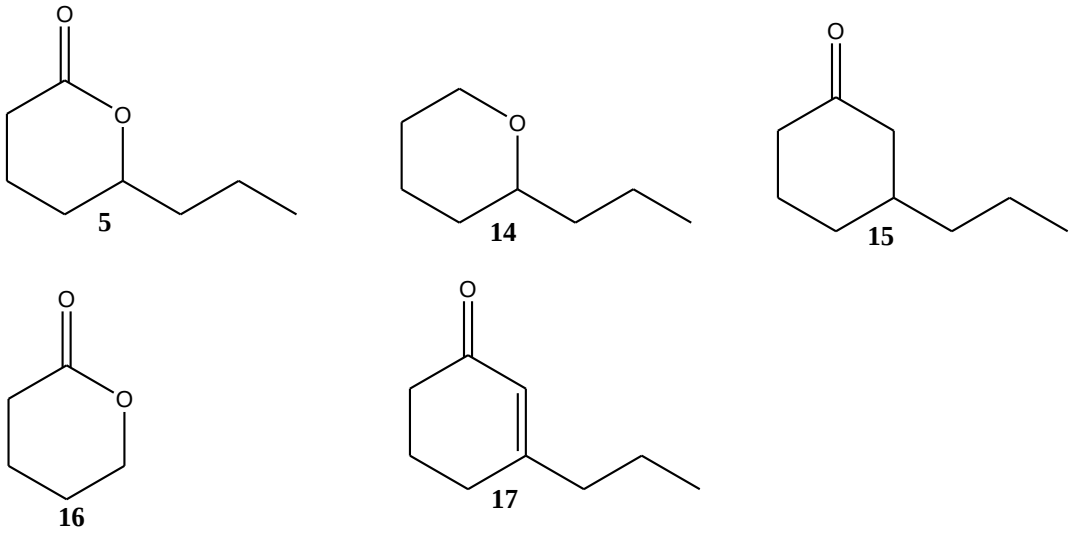
In the present study, we sought to search for more potent attractants which can be designed and be used in push-pull method as an arsenal to suppress tsetse flies population and mitigate the effects of Trypanosomiasis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Test compounds and blends

Test compounds consisted of (RS)-δ-octalactone **(5)**, a known repellent produced by tsetse refractory waterbuck and four selected analogues comprising (RS)-2-propyloxane **(14)**, (RS)-3-propylcyclohexanone **(15)**, (RS)-δ-valerolactone **(16)**, (RS)-3-propylcyclohex-2-enone **(17)**. In addition, repellency of blend R [(RS)-2-propyloxane **(14)** and (RS)-δ-valerolactone **(16)]** which was prepared by mixing an equal dosage of the constituent compounds and attractancy of blend A [(RS)-3-propylcyclohexanone **(15)** and (RS)-3-propylcyclohex-2-enone **(17)** which was prepared by blending an equal dosage of the constituent compounds to both *G. pallidipes* and *G. m. morsitans* were also studied.

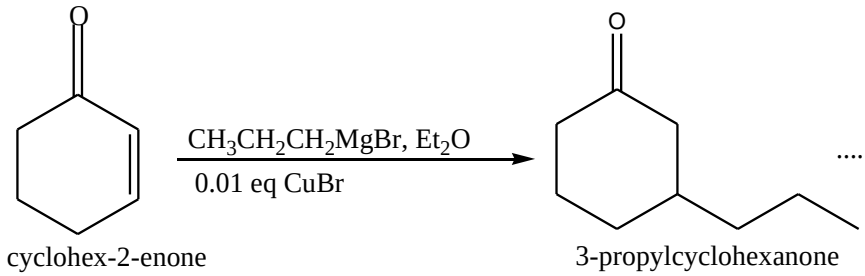


(RS)-δ-octalactone **(5)** and the analogues (RS)-2-propyloxane **(14)** and (RS)-3-propylcyclohex-2-enone **(17)** (98-99% pure), were acquired from Sigma-Aldrich, while (RS)-3-propylcyclohexanone **(15)** and (RS)-δ-valerolactone **(16)** were synthesized.

3.2. Syntheses of targeted δ-octalactone analogue

3.2.1 Synthesis of (RS)-3-propylcyclohexanone (15)

(RS)-3-propylcyclohexanone **(15)** was synthesized according to the procedure provided by (Nakamura, 2000). 5.0 ml (0.05156 moles) of 2-cyclohexen-1-one was added to a mixture of 25.8 ml (0.05156 moles) of propylmagnesium bromide and 0.074 g (0.01 eq) of copper (I) bromide contained in 40 ml of diethyl ether. 1,4-Michael addition was allowed to proceed at room temperature. The mixture was magnetically stirred and the reaction progress was monitored by TLC. The reaction went to completion after 2 hrs.



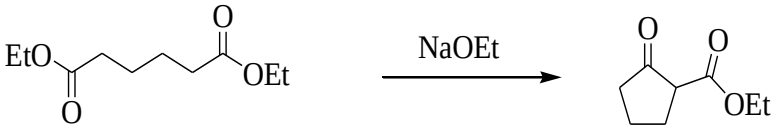
cyclohex-2-enone 3-propylcyclohexanone .....Equation 3.1

Batch extraction (5 × 5 ml) was carried out with dichloromethane. The combined DCM extract was dried with 4.0 g anhydrous sodium sulphate, filtered and the solvent evaporated in a rotor evaporator. The compound was purified by column chromatography. 10.0 g of silica gel (60 - 120 mesh-Sigma Aldrich) was weighed and mixed with the 5.0 ml of the extract using a glass rod to make a fine powder. A column of diameter 4.5 cm and height 52 cm was packed with slurry of 300 g of silica gel in hexane. The extract was slowly packed on top of the column, another 2 cm layer of silica gel added and covered with a clean cotton wool. The column was eluted with 100 ml of hexane, followed by 100 ml each of 90%, 80%, 70% and 60% hexane in ethyl acetate. 3-propylcyclohexanone **(15)**, eluted with 60% hexane in ethyl acetate to give a yield of 5.4 ml (75%).

The identity of 3-propylcyclohexanone **(15)** was confirmed by IR, GC-MS and GC-MSD spectroscopic techniques.

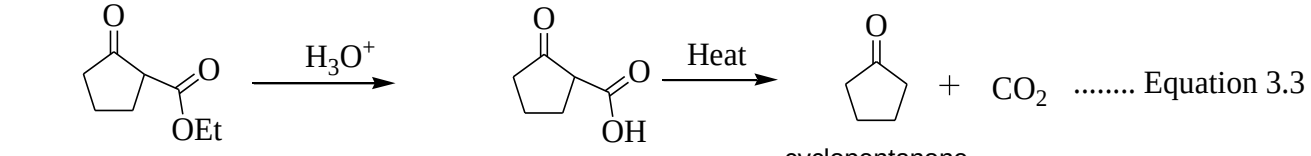
3.2.2 Synthesis of (RS)-δ-valerolactone (16)

10.0 ml (0.04939 moles) of diethyl adipate was dissolved in 20.0 ml of 99.0% absolute ethanol in a 100 ml three-necked flask. 3.50 g (0.030 moles) of sodium ethoxide was added and then the reaction mixture was stirred using a magnetic stirrer, the reaction was allowed to take place at room temperature. The reaction progress was monitored by TLC and came to an end after 2 hours. Sodium ethoxide facilitated cyclization of the diester to cyclic β-keto ester as shown in Equation 3.2.



Diethyl adipate — Ethyl-2-oxocyclopentanecarboxylate ..... Equation 3.2

The ester was heated under reflux with 62.0 ml (0.8 M HCl) for three hours at 80 °C to facilitate hydrolysis and subsequent decarboxylation of β-keto ester to cyclopentanone (Equation 3.3).

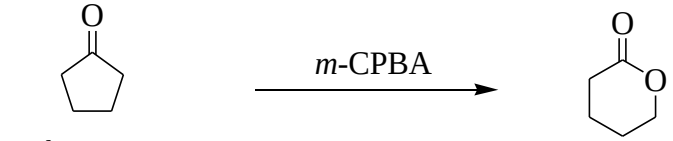


ethyl 2-oxocyclopentanecarboxylate — 2-oxocyclopentanecarboxylic acid — cyclopentanone ..... Equation 3.3

After cooling, batch extraction (6 × 5 ml) was carried out with dichloromethane. The combined DCM extract was dried with 5.0 g anhydrous sodium sulphate, filtered and the

solvent evaporated in vacuo. The compound was purified by column chromatography. 10.0 g of silica gel (Sigma Aldrich 60 - 120 mesh) was weighed and mixed with the 3.5 ml of the extract with a glass rod to make a fine powder. A column of diameter 4.5 cm and height 52 cm was packed with slurry of 300 g of silica gel in hexane. The extract was slowly packed on top of the column, another 2 cm layer of silica gel added and covered with a clean cotton wool. The column was eluted with 100 ml of hexane, followed by 100 ml each of 90%, 80%, 70% and 60% hexane in ethyl acetate. Cyclopentanone, eluted with hexane-ethyl acetate (70:30) to give a yield of 3.0ml.

The isolated cyclopentanone, was oxidized by Baeyer-villiger oxidation using meta-chloroperbenzoic acid (*m*-CPBA) for 2 hrs at room temperature as shown in Equation 3.4. This was achieved by reacting 8.52 g (0.04939 moles) of *m*-CPBA and adding to the 3.0 ml of cyclopentanone in 20.0 ml of chloroform and stirring. After three hours chloroform was removed under reduced pressure and the reaction quenched with 30 ml of saturated aqueous sodium thiosulphate. The compound was extracted by batch extraction with ethyl acetate (4×60 ml), neutralized with $NaHCO_3$, dried over anhydrous Na_2SO_4 and then solvent evaporated in vacuo. Ethyl acetate solvent was removed in vacuo and the residue was chromatographed on silica gel column to obtain 3.0 ml, 64% yield of the targeted product eluted with ethyl acetate – hexane (15:85).



cyclopentanone

tetrahydropyran-2-one
(Delta-valerolactone)

..... Equation 3.4

The identity of the synthesized compound was confirmed by IR, GC-MSD, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

3.3 Tsetse flies

Three day old male tsetse flies [females respond the same but they are not commonly used as they are involved in reproduction hence preservation of the insectary (Gikonyo *et al.,* 2003)] of *G. pallidipes* and *G. m. morsitans* were obtained from Biotechnology Research Institute insectaries. The males are distinguished by the presence of hypopygium in their abdomen which is absent in the females. Once isolated, they are placed in cubical cages (15.0 cm × 8.5 cm × 5.0 cm) in a bioassay laboratory maintained at temperatures of 25 ± 2 °C and 74 ± 2% relative humidity. On the day of the carrying out the bioassays, the flies were individually transferred into cylindrical PVC release cages (4 cm long and 3 cm in diameter) closed on one side with dark PVC gauze and the open end was plugged loosely with a piece of cotton wool.

3.4 The wind-tunnel

Behavioural tests were performed in a two-choice cuboidal plexi-glass tunnel (195 cm × 20 cm × 20 cm) with a slight adjustment to the one used by (Gikonyo *et al*., 2003). The wind tunnel (Figure 3.1) had three square windows (5 cm × 5 cm) on the top side: two rear ones (**II**) were for introducing odour dispensers while the middle one (**III**), which divided the tunnel equally into the left and right arms, was for introducing release cages. The middle window (**III**) was covered with a white plexiglass wire mesh to facilitate air flow from both arms. Each side of the tunnel had a tube (20 cm long and 3.3 cm in internal diameter) fixed with a white plexiglass wire gauze (**VI**) on the inner side to

prevent the flies from exiting the tunnel. These tubes were connected via air flow metres to an air cylinder by means of rubber tubing. Light was supplied from fluorescent tubes suspended 2 m above the tunnel giving about 1000 lux incident light. A white sheet of paper with graduated marks was fixed on the wooden frame of the tunnel to enable correct recording of fly distances during anemotaxis.

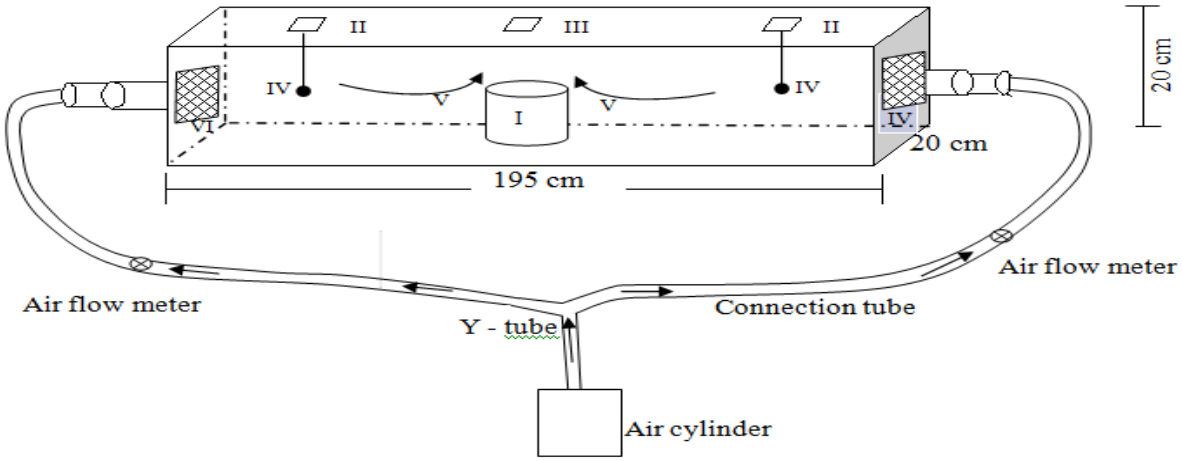


Figure 3.1: The design of the wind tunnel.
Key: Insect release cage (**I**), windows for introducing test material dispensers (**II**), window for introducing insect release cage (**III**), test material dispenser (**IV**), air flow direction (**V**) and PVC gauze (**VI**)

3.4.1 Odour dispensers

The odour dispensers comprised of a plexi-glass rod attached to the lids covering the rear windows (**II**). The rods were fixed in such a way that when the rear windows are covered by the lids, they hanged halfway inside the tunnel so as to coincide with the air flow. The rods were covered with an aluminium foil which was replaced after every experiment. A copper wire was rolled on the rod which was used for attaching a piece of clean cotton wool, containing the test odour, at the tip of the rod. 1000 µl of each dose of the test material was dispensed on the clean cotton wool on one side while the same volume of

DCM on the other side of the tunnel. The rear windows (**II**) were closed tightly and air allowed to flow for 2 minutes before the flies were introduced.

3.5 Experimental procedure for the tsetse behavioral responses

Behavioral responses of tsetse flies to the δ-octalactone analogues and blends were studied according to the procedure described by (Gikonyo *et al*., 2003). Three different doses (0.05, 0.10 and 0.20 mg/ml) of the known repellant (δ-octalactone), analogues and blends were prepared in DCM (Neutral odour). Cow urine which is a known attractant was used as a positive control for attractancy. The urine was fermented for three days to concentrate its attractancy (Saini *et al.,* 1993). Each known odour, δ-octalactone analogue or blend was tested in different days in the morning (0800 – 1200 hrs) and afternoon (1400 – 1600 hrs) to correspond with the tsetse feeding hours (Gikonyo *et al.,* 2003).

About 60 three-day-old teneral male tsetse flies were placed in cuboidal cages (15.0 cm × 8.5 cm × 5.0 cm), a few minutes to the bioassay. At the start of the bioassay, 10 flies were transferred to cylindrical release cages (**1**) (3 cm long and 3 cm diameter) by a cylindrical tube (1.5 cm diameter and 22.0 cm length) and then closed loosely with a cotton wool.

The release cage was introduced carefully through the release window at the middle of the tunnel. The cotton-plugged end of the release cage was positioned facing up. The cotton-plugged end of the release cage was opened with a pair of forceps to free the flies. Pure air from an air cylinder was allowed to flow into the tunnel from both sides via controlled flow metres at a rate of 12 cm/min. The meshed central window was closed

after the release of tsetse flies so that only pure air carrying the test odours from the dispensers passed over the release cage.

The following behaviors were observed throughout the study: activation (walking or hopping within or out of the release cage), escape behavior by the tendency of the tsetse flies attempting to fly out by gliding against wall of the tunnel, the initial direction of flight and final landing/resting position (control or treated arms of the tunnel) of each fly. The behavioural experiments were carried out using ten tsetse flies and in triplicates for each treatment. After each bioassay, the aluminium foils were removed from each dispenser and then cleaned with a cotton wool soaked in 70% ethanol. The release cages were cleaned with distilled water and then with 70% ethanol after each experiment. Pure air was allowed to flow for about five minutes at high speed in each replicate cycle and from one test compound to the other. The absence of residual effects of the previous test compounds were confirmed by carrying out blank tests. To avoid possible directional bias, the arms of the two-choice wind-tunnel were used alternately for control or test compounds/blends in successive replicates. The flies were removed out of the wind tunnel by use of an improvised suction system consisting of a pipette filler connected to a rubber tube (65 cm) and a glass tube (8 cm) at the end.

Air from the bioassay room was expelled for about one hour before another test is performed by opening the windows and door. In addition, a fan was switched on to speed up air circulation in the room.

3.6 Statistical analyses

Statistical analysis of differences in observed behaviors of tsetse flies inside the tunnel were performed non-parametrically and comparisons of proportions of tsetse flies' distribution between the treated and the control arms determined. The repellency expressed as protective efficacy at each dose of the tested odours was calculated from three replicates using the expression shown in Equation 3.5 below;

$$\% \text{ Repellency} = \left(\frac{C - T}{C + T}\right) 100 \qquad \text{..... Equation 3.5}$$

Where C = Number of flies in the control arm

T = Number of flies in the treated arm

The attractancy expressed as attractive efficacy at each dose of the tested odours was calculated from three replicates using the expression shown in Equation 3.6 below;

$$\% \text{ Attractancy} = \left(\frac{T - C}{T+C}\right) 100 \qquad \text{.....Equation 3.6}$$

Where C = Number of flies in the control arm

T = Number of flies in the treated arm

The data obtained from repellency studies was transformed and subjected to Analysis of Variance (ANOVA) using SPSS 22.0. Mean repellencies of each of the compound or blends tested at different doses were ranked using Student-Newman-Keuls test (SPSS 22.0). The percentage preferences between the number of flies in control arm and the test arm were compared using t-Test.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Syntheses and structure confirmation of targeted δ-octalactone analogues

The synthetic (RS)-3-propylcyclohexanone **(15)** was a colourless liquid. Its structure was confirmed using GC-MS, IR and GC-MSD Spectroscopic techniques. From the total ion chromatogram (Appendix - Figure 6.1), it had a peak at retention time (Rt) of = 19.63 and 20.28 min (Appendix – Table 6.1) that corresponded to 3-propylcyclohexanone **(15)** (Appendix – Table 6.2) for the R and S stereoisomers of 3-propylcyclohexanone. The GC-MSD gave *m/z* ratio for this compound as 140.00 while the *m/z* ratio calculated for molecular ion, $C_9H_{16}O$ [M^+] was 140.225 (Appendix – Figure 6.2). The IR spectrum (Appendix – Figure 6.3) had characteristic peaks at 2862.36-2931.80 cm^{-1} (C-H) and 1701.22 cm^{-1} (C=O). The spectral data was corroborated as per the table of spectral information provided by (Pretsch *et al*., 1989).

(RS)-δ-valerolactone **(16)** was a colourless liquid. Its identity was confirmed using GC-MSD, ^{13}C-NMR, ^{1}H-NMR and IR spectroscopic techniques. The GC-MSD gave *m/z* ratio for this compound as 100.00 while the *m/z* ratio calculated for molecular ion, $C_5H_8O_2$ [M^+] was 100.1158 (Appendix – Figure 6.4). The ^{13}C-NMR (Appendix – Figure6.5) (100 MHz, $CDCl_3$) revealed a total of 5 carbon atoms with the chemical shifts at δ (ppm): 19.1 (CH_2), 22.3 (CH_2), 29.0 (CH_2), 69.47 (C-O) and 171.4 (C=O). The ^{1}H-NMR (300 MHz) spectra (Appendix – Figure 6.6) gave 8 protons with the following chemical shifts δ (ppm): 1.829-1.833 (m, 4H), 1.876-1.886 (m, 2H) and 2.062-2.079 (m, 2H). The IR spectrum (Appendix – Figure 6.7) had characteristic peaks at 2974 cm^{-1} (C-H), 1732 cm^{-1}

(C=O) and 1274.9 – 1049.2 cm^{-1} (C-O). The spectral data was corroborated as per the table of spectral information provided by (Pretsch *et al*., 1989).

4.2 Behavioural responses of *G. pallidipes* to δ-octalactone analogues and blends

During the three minutes' observation of the behavioral responses of *G. pallidipes* to each of the test compounds and selected blends, the following parameters were recorded: (i) the number of the flies activated and proportions flying upwind in treated and control sides (table 4.1); (ii) upwind progression distance (table 4.2); and (iii) final resting position (table 4.3).

Table 4.1: Percentage of flies activated and numbers that selected one of the two wings in the two-choice wind-tunnel

Test odour	Dose (g/ml)	N	Percent of flies activated	Percent flies showing upwind flight preference		p-values
				Control	Test	
Blank (DCM)		30	100	48.90±2.20	50.0±1.22	0.23
Cow urine (3-days old)		30	90	20.00±1.97	73.33±3.23	0.01
δ-Octalactone (**5**)	0.05	30	90	63.33±2.11[a]	33.33±1.33[a]	0.002
	0.10	30	100	64.67±0.33[a]	30.00±2.54[a]	0.001
	0.20	30	100	67.67±1.21[a]	30.00±1.23[a]	0.01
	p-value			> 0.05	0.75	
δ-Valerolactone (**16**)	0.05	30	100	60.00±1.00[a]	37.00±0.45[a]	0.05
	0.10	30	100	61.00±1.09[a]	33.00±1.00[a]	0.03
	0.20	30	90	62.00±0.78[a]	30.00±1.87[a]	0.001
	p-value			> 0.05	> 0.05	
2-Propyloxane (**14**)	0.05	30	93.33	50.33±1.02[a]	43.33±1.82[a]	0.005
	0.10	30	90	53.00±2.27[a]	40.00±1.67[a]	0.001
	0.20	30	100	55.00±2.13[a]	36.67±3.33[a]	0.001
	p-value			> 0.05	> 0.05	
Blend R	0.05	30	100	61.00±0.40[a]	40.00±0.09[a]	0.002
	0.10	30	100	60.00±0.77[a]	36.67±3.33[a]	0.009
	0.20	30	90	66.67±1.50[a]	30.00±1.77[a]	0.01
	p-value			> 0.05	0.06	
3-Propylcyclohexanone	0.05	30	96.67	26.67±2.11[a]	59.67±0.33[a]	0.002
	0.10	30	100	24.67±3.33[a]	62.00±2.30[a]	0.001

(**15**)	0.20	30	90	23.33±1.65[a]	64.00±0.54[a]	0.01
	p-value			0.12	> 0.05	
3-Propylcyclohex-2-enone (**17**)	0.05	30	90	35.33±2.11[a]	63.00±2.24[a]	0.01
	0.10	30	93.33	33.67±3.33[a]	65.33±1.22[a]	0.01
	0.20	30	90	30.67±1.54[a]	66.00±2.17[a]	0.01
	p-value			0.65	> 0.05	
Blend A	0.05	30	90	38.00±1.77[a]	60.33±1.33[a]	0.03
	0.10	30	100	34.67±3.33[a]	64.00±1.09[a]	0.01
	0.20	30	96.67	30.00±1.17[a]	67.67±2.11[a]	0.001
	p-value			0.14	> 0.05	

N= number of tsetse flies used in the experiment; Blend A= a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**17**); Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**); analogues are R and S stereoisomers. Within columns, means (±SE) with the same small letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test) and across a given row means (±SE) are not significantly different at $p > 0.05$ (t-Test).

Generally, 90-100% of the tsetse flies were activated by all the test compounds and blends at all doses. There was no significant difference in choice selection when the blank was dispensed ($p > 0.05$). It was noted that for different doses of each compound or blend, the number of flies activated were not significantly different ($p > 0.05$) but the response behaviour of the tsetse flies toward or away from the test compound or blends depended on their structure. When δ-octalactone (**5**), 2-propyloxane **(14)**, δ-valerolactone **(16)** and blend R were dispensed respectively, more flies chose the control side than the treated side ($p < 0.05$). However, for the known attractant (urine - 3 days old), 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)** and blend A, more flies preferred the treated side than the control side ($p < 0.05$) (table 4.1). These results show that 2-propyloxane **(14)**, δ-valerolactone **(16)** and blend R elicited avoidance as compared to the δ-octalactone (**5**) (known repellent). On the other hand, **3**-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)** and blend A elicited attraction as compared to the known attractant [cow urine (3 days old)].

Table 4.2: Upwind flight progression (cm ± SE)

Test odour	Dose (g/ml)	N	Mean distance covered (Cm±SE)		p-values
			Control	Test	
Blank (DCM)		30	28.20±1.25	28.67±1.91	0.06
Cow urine (3-days old)		30	11.00±6.87	42.65±5.14	0.01
δ-Octalactone (**5**)	0.05	30	36.00±4.45[a]	20.89±3.48[a]	0.04
	0.10	30	37.57±5.08[a]	17.57±3.64[a]	0.01
	0.20	30	33.25±4.56[a]	14.17±6.22[a]	0.011
	p-value		0.074	> 0.05	
δ-Valerolactone (**16**)	0.05	30	45.00±4.24[a]	28.67±4.38[a]	0.01
	0.10	30	40.22±3.20[a]	23.50±4.59[a]	0.001
	0.20	30	37.89±2.73[a]	22.75±5.67[a]	0.001
	p-value		0.10	0.06	
2-Propyloxane (**14**)	0.05	30	44.94±4.51[a]	19.62±3.31[a]	0.01
	0.10	30	37.44±4.58[a]	15.64±4.60[a]	0.01
	0.20	30	35.12±3.13[a]	16.18±3.32[a]	0.001
	p-value		0.274	> 0.05	
Blend R	0.05	30	38.89±2.73[a]	17.75±5.67[a]	0.04
	0.10	30	34.05±4.26[a]	19.89±3.71[a]	0.01
	0.20	30	37.57±5.06[a]	17.57±3.64[a]	0.034
	p-value		0.06	> 0.05	
3-Propylcyclohexanone (**15**)	0.05	30	17.88±4.17[a]	30.38±3.05[a]	0.01
	0.10	30	24.36±4.77[a]	37.26±4.15[a]	0.01
	0.20	30	20.00±0.40[a]	37.88±8.89[a]	0.001
	p-value		0.07	> 0.05	
3-Propylcyclohex-2-enone (**17**)	0.05	30	15.38±4.49[a]	32.90±2.77[a]	0.05
	0.10	30	20.13±4.20[a]	26.80±2.98[a]	0.001
	0.20	30	19.71±2.48[a]	29.78±4.04[a]	0.001
	p-value		0.06	> 0.05	
Blend A	0.05	30	22.56±6.73[a]	34.95±5.09[a]	0.03
	0.10	30	24.89±3.48[a]	36.00±4.64[a]	0.001
	0.20	30	17.57±3.64[a]	31.13±5.12[a]	0.001
	p-value		> 0.05	> 0.05	

N= number of tsetse flies used in the experiment; Blend A= a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**17**); Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**); analogues are R and S stereoisomers. Within columns, means (±SE) with the same small letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test) and across a given row means (±SE) are not significantly different at $p > 0.05$ (t-Test).

There was no significant difference in the average upwind distance on both arms of the tunnel when DCM control was introduced ($p > 0.05$). However, in the presence of the

odour of test compound or blend, significant differences were observed. In the presence of δ-octalactone (**5**) (known repellent), and 2-propyloxane **(14)**, δ-valerolactone **(16)** and blend R at all doses, the flies flew shorter distances in the treated arm than in the control arm ($p < 0.05$). The distances moved by the flies in the tunnel arm containing δ-valerolactone **(16)** odour were shorter than those in 2-propyloxane. In addition, the distance covered by the tsetse flies in the arm of the tunnel containing δ-octalactone (**5**) (known tsetse repellant) odour were much shorter than those covered in the presence of the odours of 2-propyloxane **(14)** and δ-valerolactone **(16)** respectively (table 4.2).

When the known attractant [cow urine (3 days old)], 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)**, and blend A were dispensed respectively, the flies showed higher upwind progression at all doses on the treated side than the control side ($p < 0.05$). The distances covered in the tunnel arm having the odour of 3-propylcyclohex-2-enone **(17)**, blend A and 3-propylcyclohexanone **(15)** respectively, were longer than in the control arms as compared to known attractant [cow urine (3 days old)] (table 4.2).

Table 4.3: Percent flies in the final resting position

Test odour	Dose (g/ml)	N	Percent flies in the final resting position		p-values
			Control	Test	
Blank (DCM)		30	46.67±3.33	47.00±1.77	0.06
Cow urine (3-days old)		30	15.00±0.30	85.67±3.33	0.01
δ-Octalactone (**5**)	0.05	30	70.00±0.76[a]	25.00±0.87[a]	0.01
	0.10	30	76.67±1.33[a]	22.33±0.33[a]	0.02
	0.20	30	79.00±2.55[a]	18.00±2.44[a]	0.001
	p-value		> 0.05	0.65	
2-Propyloxane (**14**)	0.05	30	60.00±2.33[a]	35.00±3.54[a]	0.01
	0.10	30	62.00±1.00[a]	33.00±1.09[a]	0.001
	0.20	30	64.00±2.22[a]	31.00±2.33[a]	0.01
	p-value		0.21	0.10	
δ-Valerolactone (**16**)	0.05	30	56.67±4.82[a]	27.33±2.28[a]	0.01
	0.10	30	57.33±2.34[a]	25.67±0.33[a]	0.02
	0.20	30	58.67±1.67[a]	23.67±3.33[a]	0.01
	p-value		0.06	0.12	
Blend R	0.05	30	64.00±0.64[a]	26.00±2.87[a]	0.01
	0.10	30	70.00±0.76[a]	23.00±0.20[a]	0.01
	0.20	30	76.67±1.52[a]	20.33±0.33[a]	0.01
	p-value		> 0.05	0.10	
3-Propylcyclohexanone (**15**)	0.05	30	30.67±1.45[a]	62.60±1.12[a]	0.01
	0.10	30	28.67±2.54[a]	64.67±2.21[a]	0.04
	0.20	30	23.33±3.33[a]	72.00±2.23[a]	0.001
	p-value		0.13	0.441	
3-Propylcyclohex-2-enone (**17**)	0.05	30	33.33±1.67[a]	64.00±2.24[a]	0.01
	0.10	30	31.67±2.20[a]	66.33±0.20[a]	0.01
	0.20	30	26.33±3.32[a]	66.67±1.89[a]	0.01
	p-value		0.36	0.24	
Blend A	0.05	30	32.00±2.25[a]	64.00±0.77[a]	0.001
	0.10	30	28.00±1.87[a]	69.00±0.88[a]	0.001
	0.20	30	23.33±3.33[a]	70.67±0.33[a]	0.001
	p-value		> 0.05	> 0.05	

N= number of tsetse flies used in the experiment; Blend A= a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**17**); Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**); analogues are R and S stereoisomers. Within columns, means (±SE) with the same small letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test) and across a given row means (±SE) are not significantly different at $p > 0.05$ (t-Test).

At the end of three minutes' observation, the resting position of the tsetse flies was dependent on the nature of the test odour ($p < 0.05$) irrespective of the dosage ($p > 0.05$).

There was no significant difference in fly choice direction when the control (DCM) was dispensed on both arms ($p > 0.05$). It was also noted that the percentage of flies resting on the treated side were more than the control side when known attractant [cow urine (3 days old)], 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)** and blend A were dispensed ($p < 0.05$). Comparison of the percentage of flies resting in either control or treated arms indicated that fewer flies rested on the treated side than control side when δ-octalactone (**5**), 2-propyloxane **(14)**, δ-valerolactone **(16)** and blend R were dispensed respectively ($p < 0.05$) (table 4.3).

Generally, the behaviour of flies in the treated arm of the tunnel containing odours of 2-propyloxane **(14)**, δ-valerolactone **(16)**, δ-octalactone (**5**) or blend R was characterised by sharp U-turn in flight perhaps an attempt to escape from the tunnel. No such behaviour was noted when 3-propylcyclohex-2-enone **(17)**, 3-propylcyclohexanone **(15)**, blend A or [cow urine (3 days old)] was dispensed; instead the flies chose to walk, hop or fly within the treated arm of the tunnel. The consecutive behaviors of the flies that flew upwind after interacting with the odour were also in tandem with the repulsive or attractive nature of test odours. In the presence of odours of 2-propyloxane **(14)**, δ-valerolactone **(16)**, δ-octalactone (**5**) or blend R respectively, the flies moved downwind and settled mostly in the odourless control arm of the tunnel. In the presence of 3-propylcyclohex-2-enone **(17)**, 3-propylcyclohexanone **(15)** and blend A respectively, the flies flew upwind and settled on the treated side of the tunnel while flapping their wings, similar to a previous observation reported by (Gikonyo *et al*., 2003; Wachira *et al*., 2016).

4.2.1 Relative repellency of the synthetic analogues against *G. pallidipes* using a wind tunnel assay

The results of the relative repellency of the odours of δ-octalactone (**5**), δ-valerolactone **(16)**, 2-propyloxane **(14)** and blend R (2-propyloxane and δ-valerolactone) against 3-day old teneral tsetse flies, *G. pallidipes*, in a wind tunnel assay apparatus are summarised in the table 4.4

Table 4.4: Relative repellency of individual compounds and blend R

Doses (g/ml)	δ-Octalactone (**5**)	δ-Valerolactone (**16**)	2-Propyloxane (**14**)	Blend R	p-values
0.05	47.9 ± 4.01^{aA}	33.73 ± 2.21^{aB}	26.33 ± 1.64^{aB}	42.22 ± 0.07^{aA}	0.018
0.10	54.89 ± 6.67^{aA}	39.02 ± 0.33^{aB}	30.70 ± 4.06^{aB}	50.53 ± 1.01^{aA}	0.040
0.20	62.00 ± 3.34^{abA}	43.21 ± 2.22^{abB}	34.74 ± 4.63^{aB}	58.33 ± 6.67^{abA}	0.006
p-values	0.06	0.160	0.215	0.205	

Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**). Within columns, means (±SE) with the same small letter(s), and across a given row, means with the same capital letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test), respectively.

After three minutes of observation, there was no significant difference between percentages of repellency caused by different doses of δ-octalactone (**5**) ($p = 0.06$), 2-propyloxane **(14)** ($p = 0.215$) δ-valerolactone **(16)** ($p = 0.160$) and blend R ($p = 0.205$). It was also found that, the repellency of δ-octalactone (**5**) was comparable to that of blend R ($p > 0.05$). The repellency caused by the three doses of 2-propyloxane **(14)** and δ-valerolactone **(16)** were not significantly different ($p > 0.05$)

Table 4.5: Attractancy of synthetic analogues (3-propylcyclohexanone and 3-propylcyclohex-2-enone and blend A)

Dose (g/ml)	3-Propylcyclohex-2-enone (**17**)	3-Propylcyclohexanone (**15**)	Blend A	p-values
0.05	34.96 ± 2.54^{aA}	31.26 ± 1.52^{aA}	33.33 ± 1.55^{aA}	0.69
0.10	39.23 ± 0.34^{aA}	36.07 ± 1.67^{aA}	42.26 ± 0.70^{aA}	0.13
0.20	51.44 ± 1.14^{abA}	43.67 ± 0.23^{abA}	50.93 ± 1.83^{abA}	0.55
p-value	0.064	0.228	0.079	

Blend A = a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**17**). Within columns, means (±SE) with the same small letter(s), and across a given row, means (±SE) with the same capital letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test), respectively.

It was observed that there was no significant difference in attractancy for the three doses across the test compounds and blend A ($p = 0.13$, 0.55 and 0.69). At the end of the three minute observation period, there was no significant difference between percentages of attractancy caused by different doses of 3-propylcyclohex-2-enone **(17)** ($p = 0.064$), 3-propylcyclohexanone **(15)** ($p = 0.228$) and blend A ($p = 0.079$).

It can be summarised that the structural modification of δ-octalactone to obtain analogues with oxygen in the ring, 2-propyloxane **(14)** (without the carbonyl group) and δ-valerolactone **(16)** (without the propyl side chain) resulted in milder repellency (table 4.4). On the other hand, the analogues without oxygen in the ring [3-propylcyclohexanone **(15)** and 3-propylcyclohex-2-enone **(17)** (having a double bond between 2^{nd} and 3^{rd} position in the ring] reversed the behaviour from repellency to attractancy. It was also noted that the response of the tsetse flies to the test odours was dose-independent for the dose range that was applied (table 4.1, 4.2 and 4.3). The repellency of 2-propyloxane **(14)** and δ-valerolactone **(16)** were comparable. Synergistic effect was observed in blend R [a mixture of 2-propyloxane **(14)** and **δ-**valerolactone

(16)] whose repellency was comparable to δ-octalactone (**5**) (Table 4.4). Blend A [a mixture of 3-propylcyclohexanone **(15)** and 3-propylcyclohex-2-enone **(17)**] did not cause synergistic effect as the attractancy was not significantly different from that of the individual test compounds (table 4.5).

4.3 Behavioural responses of *G. m. morsitans* to δ-octalactone analogues and blends

During the three minutes' observation of the behavioral responses of *G. m. morsitans* to each of the test compounds and selected blends, the following parameters were observed and recorded: (i) the number of the flies activated and proportions flying upwind in treated and control sides (table 4.6); (ii) upwind progression distance (table 4.7); and (iii) final resting position (table 4.8).

Table 4.6: Percentage of flies activated and those showing upwind flight in the two-choice wind-tunnel.

Test compound	Dose (g/ml)	N	Percent of flies activated	Percent flies showing upwind flight preference		p-values
				Control	Test	
Blank (DCM)	0.00	30	100	51.0±2.12	48.0±1.11	0.06
Urine (3-days old)	0.00	30	100	14.0±1.34	82.0±3.12	0.01
δ-Octalactone (**5**)	0.05	30	100	55.7±2.11[a]	36.3±3.12[a]	0.01
	0.10	30	96.67	60.6±1.11[a]	37.4±1.09[a]	0.01
	0.20	30	96.67	61.7±1.21[a]	35.3±1.55[a]	0.001
	P-value			> 0.05	0.10	
2-Propyloxane (**14**)	0.05	30	100	53.22±2.98[a]	35.7±3.21[a]	0.01
	0.10	30	100	54.0±2.56[a]	40.0±2.87[a]	0.01
	0.20	30	100	56.6±2.11[a]	41.42±1.98[a]	0.01
	P-value			0.07	> 0.05	
δ-Valerolactone (**16**)	0.05	30	100	54.0±1.76[a]	37.0±2.86[a]	0.001
	0.10	30	100	55.0±1.54[a]	38.0±2.65[a]	0.001
	0.20	30	100	58.3±2.65[a]	39.7±2.87[a]	0.01
	P-value			0.33	0.23	
Blend R	0.05	30	100	60.0±2.98[a]	37.0±1.22[a]	0.01
	0.10	30	96.67	62.7±1.68[a]	36.3±1.21[a]	0.01
	0.20	30	96.67	64.7±1.66[a]	32.3±1.97[a]	0.01

	p-value			> 0.05	0.91	
3-Propylcyclohexanone (**15**)	0.05	30	96.67	41.7±2.12[a]	56.7±1.33[a]	0.01
	0.10	30	96.67	44.7±2.45[a]	54.3±2.79[a]	0.01
	0.20	30	96.67	33.7±3.14[a]	62.3±4.33[a]	0.001
	p-value			0.47	> 0.05	
3-Propylcyclohex-2-enone (**17**)	0.05	30	100	35.7±0.17[a]	63.3±1.23[a]	0.02
	0.10	30	96.67	30.3±1.09[a]	66.7±1.87[a]	0.04
	0.20	30	100	36.6±1.13[a]	63.7±1.04[a]	0.01
	p-value			0.22	0.87	
Blend A	0.05	30	100	33.7±2.23[a]	65.3±1.45[a]	0.01
	0.10	30	100	34.7±1.43[a]	64.3±1.23[a]	0.01
	0.20	30	100	30.3±1.90[a]	66.7±2.22[a]	0.001
	p-value			> 0.05	> 0.05	

N= number of tsetse flies used in the experiment; Blend A= a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**17**); Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**); analogues are R and S stereoisomers. Within columns, means (±SE) with the same small letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test) and across a given row means (±SE) are not significantly different at $p > 0.05$ (t-Test).

Generally, 96.67-100% of the flies were activated by all the test compounds and blends at all doses. There was no significant difference in choice selection when the blank was dispensed ($p > 0.05$). It was noted that for different doses of each compound or blend, the number of flies activated were not significantly different ($p > 0.05$) but the response behaviour of the tsetse flies toward or away from the test compound or blends depended on their structure. When δ-octalactone (**5**), 2-propyloxane **(14)**, δ-valerolactone **(16)** and blend R were dispensed, more flies chose the control side than the treated side ($p < 0.05$). However, for the known attractant (urine - 3 days old), 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)** and blend A, more flies preferred the treated side than the control side ($p < 0.05$) (table 4.6). These results show that 2-propyloxane, δ-valerolactone and blend R elicited avoidance as compared to the δ-octalactone (**5**) (known repellent), on the other hand, 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)** and blend A caused attraction as the known attractant [cow urine (3 days old)].

Table 4.7: Upwind-flight progression

Test odour	Dose (g/ml)	N	Mean distance covered (CM±SE)		p-values
			Control	Test	
Blank (DCM)		30	48.20±5.25	48.67±6.91	0.06
Urine (3-days old)		30	21.00±2.87	62.65±1.14	0.01
δ-Octalactone (**5**)	0.05	30	48.00±4.75[a]	28.89±2.52[a]	0.01
	0.10	30	45.57±5.08[a]	26.57±3.64[a]	0.002
	0.20	30	53.25±4.56[a]	22.17±6.22[a]	0.05
	p-value		> 0.05	0.10	
2-Propyloxane (**14**)	0.05	30	48.00±4.14[a]	34.62±1.03[a]	0.001
	0.10	30	53.22±2.19[a]	34.64±1.59[a]	0.01
	0.20	30	64.89±2.45[ab]	31.23±1.67[a]	0.01
	p-value		0.91	0.65	
δ-Valerolactone (**16**)	0.05	30	55.67±3.12[a]	33.67±1.44[a]	0.05
	0.10	30	56.14±2.58[a]	29.50±1.60[a]	0.05
	0.20	30	57.45±3.13[a]	24.75±3.12[a]	0.05
	p-value		> 0.05	0.55	
Blend R	0.05	30	46.19±2.44[a]	32.75±5.67[a]	0.03
	0.10	30	46.05±4.16[a]	28.89±3.21[a]	0.05
	0.20	30	43.22±1.22[a]	23.57±1.22[a]	0.012
	p-value		> 0.05	> 0.05	
3-Propylcyclohexanone (**15**)	0.05	30	26.38±4.29[a]	38.90±1.37[a]	0.05
	0.10	30	21.30±2.20[a]	39.44±0.98[a]	0.01
	0.20	30	19.33±1.22[a]	40.22±1.05[a]	0.01
	P-value		0.70	0.11	
3-Propylcyclohex-2-enone (**17**)	0.05	30	26.18±4.23[a]	41.22±1.05[a]	0.01
	0.10	30	33.30±1.67[a]	47.30±1.15[a]	0.01
	0.20	30	41.00±2.40[ab]	48.88±1.34[a]	0.001
	p-value		0.34	> 0.05	
Blend A	0.05	30	35.72±6.73[a]	51.95±5.09[a]	0.05
	0.10	30	32.33±3.48[a]	47.22±2.13[a]	0.01
	0.20	30	22.34±1.11[a]	52.33±2.01[a]	0.01
	p-value		> 0.05	> 0.05	

N= number of tsetse flies used in the experiment; Blend A= a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**17**); Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**); analogues are R and S stereoisomers. Within columns, means (±SE) with the same small letter(s) are not significantly different at p > 0.05 (Student-Newman-Keuls test) and across a given row means (±SE) are not significantly different at p > 0.05 (t-Test).

There was no significant difference in the average upwind distance on both arms of the tunnel when DCM control was introduced (p > 0.05). However, in the presence of the

odour of test compound or blend, significant differences were observed. In the presence of δ-octalactone (**5**) (known repellent), 2-propyloxane **(14)**, δ-valerolactone **(16)** and blend R at all doses, the flies flew shorter distances in the treated arm than in the control arm ($p < 0.05$). The distances moved by the flies in the tunnel arm containing δ-valerolactone **(16)** odour were shorter than those in 2-propyloxane. In addition, the distance covered by the tsetse flies in the arm of the tunnel containing δ-octalactone (**5**) (known tsetse repellant) odour was much shorter than those covered in the presence of the odours of 2-propyloxane **(14)** and δ-valerolactone **(16)** respectively (table 4.7).

When the known attractant [cow urine (3 days old)], 3-propylcyclohexanone **(15)**, **3-**propylcyclohex-2-enone **(17)**, and blend A were dispensed respectively, the flies showed higher upwind progression at all doses on the treated side than the control side ($p < 0.05$). The distances covered in the tunnel arm containing the odour of attractants [3-propylcyclohex-2-enone **(17)**, blend A, 3-propylcyclohexanone (**12**)] were longer than in the control arms as compared to known attractant [cow urine (3 days old)] (table 4.7).

Table 4.8: Percent of flies in final resting position

Test odour	**Dose (g/ml)**	**N**	**Percent flies in the final resting position**		**p-values**
			Control	**Test**	
Blank (DCM)		30	48.0±1.10	49.0±0.98	0.06
Cow urine (3-days old)		30	17.1±2.11	79.9±2.17	0.01
δ-Octalactone (**5**)	0.05	30	79.2±1.77[a]	20.8±0.33[a]	0.01
	0.10	30	80.7±1.01[a]	18.3±1.65[a]	0.01
	0.20	30	81.6±1.53[a]	17.4±1.32[a]	0.01
	p-value		> 0.05	> 0.05	
2-Propyloxane (**14**)	0.05	30	55.1±1.26[a]	33.9±1.99[a]	0.01
	0.10	30	58.0±1.82[a]	32.0±1.54[a]	0.002
	0.20	30	60.2±1.55[a]	31.8±1.53[a]	0.001

	p-value		> 0.05	> 0.05	
δ-Valerolactone (**16**)	0.05	30	58.5±2.55[a]	32.5±1.22[a]	0.001
	0.10	30	66.4±3.12[a]	29.6±1.89[a]	0.01
	0.20	30	71.9±2.56[ab]	26.1±2.23[a]	0.02
	p-value		0.54	> 0.05	
Blend R	0.05	30	69.7±1.33[a]	29.3±0.01[a]	0.01
	0.10	30	70.5±2.98[a]	27.5±1.76[a]	0.01
	0.20	30	72.1±1.38[a]	26.9±1.54[a]	0.01
	p-value		0.56	0.98	
3-Propylcyclohexanone (**15**)	0.05	30	27.3±1.87[a]	70.7±2.99[a]	0.01
	0.10	30	26.6±2.97[a]	71.4±2.54[a]	0.01
	0.20	30	25.1±2.09[a]	72.9±1.43[a]	0.01
	p-value		0.78	0.10	
3-Propylcyclohex-2-enone (**17**)	0.05	30	26.9±2.11[a]	71.1±0.87[a]	0.002
	0.10	30	25.2±0.56[a]	72.8±1.21[a]	0.012
	0.20	30	24.7±2.09[a]	73.3±0.78[a]	0.041
	p-value		> 0.05	0.23	
Blend A	0.05	30	23.0±2.54[a]	74.0±0.24[a]	0.001
	0.10	30	21.8±0.56[a]	75.2±1.76[a]	0.001
	0.20	30	20.4±1.63[a]	76.6±2.53[a]	0.001
	p-value		0.46	0.22	

N= number of tsetse flies used in the experiment; Blend A= a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**17**); Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**); analogues are R and S stereoisomers. Within columns, means (±SE) with the same small letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test) and across a given row means (±SE) are not significantly different at $p > 0.05$ (t-Test).

At the end of three minutes of observation, the resting position of the tsetse flies was dependent on the nature of the test odour ($p < 0.05$) irrespective of the dosage ($p > 0.05$). There was no significant difference in fly choice direction when the control (DCM) was dispensed on both arms ($p > 0.05$). It was also noted that the percentage of flies resting on the treated side were more than the control side when known attractant [cow urine (3 days old)], 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)** and blend A were dispensed ($p < 0.05$). Comparison of the percentage of flies resting in either control or treated arms indicated that fewer flies rested on the treated side than control side when

δ-octalactone (**5**) (known repellent), 2-propyloxane **(14)**, δ-valerolactone **(16)** and blend R were dispensed respectively ($p < 0.05$) (table 4.8).

Generally, the behaviour of flies in the treated arm of the tunnel containing odours of 2-propyloxane **(14)**, δ-valerolactone **(16)**, δ-octalactone (**5**) or blend R was characterised by sharp U-turn in flight perhaps an attempt to escape from the tunnel. No such behaviour was noted when 3-propylcyclohex-2-enone **(17)**, 3-propylcyclohexanone **(15)**, blend A or [cow urine (3 days old)] was dispensed; instead the flies chose to walk, hop or fly within the treated arm of the tunnel. The successive behaviors of the flies that flew upwind after interacting with the odour were also in tandem with the repulsive or attractive nature of test odours. In the presence of 2-propyloxane **(14)**, δ-valerolactone **(16)**, δ-octalactone (**5**) or blend R respectively, the flies moved downwind and settled mostly in the odourless control arm of the tunnel. In the presence of 3-propylcyclohex-2-enone **(17)**, 3-propylcyclohexanone **(15)** or blend A respectively, the flies flew upwind and settled on the treated side of the tunnel while flapping their wings, similar to a previous observation reported by (Gikonyo *et al*. 2003; Wachira *et al.,* 2016).

4.3.1 Relative repellency of the synthetic analogues against *G. m. morsitans* using a wind tunnel assay

The results of the relative repellency of the odours of δ-octalactone (**5**), δ-valerolactone **(16)**, 2-propyloxane **(14)** and blend R [2-propyloxane **(14)** and δ-valerolactone **(16)**] against 3-day old teneral tsetse flies, *G. m. morsitans*, in a wind tunnel assay apparatus are summarised in the table 4.9.

Table 4.9: Repellency of individual compounds and blend R

Doses (g/ml)	δ-Octalactone (**5**)	2-Propyloxane (**14**)	δ-Valerolactone (**16**)	Blend R	p-values
0.05	60.00 ± 2.11^{aA}	25.00 ± 1.44^{aC}	28.45 ± 1.23^{aC}	40.65 ± 2.19^{aB}	0.01
0.10	63.13 ± 2.34^{aA}	28.44 ± 1.34^{aC}	36.28 ± 3.43^{aC}	44.44 ± 2.43^{aB}	0.023
0.20	65.44 ± 2.87^{aA}	31.65 ± 2.02^{aC}	46.66 ± 3.22^{abB}	46.22 ± 2.68^{aB}	0.019
p-values	0.15	0.13	0.13	0.06	

Blend R = a blend of 2-propyloxane (**14**) and δ-valerolactone (**16**). Within columns, means (±SE) with the same letter(s), and across a given row, means (±SE) with the capital same letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test), respectively.

After three minutes of observation, there was no significant difference in repellency among percentages of repellency at all doses caused by 2-propyloxane **(14)** ($p = 0.13$), δ-valerolactone **(16)** ($p = 0.13$), δ-octalactone (**5**) ($p = 0.15$) and blend R ($p = 0.06$) respectively. It was also found that, there was significant difference in the repellency of the individual compounds and blend R across all the three doses ($p < 0.05$). However, there was no significant difference in the mean percentage repellency caused by 0.05 g/ml and 0.20 g/ml doses of 2-propyloxane **(14)** and δ-valerolactone **(16)** ($p > 0.05$). In contrast, there was a significant difference in the repellency at 0.20 g/ml ($p > 0.05$). The repellency of δ-valerolactone **(16)** and blend R were comparable at 0.20 g/ml ($p > 0.05$) (table 4.9).

Table 4.10: Attractancy of synthetic analogues (3-propylcyclohexanone and 3-propylcyclohex-2-enone and blend A)

Dose (g/ml)	3-Propylcyclohex-2-enone (**17**)	3-Propylcyclohexanone (**15**)	Blend A	p-value
0.05	46.12 ± 1.55^{aA}	44.16 ± 2.77^{aA}	52.00 ± 3.46^{aA}	0.91
0.10	48.00 ± 0.24^{aA}	46.44 ± 1.56^{aA}	56.37 ± 5.19^{aA}	0.77
0.20	50.33 ± 1.67^{aA}	48.11 ± 2.63^{aA}	58.33 ± 6.43^{aA}	0.95
p-value	0.13	0.21	0.11	

Blend A = a blend of 3-propylcyclohexanone (**15**) and 3-propylcyclohex-2-enone (**16**). Within columns, means (±SE) with the same letter(s), and across a given row, means (±SE) with the same capital letter(s) are not significantly different at $p > 0.05$ (Student-Newman-Keuls test), respectively.

From these results all odours, 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)** and blend A, showed attractancy for *G. m. morsitans.* There was no significance difference in attractancy among the test compounds for all the three doses (p = 0.91, 0.77 and 0.95). It was also noted that for every test compound there was no significant difference in attractancy for all the three doses (p = 0.13, 0.21 and 0.11) (table 4.10).

It can be reported that the structural modification of δ-octalactone to obtain analogues with oxygen in the ring, 2-propyloxane **(14)** (without the carbonyl group) and δ-valerolactone **(16)** (without the propyl side chain) resulted in reduced repellency as compared to the δ-octalactone (**5**) (Table 4.9). On the other hand, the analogues without oxygen in the ring, 3-propylcyclohexanone **(15)** (without lactone oxygen) and 3-propylcyclohex-2-enone **(17)** (having a double bond between 2^{nd} and 3^{rd} position in the ring), reversed the behaviour from repellency to attractancy. It was also noted that the response of the tsetse flies to the test odours was dose-independent for the dose range applied (table 4.6, 4.7 and 4.8). The repellency of 2-propyloxane **(14)** and δ-valerolactone **(16)** were comparable and the repellency of their blend was lower than that of δ-octalactone (**5**) (Table 4.9). The attractancy of 3-propylcyclohexanone **(15)** and 3-propylcyclohex-2-enone **(17)** were comparable and no synergistic effect in the attractancy of their blend was observed (table 4.10).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The synthesis of 3-propylcyclohexanone **(15)** was not involving, and therefore the method adopted was relatively effective as it led to a yield of 75% (5.4ml). On the hand, the yield of δ-valerolactone **(16)** was relatively lower 64% (3ml) due to the longevity of the procedure adopted.

Based on the analysis of the results, it can be concluded that the structural modification of δ-octalactone affects the repellency and even reverses the behaviour of tsetse from repulsion to attraction. 2-Propyloxane **(14)** and δ-valerolactone **(16)** manifested avoidance whereas 3-propylcyclohexanone **(15)** and 3-propylcyclohex-2-enone **(17)** elicited attraction.

Synergistic effect on repellency was noted when blending 2-propyloxane **(14)** and δ-valerolactone **(16)** compared with individual compounds when *G. pallidipes* was deployed. However, there was no significant change when *G. m. morsitans* was deployed. It was also noted that *G. m. morsitans* responded more actively to the known odours and synthetic analogues than *G. pallidipes*.

This study lays down useful groundwork for further structure-activity studies for identifying more potent attractants and repellents for the control of *G. m. morsitans, G. pallidipes* and other tsetse species by ‘push’, ‘pull’ and ‘push-pull’ tactics.

5.2 Recommendations

The following are recommended from this research:

i. Other methods need to be explored in the synthesis of δ-valerolactone **(16)** so as to minimize procedural steps and maximize the yield.

ii. The effect of modifying the alkyl chain and the ring of 3-propylcyclohexanone **(15)** and 3-propylcyclohex-2-enone **(17)** on their attractancy need to be carried out.

iii. The responses of other tsetse species to these analogues and blends also need to be studied.

iv. Field work should be carried out to compare the attractancy of 3-propylcyclohexanone **(15)**, 3-propylcyclohex-2-enone **(17)**, and their blend to see if they can complement currently used attractants (cow urine and acetone).

v. Studies on the other proportions of the two constituents of blend A [3-propylcyclohexanone **(15)** and 3-propylcyclohex-2-enone **(17)**] need to be undertaken because different compounds interact with different neuro-receptors in the tsetse antennae and therefore a blend might have an additive effect hence greater attraction.

vi. Development and deployment of 'push-pull' tactic in eliminating tsetse flies using the putative attractants and known repellants.

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APPENDIX

APPENDIX 1: CHROMATOGRAMS AND TABLES FOR STRUCTURAL CONFIRMATION

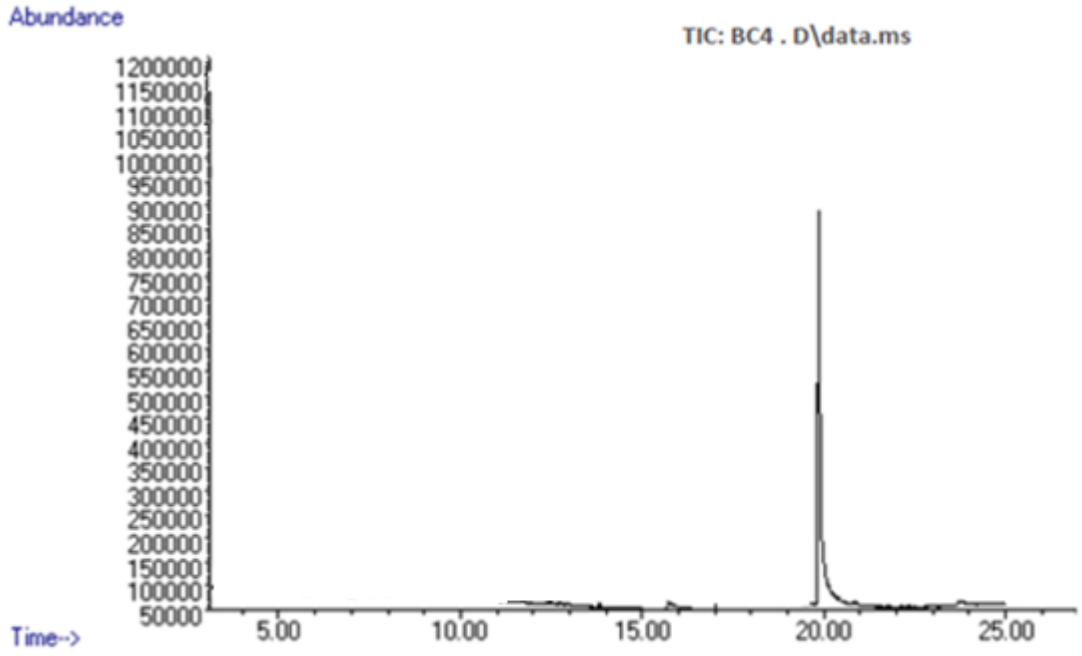

Figure 6.1: Total ion chromatogram for (RS)-3-propylcyclohexanone **(15)**

[contents]							
count=1							
Name=	C:\msdchem\1\DATA\Ronald\july\BC4.D						
1=	PBM Apex minus start of peak						
[PBM Apex minus start of peak]							
Time=	Tue Jun 24 06:33:11 2014						
Header=	PK	RT	Area Pct	Library/ID	Ref	CAS	Qual
61=	61	18.6306	0.6565	28.37 Selinene<beta->	420	017066-67	99
62=	62	18.7426	1.2535	28.74 Selinene<alpha->	421	000473-13	99
63=	63	19.0337	0.3461	N-Acetyl-3-ethoxyamphetamine	75385	1000122-5	35
64=	64	19.1905	0.4093	Benzene, 1-ethyl-3-(phenylmethyl)-	56627	028122-24	30
65=	65	19.4593	0.132	1H-Pyrrole-2-carboxylic acid	6017	000634-97	22
66=	66	19.6384	9.3773	3-Propylcyclohexanone, R	71286	002050-20	52
67=	67	20.288	0.4447	3-Propylcyclohexanone, S	15228	002895-21	43
68=	68	20.5791	0.1338	Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenyl	208547	056114-62	50
69=	69	20.6687	0.1906	2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-naphthaler	62222	124957-09	49
70=	70	20.8255	0.2445	2-Dimethylaminomethyl-4-chloro-1-naphthol	86125	167163-22	22

Table 6.1: GC-MS run of (RS)-3-propylcyclohexanone **(15)**

Data File : BC4.D
Acq On : 23 Jun 2014 01:30
Operator :
Sample : BC4
Misc : BC4
ALS Vial : 22 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : C:\msdchem\1\METHODS\DEFAULT-USER1.M

Signal : TIC: BC4.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
61	18.639	693	694	697	VV 2	75430	3048543	4.24%	0.657%
62	18.753	697	699	710	VV 2	76144	5820632	8.10%	1.254%
63	19.039	710	712	718	VV 7	20444	1607216	2.24%	0.346%
64	19.197	718	719	729	VV 8	14142	1900605	2.65%	0.409%
65	19.451	729	731	734	VV 4	10310	612753	0.85%	0.132%
66	19.633	734	739	766	VV	740203	43541918	60.61%	39.778%
67	20.284	766	768	778	VV 8	16564	2065088	2.87%	14.145%
68	20.584	778	781	783	VV 2	12399	621377	0.86%	0.134%
69	20.669	783	785	789	VV 5	13470	885250	1.23%	0.191%
70	20.821	789	792	801	VV 9	8713	1135468	1.58%	0.245%

Table 6.2: GC-MS library for identification of (RS)-3-propylcyclohexanone **(15)**

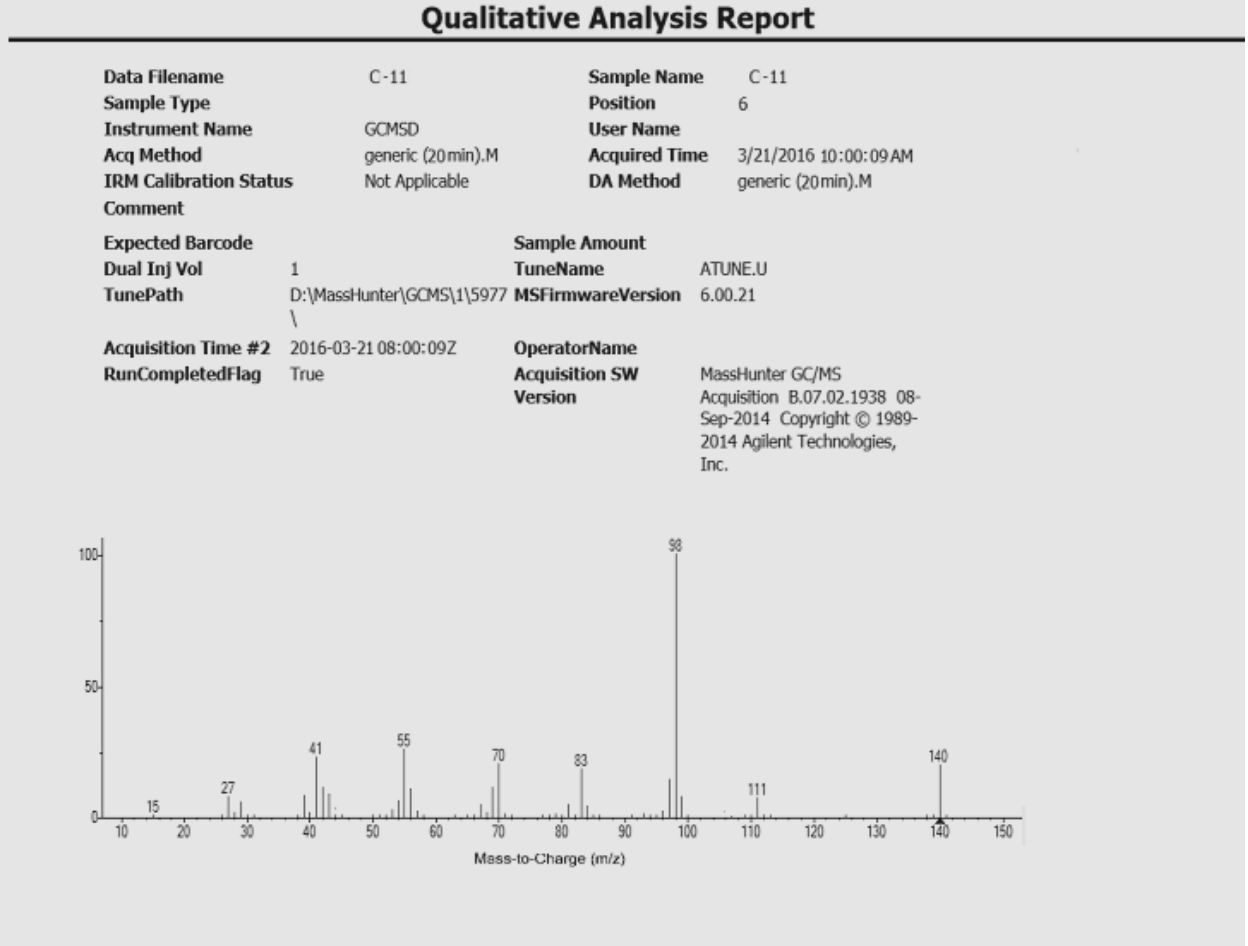

Figure 6.2: GC-MSD spectrum of (RS)-3-propylcyclohexanone **(15)**

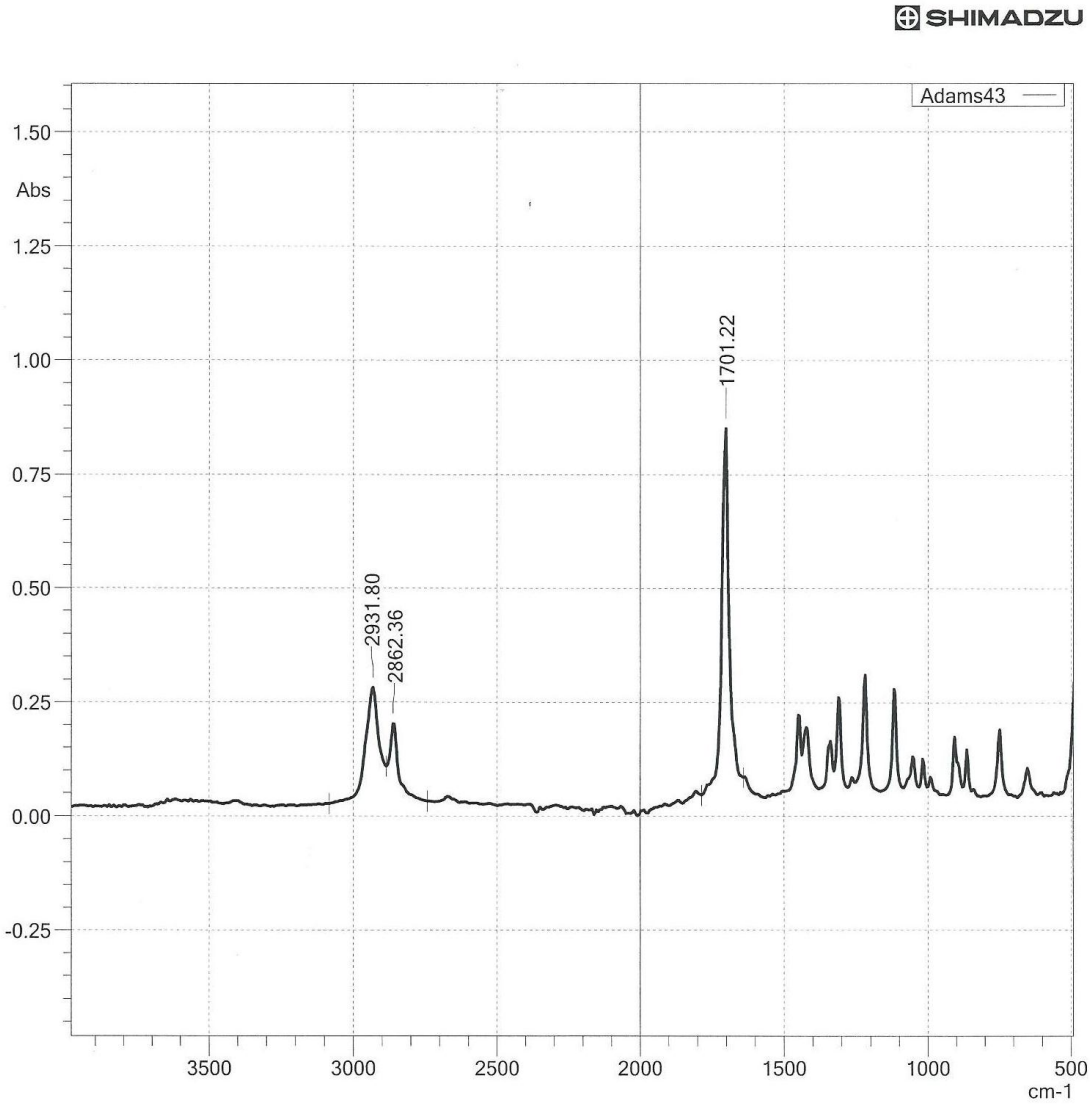

Figure 6.3: IR spectrum of (RS)-3-propylcyclohexanone **(15)**

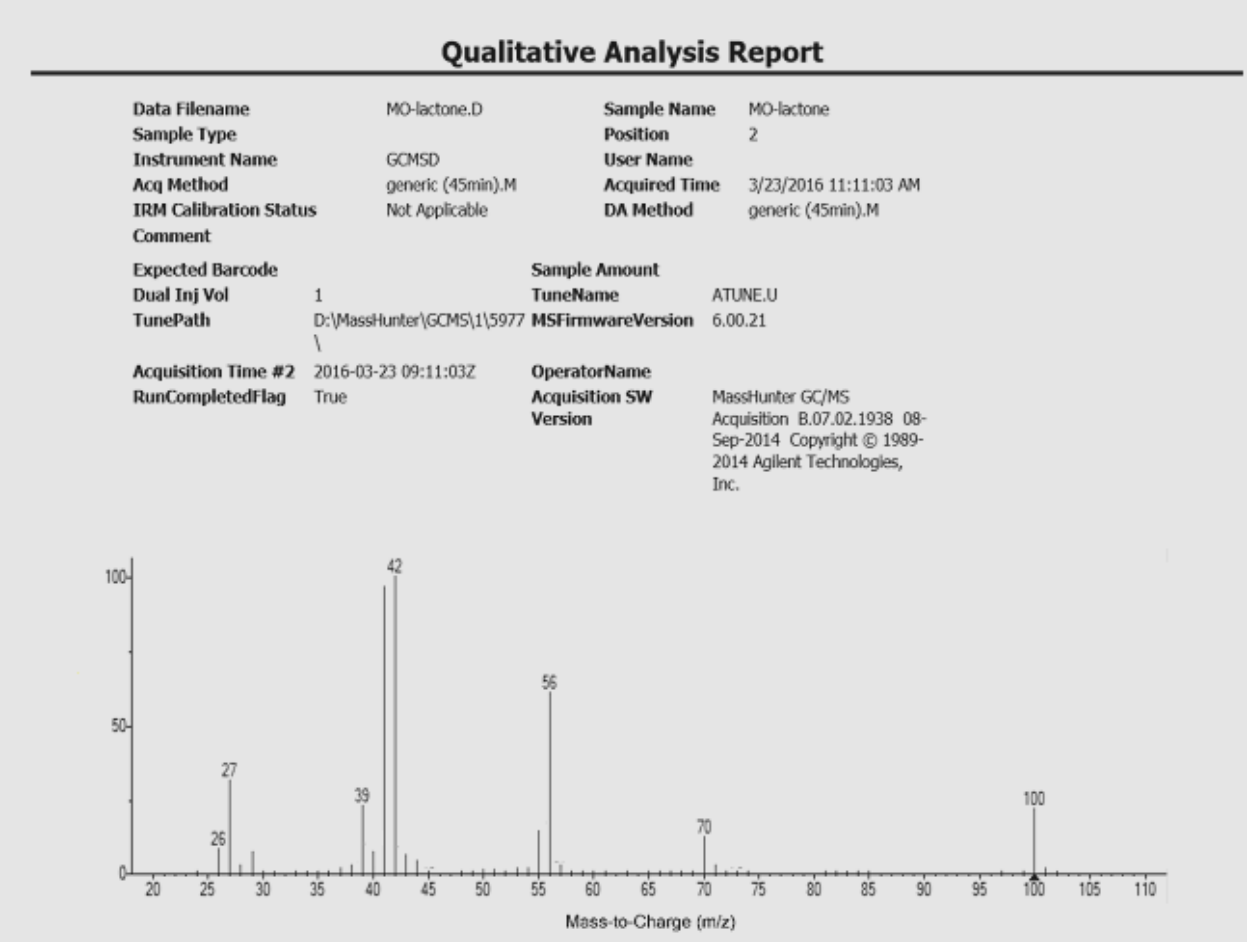

Figure 6.4: GC-MSD spectrum of (RS)-δ-valerolactone **(16)**

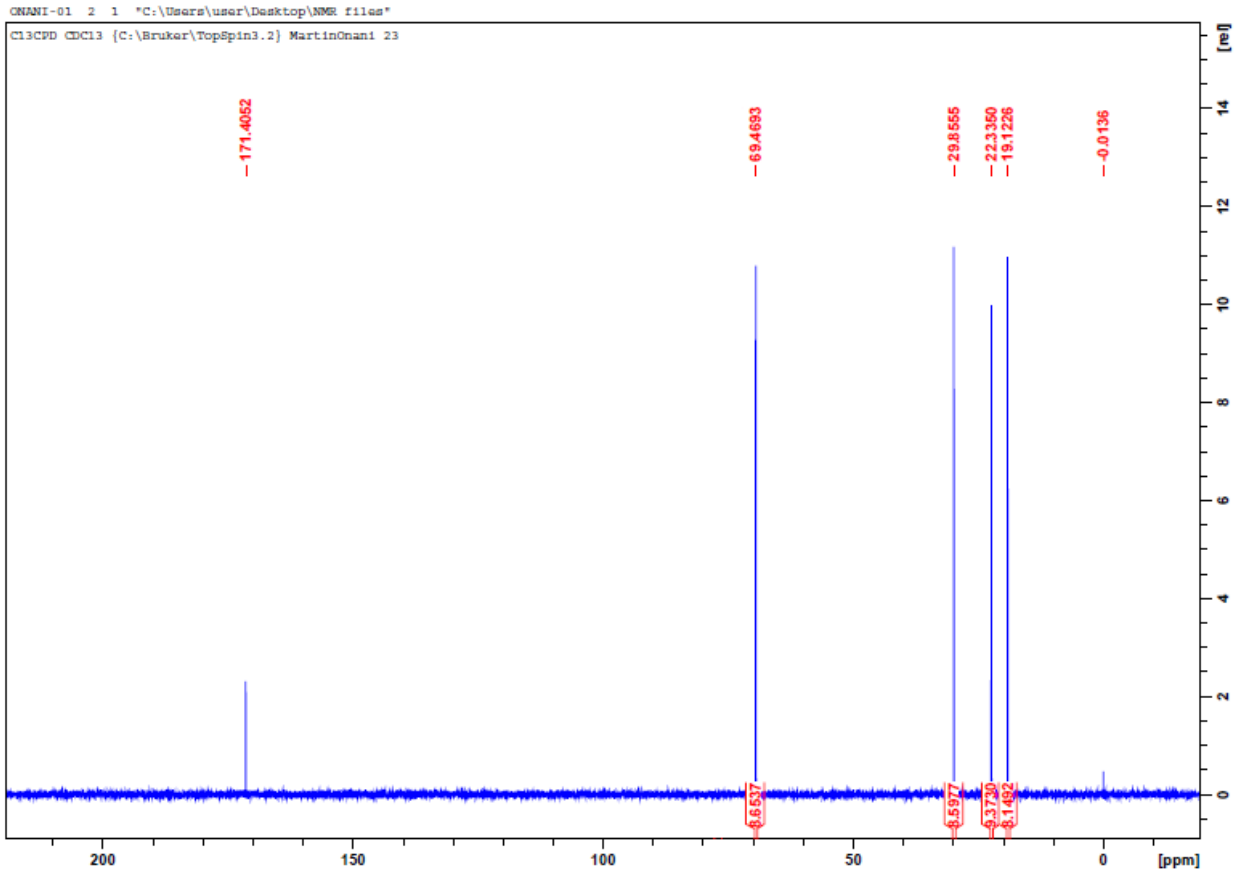

Figure 6.5: ^{13}C – NMR spectrum of (RS)-δ-valerolactone **(16)**

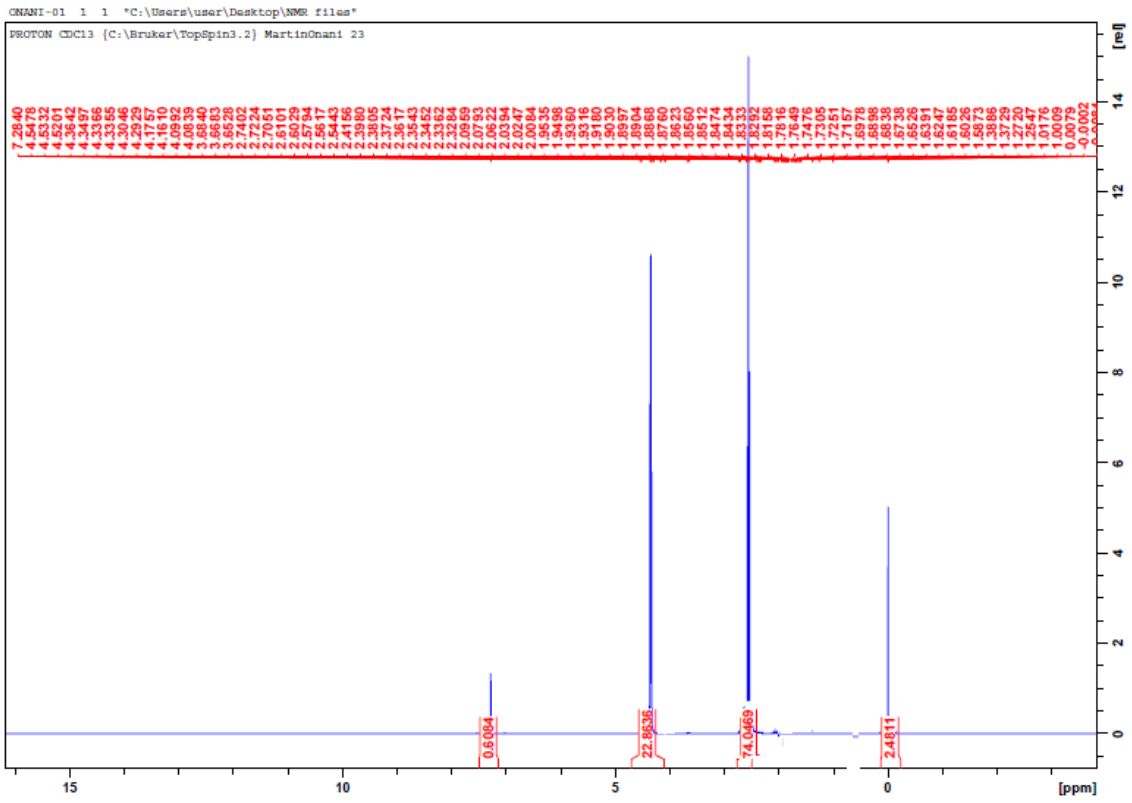

Figure 6.6: ^{1}H – NMR spectrum of (RS)-δ-valerolactone **(16)**

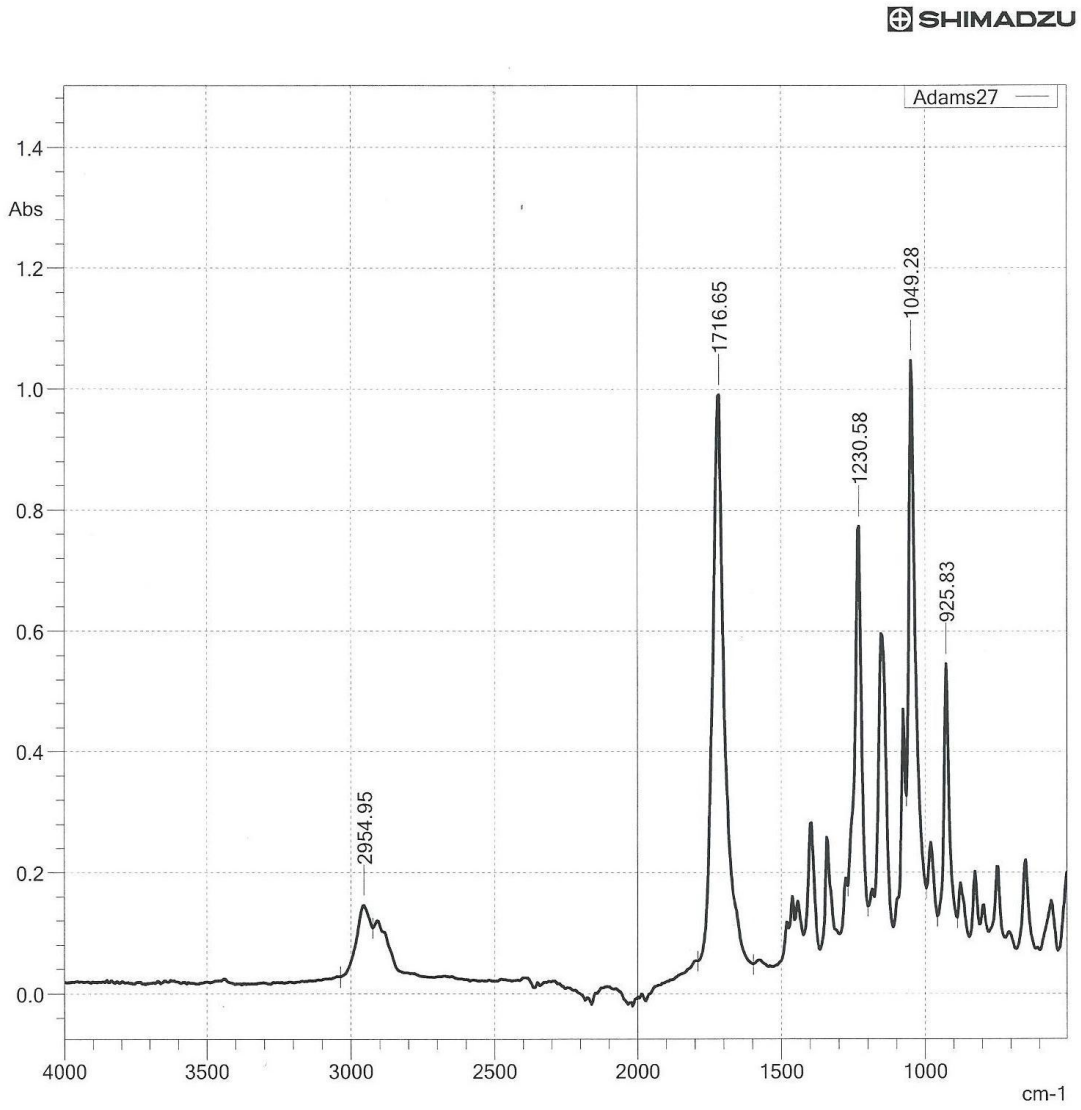

Figure 6.7: IR spectrum of (RS)-δ-valerolactone **(16)**