ENANTIOSELECTIVE SYNTHESIS OF (R)- AND (S)- δ-OCTALACTONE AND THEIR EFFECTS ON *GLOSSINA MORSITANS MORSITANS* HOST SEEKING BEHAVIOUR

BY

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DECLARATION

I declare that the work reported in this thesis is my original work excluding any work done in collaboration and has never been presented for any academic award in any other University/institution.

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DEDICATIONS;

To: Mrs. Mary Wambui (an ideal and supportive mother for your timely support).

&

David Mwangi (Born on my 24th birthday).

List of plates, figures and tables

List of abbreviations

AAA	Asymmetric Allylic Alkylation
AAT	African Animal Trypanosomosis
AI	Asymmetric Induction
AT	Animal Trypanosomosis
^{13}C-NMR	Carbon 13 Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform.
CIPE	Complex Induced Proximity Effect
DDT	Dichlorodiphenyltrichloroethane
DEET	N,N-Diethyl-*m*-toluamide
DEPT	Distortionless Enhancement Polarization Transfer
DIBALH	Diisobutyl aluminium hydride
DIP	Direct insertion probe
EAD	Electroantennal detector
ECF	East Coast Fever
ee	Enantiomeric excess
EI-MS	Electron Impact Mass Spectrometry
eV	Electron volt
FID	Flame Ionization Detector
GC	Gas Chromatography
GNP	Gross National Product
HAT	Human Animal Trypanosomosis
HLE	Horse liver esterase
HMPA	Hexamethylphosphoric acid
HPLC	High Performance Liquid Chromatography.
Hz	Hertz
i.d	Internal diameter
ILCA	International Livestock Centre for Africa
ILRAD	International Laboratory for Research in Animal Diseases

ILRI	International Livestock Research Institute
$IpcBH_2$	Isopinocamphenyl Borane
Ipc_2BH	Diisopinocamphenyl Borane
mCPBA	*m*-Chloroperbenzoic Acid
m.p.	Melting Point.
MS	Mass spectrometer
NMR	Nuclear Magnetic Resonance
NTOs	Non-target organisms
PLE	Pig liver esterase
PMB	*p*-Methoxybenzyl
PPL	Pig pancrease lipase
ppm	Parts per million
pTSA	*p*-Toluene sulphonic acid
PVC	Polyvinyl chloride
RAMP	(R)-1-amino-2-methoxymethylpyrrolidine
R_f	Retention factor
R_t	Retention time
SAMP	(S)-1-amino-2-methoxymethylpyrrolidine
SNOPAD	Standardized nomenclature of parasitic diseases
TBDMS	tert-Butyldimethylsilyl
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
VSG	Variant Surface Glycoproteins
WHO	World Health Organization

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PROLOGUE

"The struggle between man and insects began long before the dawn of civilization, continued without cessation to the present time and will continue, no doubt, as long as human race endures (Metcalf *et al*, 1962) basically due to the fight of this pair for a common target or goal. Its intensity owing to the importance, to both, of the targets they struggle for and its long continuance is due to the fact that the contestants are so equally matched. We commonly think of ourselves as the lords and conquerors of nature, but insects had thoroughly mastered the world and taken full possession of it long before man began the attempt. Consequently, they had all the advantage of a possession of the field long before the contest began. They have disrupted every step of our invasion of their original domain so persistently and so successfully that we can even scarcely flatter ourselves that we have gained any very important advantage over them. Here and there a truce has been declared, a treaty made, and even a partnership, advantageous to both parties of the contract, established - as is the case with bees and silkworms. But wherever their interest and ours are diametrically opposed, the war still goes on and neither side can claim a final victory. If they want our crops, they still help themselves to them. If they wish the blood of our domestic animals, they pump it out of our cattle and horses at their leisure and under our very own eyes. If they wish to take their abode with us, we cannot wholly keep them out of the houses we live in. We cannot even protect our very persons from their annoying and pestiferous attacks, and since the world began, we have never yet exterminated so much as a single insect species-we probably never shall. They have in fact inflicted upon us for ages the most serious evils without our even knowing it" (Forbes, 1915).

ABSTRACT

African trypanosomosis is a complex disease of man and livestock that smoulders silently in the rural sub-Saharan Africa wrecking great havoc to the poor communities and causing loss of life, properties, and migration from villages or regions. To the victims, it causes a reversal of the circardian rhythms (sleep-wake cycle) and hormonal secretion. About 55 million people, 56 million cattle, 32 million goats and sheep and >260 million other small ruminants are at risk of this disease in Africa. Tsetse flies are its primary cyclic vectors thus a threat in >40 countries south of the Sahara. The economic effects of trypanosomosis places it second to malaria among vector borne diseases of importance to Africa. Thus, the OAU dedicated year 2001 to the control of the tsetse fly.

The use of semiochemical-derived products in disease vector control is environmentally friendly and has low risks of resistance development. Unfortunately, semiochemicals occur in minute quantities in nature thus cheap synthetic samples become handy tool in their use. δ-Octalactone was isolated from a non-preferred host of the tsetse fly, the waterbuck, among other electrophysiologically active compounds. The electrophysiological activity studies and synthesis of the racemic mixture was done. Behavioural bioassay of the racemate was not done nor were the enantiomers synthesized separately.

The current work involved the enantioselective synthesis of δ-octalactone and investigation of an economically viable route to the active isomer(s). We have discovered an efficient and economical synthetic route to the racemate and both isomers. The CIPE in asymmetric induction reactions was exploited efficiently in the chiral synthesis. Absolute configuration was assigned based on Cram's rule, CIPE and basic chemical thermodynamic theory. The bioassays of the synthetic products using 3-day starved teneral tsetse flies (*G. morsitan*) revealed that stereochemistry has no role to play in the repellent properties of this lactone (no stereospecific antennal responses). The compounds were identified by conventional analytical methods. Results indicate that the molecule is a repellent with a high potential in tsetse fly control. From this work, a new route to δ-lactones utilizing organomettalic reagents is being reported for the first time.

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CHAPTER 1: INTRODUCTION

Over 170 years ago the tsetse fly was first described in Africa and scientifically named, yet it continues to demand our attention to this day. This is mainly because they transmit nagana to livestock and sleeping sickness to man, making them insects of major medical and veterinary importance. Despite the voluminous work done on the tsetse fly and trypanosomosis (Brown *et al*, 2001), they are still a menace in 40 African countries (Leak, 1998). The infested areas lie between latitudes 15°N and 20°S (Service, 1986), covering about 11 million km^2 (Hagan, 1975). In East Africa half of Tanzania, a third of Uganda and a quarter of Kenya is infested (ILRAD, 1990). The affected areas have good rainfall and high agricultural potential (WHO, 1996).

It is amazing that, although developing countries hold 70% of the world's population (50% working in agriculture), they produce only 29% and 23% of the world's meat and milk respectively, while developed countries with 5% of their population in agriculture produce the balance. Meat and milk consumption in Africa is low, with average per-capita of 7.5 kg and 15.2 kg, respectively (Swallow, 1997). This is due to low livestock productivity attributable to livestock diseases of which trypanosomosis and ECF are of major concerns. Although livestock contribution to GNP is low, it is important as a protein supply and a significant contributor to crop production in Africa. The continent already shows a high deficit in meat production and a much higher one in dairy products. Presence of tsetse fly, alongside ticks, is an overwhelming feature in livestock keeping in sub-Saharan Africa (Leak, 1998).

African trypanosomiasis (AT) (trypanosomosis)* remains a major constraint to livestock productivity and continues to impede intensification of crop-livestock systems across vast areas of the humid and sub-humid zones that hold the greatest potential for agricultural production (Brown *et al*, 2001). Surprisingly, the disease has not been accorded adequate attention mainly due to its prevalence among the rural poor and therefore has low political

* "trypanosomosis" (Latin) has been recommended by the SNOPAD in preference to "trypanosomiasis" (Greek) (Kassai *et al*, 1994.

visibility and priority. This is exemplified by the trend the disease is taking after the ban on DDT, the future being headed for the worst as evident in a case study of central Africa between 1926 and 1994 (Plate 1).

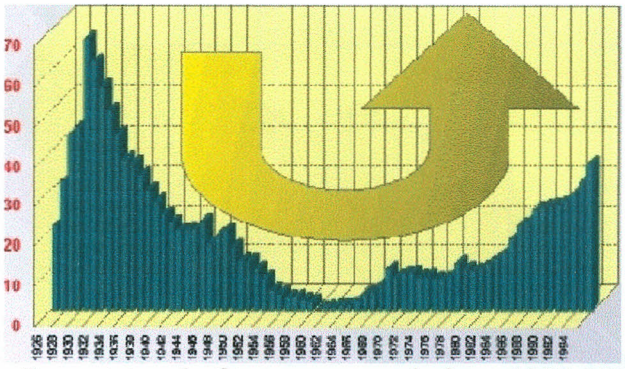


Plate 1: Current trend of trypanosomosis from 1926-1994 (source: www.brown.edu/courses/bio_160/projects1999/trypanosomes/burden.html)

The main direct economic impact of trypanosomosis is on cattle. Annual losses amount to ~3 million deaths, mainly of young stock, with up to 25% mortality in pre-weaning calves. Mortality rates are compounded by lower reproduction, low milk yield and weight loss. With nearly 50 million cattle (94% of the total African cattle population) distributed at the fringes of the continental tsetse belt, direct losses were estimated at Kshs. 48-96 billion annually. The distorted cattle distribution also affects crop production. In the absence of cattle there is no draught power for ploughing, less manure to use as fertilizer, hence less feeding of animals with crop residues and by products (Leak, 1998). The productivity of the land therefore remains sub-optimal. In Burkina Faso, animal african trypanosomosis (AAT) was found to be the major constraint in agro-pastrolism. Use of curative drugs, pour-ons and targets reduced *Glossina tachinoides* and *G. morsitans* by more than 90% in less than 7 months, while AAT incidences dropped below 5%. This translated into an increased gross income of about US$ 3/day for the local women (Bauer *et al*, 1999).

The flies confine man and his animals to specific settlement areas thus greatly influencing settlement patterns and the development of farming in tropical Africa (Deshler, 1960). In Tanzania, cattle mortality was reduced by 66% with 90% reduction in tsetse density (Leak,

1998). This emphasizes the fact that trypanosomosis is arguably the main constraint in livestock and agricultural production in Africa (Owen, 1991). The impact of tsetse fly and trypanosomosis is greater than suggested figures, because many of these infested areas are potentially the most agriculturally productive in Africa (McKelveyjr, 1973) (Plate 2).

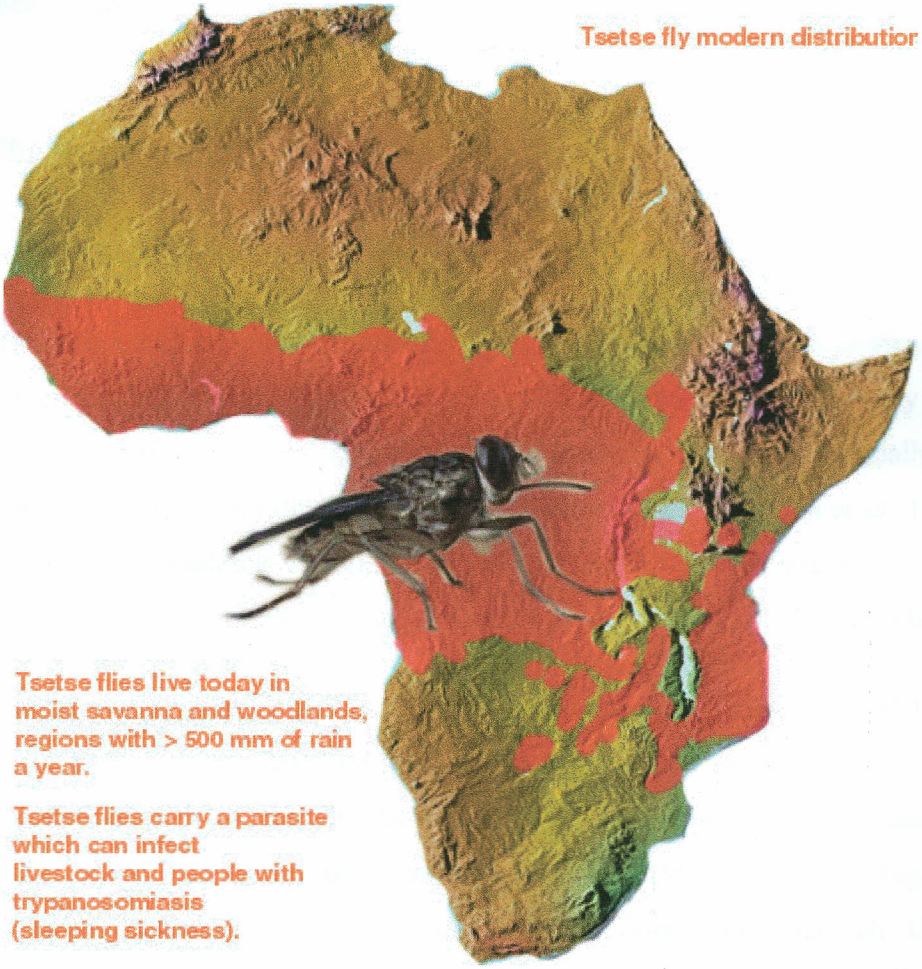


Plate 2: Tsetse fly distribution (Source: www.indiana. edu/ ~origins/teach/ p380/p380Africainfo.html)

Globally, 55 million people are at risk of being infected with sleeping sickness. In 1994, sleeping sickness caused 20,000 (66.67%) deaths of the 30,000 reported cases in the Congo basin due to lack of effective treatment (WHO, 1996). The number of deaths from sleeping sickness in Africa is estimated at 55,000 per annum (World Bank, 1993). In Africa, ~50 million cattle, >32 million goats and sheep plus >260 million small ruminants are at risk from nagana (ILRAD, 1991). This is exacerbated by losses in milk yield, tractive power,

waste products (natural fuel and fertilizer) and secondary products like hides (ILRAD, 1986). As the population grows at 3% and food production at 2% per annum, an annual shortage of 250 million tons of food is expected by year 2020 (World Bank, 1993). This can be partly addressed by implementing effective, cheap and superior measures to manage livestock diseases.

1.1.The diseases

1.1.1. Sleeping sickness

The disease is a major threat to human beings in the tropics and occurs in about 200 distinct foci in tsetse-infested areas where it has always occurred sporadically for very long periods. Although relatively few new cases of the disease are diagnosed annually, 55 million inhabitants are at risk with only 3 million under surveillance (Knudsen *et al*, 1992). Despite absence of recent epidemic situations, the disease should not be ignored. WHO estimates the number of deaths from this disease at 55,000 people per annum in Africa (WHO, 1996).

The disease is normally fatal though healthy carriers have been reported (Leak, 1998). Patients are "sleepy by day and restless by night" due to serotogenic raphe nuclei-suprachiasmatic nuclei liaison in the reversible disturbance of the circadian rhythms of sleep-wake cycle and hormonal secretions (Buguet, 1999). In early stages, the main symptoms are high fever, weakness and headaches, joint pains and itch. Gradually, the initial symptoms become more pronounced and other manifestations such as anaemia, behavioural changes, loss of concentration, cardiovascular and kidney system (cus) problems appear. Sudden completely unpredictable mood changes become increasingly frequent. It causes immense mental suffering, with early symptoms such as sleep disturbances and psychosis. Extreme topper, insomnia and exhaustion then overcome the patient, leading to deep coma and eventually death. When untreated, the disease continues until it gives no respite for suffering, day or night, and ends in death (WHO, 1996). When the transmission is high the effect is dramatic and whole villages may be abandoned. Exclusively a rural disease, it smoulders steadily except for occasional outbreaks.

Two types of the disease occur with each being caused by different parasite subspecies. The West African type is a mild form and kills the victims several years after infection. The east and southern African type is a more virulent form killing victims after only a week to one year (WHO, 1996). Control basically relies on surveillance and treatment of the disease to reduce the human reservoirs, prevention of epidemics through rapid diagnosis and treatment of all identified cases coupled with vector control to break the disease transmission cycle.

1.1.2 Nagana

"Nagana" is derived from a Zulu word meaning "a state of depressed spirits." Knowledge of the disease dates from mid 15th century (Lancaster *et al*, 1986). The disease is caused by *Trypanosoma brucei* (*T.b.*), *T.vivax*, *T. cheleiri* and *T. evansi* (Owen, 1991). Although each is a pathogen in its own right, mixed infections normally occur under natural field conditions (ILRAD, 1990). Tsetse flies are known to cyclically transmit trypanosomes although other insects do so mechanically but with a lower impact.

The disease manifests itself in such pathological features like anaemia, lymphoid cell proliferation, immuno-suppression and circulatory disturbances (Leak, 1998). In acute form of the disease; there is high temperature, quickly followed by anaemia, progressive weakness and eventually death. In the most common form; there is fluctuation in body temperature, a dry coat, gradual waste of the animal, extreme weakness and death usually after three months (Deshler, 1960). It also interferes with the reproductive cycle of the animal (ILRAD, 1987). Nagana may be acute or chronic and may lead to loss of conditions, oedema of dependent parts, progressive emaciation, intermittent fever and sunken eyes (Lancaster *et al*, 1986). Abortion, bloody diarrhoea and death in naturally transmitted infections have been reported in a number of cases. All these engender physiological changes in the host animal such as serum amino acid and various urinary catabolite levels. Although environmental factors such as drought (which cause malnutrition) have a great influence on the dynamics of trypanosomosis, man is a major contributing factor in the African trypanosomosis scene through population growth and socio-economic activities.

Nagana is transmitted by several species of the tsetse flies. The population of flies infected with nagana is comparatively higher than the human sleeping sickness trypanosomes (ILRAD, 1991). *Glossina morsitans* (*G.*m) are deadly to cattle while *G. pallidipes* are inefficient vectors (Sidiga, 1982). The primary mode of transmission is through the tsetse fly where the trypanosomes undergo changes, which include shape and infectivity.

The control of the disease depends mainly on the use of curative and prophylactic drug regimes and vector control (ILRAD, 1987). However, chemotherapy alone is not adequate to allow susceptible livestock to be raised on a permanent basis in fly infested belts except when combined with vector control.

1.2 The tsetse fly

The word "tsetse" originates from the Sechuana language (Botswana) and signifies "a fly destructive to cattle." It is probably named from the noise the fly makes while resting (Langridge *et al*, 1971). Some geographical barriers such as highlands, arid and semi-arid areas where climate is a limiting factor, inhibit spread of the fly. If some species were able to pass these natural barriers, they could spread to other areas where conditions are suitable.

Tsetse flies belong to genus *Glossina* (Diptera) with 31 species and subspecies. Twenty-three (23) species are found in sub-saharan Africa (Leak, 1998). Few (*G. pallididpes, G. morsitans, G. fuscipes, G. midecorum, G. palpalis* and *G. swynnertonni*) have been extensively studied due to their economic importance. The tsetse fly (Plate 3) is a small insect resembling the common housefly, though slightly bigger.

The genus *Glossina* has a distinct feathered arista– the long bristle on each antenna (Burgress *et al*, 1993). Amazingly, it is the only insect known to be capable of transmitting sleeping sickness cyclically (WHO, 1979), others do so mechanically and with a lower vectorial capacity. However, other insect vectors are capable of transmitting other trypasoma species except those responsible for human sleeping sickness (Cattard, 1994).

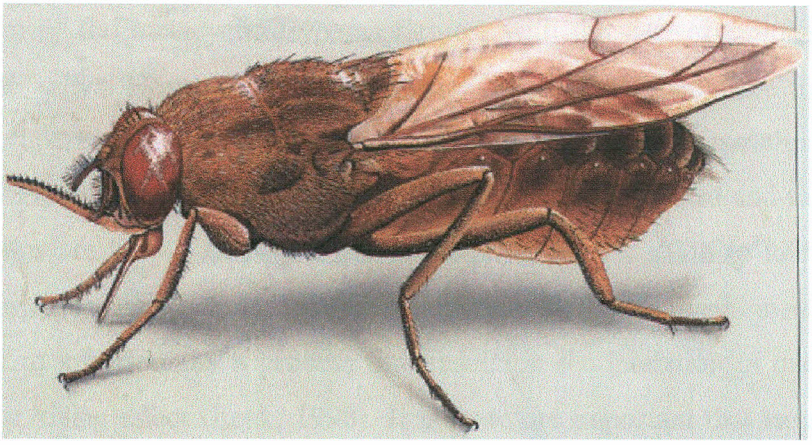


Plate 3: Tsetse fly (source: www.cdfound.to.it/HTML/trypsa.htm)

1.3 The parasite

Human African trypanosomosis (HAT) and AAT are both caused by protozoa of the *Trypanosoma* genus. Human sleeping sickness is caused by *T. brucei rhodesiense* and *T. brucei gambiense.* These two are distributed in two distinct African regions, *T.b. rhodesiense* in eastern African and *T. b gambiense* in West Africa. The latter causes a milder form of the disease as compared to the former. In livestock, *T. vivax, T. b. brucei, T. evansi* and *T. congolense* are the most common disease causing species. In birds *T. bakeri, T. bouffardi* and *T. everetti* cause diseases (Leak, 1998).

Trypanosomes are single celled parasites adapted to live in the bloodstream of the vertebrate host. In Africa they are largely parasites of the game animals, which have generally become immune and act as reservoirs, from where they find their way to man and domestic animals. Tsetse fly acquires trypanosomes (amestigotes) from the host during a blood meal (Burgress *et al*, 1993). The trypanosomes undergo crucial development once in the fly spearheaded by high mitochondria development due to change from a non-Krebs to a Krebs cycle. They then enlarge and multiply either in the midgut, proventriculus, salivary gland or mouthparts of the fly. Various trypanosoma species take different routes in the course of development once in the fly requiring 18-34 days to develop into the infective form (Service, 1986; Burgress *et al*, 1993). Both the blood stream and midgut forms of the trypanosomes are free swimming, but on reaching the salivary gland the mesocyclic forms attach and multiply as attached epimastigotes. Attachment is maintained during the

differentiation of the metacyclic trypamastigotes, with the latter becoming free again for infection with saliva. However, the trypanozoons and nannommas have to cross from the gut to the ectoperitropic space for maturation before passing to the mouthparts (Burgress *et al*, 1993). For a fly to be infective an infection has to be established and the infection must mature. Lectins are known to play a role in determining refraction to infections by killing procyclic trypanosomes, while symbiotic bacteria are involved in determining the susceptibility to infections by a process that results in the inhibition of midgut lectins thus reducing their killing effect (Leak, 1998). It is therefore important that tsetse fly control be taken more seriously if the spread of trypanosomosis has to be tamed.

1.4 Control strategies

There is a large cornucopia of theoretically viable trypanosomosis control strategies. There are those that target the elimination or control of the parasites via their lifecycle, morphology and/or genetic map. Alternatively, there are those that rely on protecting the victims from these deadly disease-causing protozoa. Since these parasites are vector-borne, there are those approaches that target the avoidance of the host-vector contact. This approach has proved quite promising and is the method of choice against biting flies (WHO, 1996). However, the control of trypanosomosis has been difficult due to the presence of well-established wild game trypanosome reservoirs. Elimination of the wild game or the vector seems an immediate solution. However, neither of the two is practically or ethically feasible. Despite the shortcomings, three main approaches have been employed in control of the disease.

1.4.1 Vaccine development

ILRI* started vaccine development program in 1978 inspired by some immunity to the disease observed in the N'dama (West African) and Boran (Eastern Africa) cattle, giving a hint that a vaccine was a possible route to trypanosome control. However, this has been tried for a long period of time (Bevan, 1928, Schilling, 1935) without success; the main obstacle being the phenomenon of antigenic variations in trypanosomes. This is basically because a dense coat of variant glycoproteins (VSGs) covers the parasite and stimulates

*Formed from merger of ILRAD (Nairobi) and ILCA (Addis Ababa).

antibody production in the host. The surface coat changes successfully during the course of an infection, thus avoiding the immune response of the host. Apparently, the prospects of developing a vaccine would be very much improved if a drug could be devised to interfere with antigen switching mechanism (Leak, 1998). Otherwise, there seems to be a remote possibility of developing an effective vaccine in the near future.

Fimmen *et al* (1982) observed that young animals are naturally protected, to some extent, from trypanosomosis by maternal antibodies in the collostrum and this influences the response of the newborns to experimental infections (Whitelaw *et al*, 1985). The protection given by these antibodies is unclear, but it is specific to trypanosome strains to which the dam had been exposed (Leak, 1998). Innate resistance has been recognized since 1906 in some African cattle (Pierre, 1906) such as *B. taurus* sub types (N'dama and Baoule) (Leak, 1998) and *B. indicus* breeds like the Boran (Njogu *et al*, 1985), Maasai Zebu (Mwangi *et al*, 1993) and in Zebu/N'dama crosses (Chandler, 1952; 1958). In a bid to exploit this trait the production of transgenic animals is seen as a possible means of rapid multiplication, using multiple ovulation and embryo transfer techniques (Jordt *et al*, 1990). However, the biggest obstacle to the possible use of this trait is the low percentage of trypanotolerant African cattle (5%). If there is nothing wrong with these cattle, why has the innate resistance not helped them in becoming widespread throughout the continent in the last 10,000 years? The possibility of exploitation of the trypanotolerant trait has had little impact in control of AAT (ILRAD, 1991). However, both innate and acquired resistance to African trypanosomosis can occur in cattle (Leak, 1998).

1.4.2 Chemotherapy

The use of curative and prophylactic drug regimes has also been employed (Ruppol *et al*, 1977). However, the drugs currently recommended for chemotherapy of AAT come from three closely related groups. These include; homidium and isometamidium, phenanthridines, the aromatic diamidine and diminazine (Leach *et al*, 1981). Drug-resistance incidences are on the increase (Peregrine, 1994; Gray *et al*, 1968) leaving the main means of controlling the disease under threat. Chemoprophylactics can be useful under high challenge situations and enable cattle to remain productive (Trail *et al*, 1985).

However, this is unfit for the fragile African economies since it involves the use of scarce foreign currencies in the purchase of the drug regimes and donors are only willing to assist in the presence of a revolving fund (Connor, 1989). Chemotherapy alone cannot achieve anything permanent since it is limited in the field by the fact that animals in contact with the flies are re-infected. Drugs for human beings are expensive and unaffordable to the resource poor rural pastoral communities. For instance, diflouromethyl-ornithine (DFMO) ("resurrection drug"), which is the most effective and safest, costs Kshs. 19,000 per dose. Unfortunately, the drug is needed in large doses for effective treatment.

The WHO has recommended a control strategy which involves identification and treatment of all cases of trypanosomosis and keeping the vector density low using screens and traps (WHO, 1996). It is therefore sensible to conclude that the effective control of trypanosomosis should be based on reducing human and bovine protozoa reservoirs, prevention of epidemics and vector control.

1.4.3 Vector control

Vectors can be controlled with insecticides (Allsopp, 1984), traps baited with attractants (Vale *et al*, 1985a; Laveissiere *et al*, 1990) or the use of repellents (WHO, 1996). However, insecticides are sometimes quite efficient but of all the insecticides tried, synthetic pyrethroids (allethrin and bioresmethrin) were found to be the most effective (Leak, 1998., Elliots, 1983). Tsetse flies are highly susceptible to pyrethroids that disrupt cationic conductance, leading to excitation of the nervous system thus inducing knockdown and paralysis (Elliots, 1983). Sub-lethal doses of these insecticides can reduce lipid accumulation from a blood meal, rendering flies more susceptible to starvation (Leak, 1998). However, they have their disadvantages, including undesirable effects on non-target organisms (NTOs). Organochloride insecticides (DDT and analogues) are also known to be effective against the tsetse fly. However, these are environmentally unfriendly besides the undesirable effects on NTOs as demonstrated by spraying dieldrin in an isolated peninsula in Homabay district (Kenya) resulting in the death of bushbucks due to toxication (Fieldler *et al*, 1954). Other cases have been reported where predators of tsetse flies are killed in the process of trying to eliminate it (Leak, 1998). Control methods that kill predators are

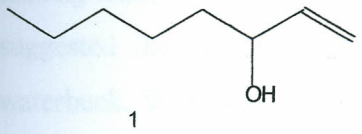
hazardous since absence of predators may result in higher populations. One major set back in the tsetse eradication campaign is that, unlike other insects that are ϒ-strategists, tsetse flies are κ-strategists. They show high-level parental "care" ensuring a high probability of survival. This is basically because eggs are produced, fertilized hatched and nurtured up to 3rd stage larvae inside the female.

1.5. Host recognition and semiochemisty in tsetse fly control

The process by which tsetse fly obtains a blood meal involves a series of behavioural rhythms/patterns. Tsetse flies recognise potential hosts by visual (physical) and olfactory (chemical) characteristics. The olfactory and mechanical stimulation will activate them and initiate host oriented responses. The fly first detects host odour and follows it upwind until the host is in sight. Heat stimulation after landing may then cause a probing response and subsequent feeding. Approach to a stationary host appears to be by upwind flight modulated by olfactory stimuli (Leak, 1998; Warnes, 1990a; Chapman, 1961). It has been shown that both endogenous factors (level of starvation, age, sex, pregnancy status and a circadian rhythm of activity) (Colvin *et al*, 1992; Brady 1972abc; 1975) and exogenous factors (temperature, vapour pressure deficit, visual and olfactory stimuli) (Bursell, 1957; Vale, 1974b; Huyton *et al*, 1975) play a role in host seeking behaviour.

1.5.1 Attractants

Over 88 years since the use of attractants was recognized as a potential tool in tsetse control (Balfour, 1913). A vital development in the 70's and 80's in tsetse fly research was the identification and synthesis of odour attractants that greatly increased the efficacy of traps and targets for some species of tsetse fly, making them a feasible alternative to insecticides (Leak, 1998; Hall *et al*, 1986). Examples of such attractants include octenol (**1**) isolated from ox breath (Vale *et al;* 1985b), four phenols and derivatives (**2-6**), isolated from oxen body sebum and urine (Warnes, 1995; Hassanali *et al;* 1986; Owaga; 1985; Bursell *et al;* 1988) among others. Potent odour baits, scattered throughout an area (without any target, trap or insecticide - chaive traps) might control the tsetse fly because the fly would respond to the baits by flying around it thus using their energy reserve without finding a host and die of starvation (Leak, 1998).



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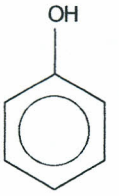

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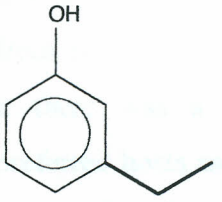

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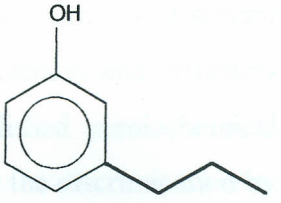

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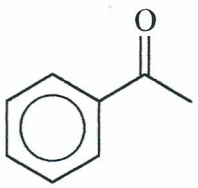
1.5.2 Repellents/allomones

Another possible tsetse control strategy would be the use of repellents, which is one of the most efficient ways of protection from biting flies (WHO, 1996). Some repellents from natural tsetse hosts have been identified accidentally while searching for attractants. Examples of tsetse repellents include acetophenone (**7**) (Vale, 1980), 2-methoxyphenol (**8**) (Hall *et al*, 1990) and lactic acid (**9**) (from human beings) (Hargrove, 1976; 1991). These, therefore, partially protect animals next to human beings.



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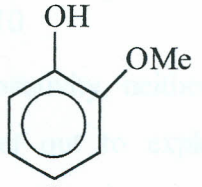

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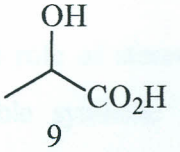

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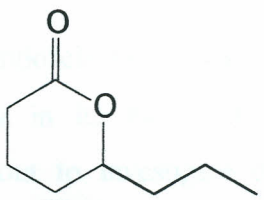
Studies based on the blood meal analysis on the tsetse fly have shown varying degrees of specialization on different vertebrate hosts (Weitz, 1963; Moloo, 1993; Clausen *et al*, 1998) irrespective of their populations in the considered ecosystem (Vale, 1974a; Turner, 1987; Snow *et al*, 1988). Gikonyo *et al* (2000) studied the effects of the waterbuck (non-preferred host) body surface chemicals on the feeding behaviour of the tsetse fly. Experiments on the waterbuck body and feeding membranes treated with different doses of the body sebum

showed significant reluctance to feed. There were significant delays in the initiation of feeding relative to a preferred host (ox) and untreated zones of the feeding membranes. This suggested the presence of deterrent signals or aversive constituents (allomones) in the waterbuck. With this cue at hand, the Hassanali group sought a semiochemical explanation of this behaviourally ascertained fact. They hypothesized (and latter proved) a semiochemical explanation to this and therefore sampled out two preferred (ox and buffalo) and a non-preferred host (waterbuck). This work culminated in the identification of several electrophysiologically active compounds from the study of surface (sebum and volatiles) chemistry. It also clearly indicated that there was a well-established semiochemical difference between the preferred and non-preferred hosts as shown by the discrimination by the fly. However, it was interesting to observe that the waterbuck (*Kobus defassa*) also contained the kairomonal aspects found in the preferred hosts (Gikonyo, 1999). δ-Octalactone (**10**) was one of the identified compounds eliciting electroatenographic activity. The compound was authenticated by both spectral and GC (co-injection) analysis of the volatiles and an authentic synthetic sample. Blends of the compounds present in the waterbuck but absent in the ox and buffalo, containing δ-octalactone, showed repellent activity in a choice wind tunnel (Gikonyo, 1999).



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The compound was not tested for bioactivity, neither was the role of stereochemistry in activity investigated. We therefore set out to explore suitable synthetic routes to δ-octalactone and the enantiomers and to investigate the role of stereochemistry in its bio-activity.

1.6 Justification

Unlike other human disease vectors such as the mosquito, little is known about tsetse fly repellents. The losses incurred due to tsetse infestations are too much to be ignored. The

pain caused by sleeping sickness and the millions of productive man-hours lost by victims make the control of the disease a vital and a long awaited endeavour. The low political visibility and priority that this disease "enjoys" is yet another reason why these studies are necessary. Since wild host reservoir elimination is impossible, vaccine development a mirage and chemotherapy expensive, ineffective and sometimes toxic, vector control is the only other affordable, safe and environmentally-friendly approach to trypanosomosis management. Personal protection against vector bites is a reasonably good strategy towards the control of trypanosomosis. The high number of livestock at risk and the potential loss if human and livestock trypanosomosis epidemic occurred is too much for the fragile sub-Saharan economies. This project therefore sought to generate basic knowledge and improve tools needed for effective vector control and reduction of trypanosomosis incidences through understanding the role of δ-octalactone in tsetse host seeking behaviour.

1.6 Objectives

The general objective was to investigate presumed bioactivity of δ-octalactone and the role of stereochemistry by synthesis of pure stereoisomers and racemate through viable abbreviated route(s).

The specific objectives were to enantioselectively synthesize (R)- and (S)-δ-octalactone and to establish the role of chirality in its bioactivity through electrophysiological and behavioural assays. We also set out to investigate the most viable and efficient route towards the synthesis of the behaviourally important isomer(s) or racemate.

CHAPTER 2: TECHNIQUES IN SEMIOCHEMISTRY

2.1 Introduction

Semiochemistry (chemical communication) covers a large area of communication including between species, host-predator, habitat-predator and different sexes within species among other relations (Figure 1). It is interesting to note that semiochemics are not only found in the lower forms of the animal kingdom but also the highly evolved ones like the primates. Belcher *et al* (1986) noted that the tamarin (*Saguinus fuscicollis*) has scent marking with specialized skin glands. Schilling (1979) observed that wide spread use of chemical signals appear to be in the communication of information on the identity of species, subspecies, gender and individual, and the reproductive, social and emotional state of the individual.

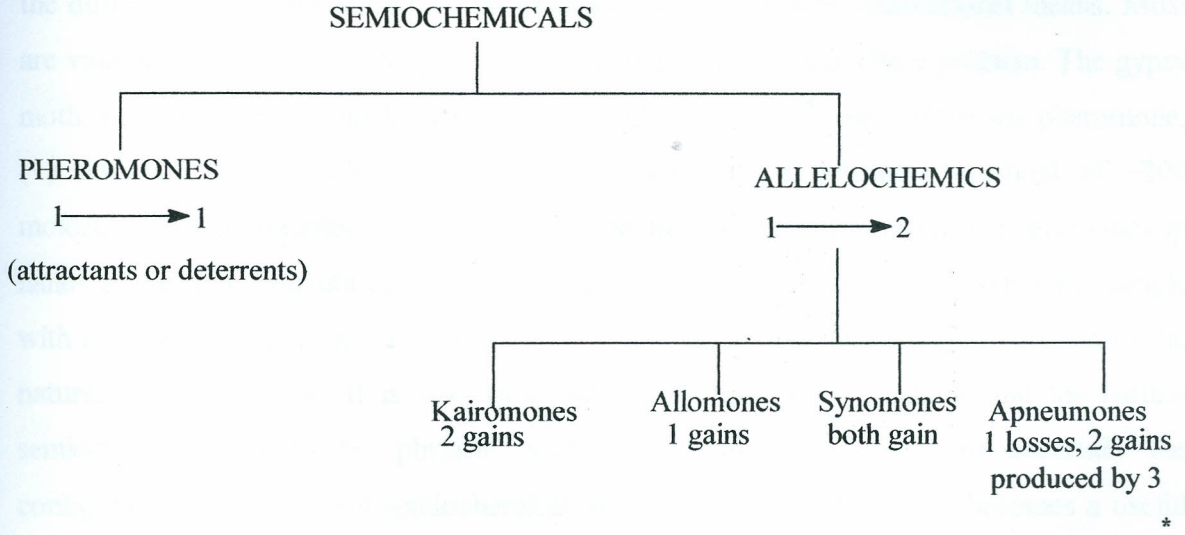


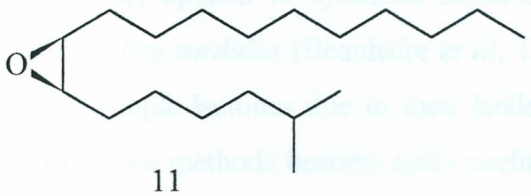
Figure 1: Semiochemistry.

Among the semiochemics, pheromones seem to be the most studied in terms of structure-activity relationships. As early as 1960's chiral pheromones had been identified (Mori, 1997). However, from a general point of view, the absolute configuration of a semiochemical has to be studied to elucidate the stereo-structure of the naturally occurring material and also clarify the relationship between chirality and activity. Mori (1973; 1974) pioneered this when he successfully identified the absolute configuration of an insect pheromone by enantioselective synthesis. The discovery that the absolute configuration of a semiochemical is important to the expression of their bio-activity, accelerated

* 1 and 2 are different species, while 3 is a material (non living) on which 1 is found.

stereochemical studies on pheromones and later other semiochemicals. This is further supported by the biological observation that olfactory information about different semiochemical enantiomers is received by separate receptor neurons in the antenna. The ability of these neurons to discriminate between enantiomers has been studied for various insects (Wibe *et al*, 1998; Ritter, 1979; Satelle *et al*, 1980; Mustaparta *et al*, 1980; Wadhams *et al*, 1982; Hansen *et al*, 1983). Synthesis of the enantiomers could solve the stereochemical problems in semiochemistry if proper methods are available for the differentiation of the enantiomers.

Unfortunately, the naturally occurring semiochemicals are released in trace amounts thus the difficulty in determination of their absolute configuration by conventional means. Most are volatile liquids and therefore acquisition in large quantities is also a problem. The gypsy moth, *Lymetria dispar,* female produces ~1 ng/gland (4×10^{-12} mol) of the sex pheromone, (+) dispalure (**11**), which is sensed by the male with a detection threshold of ~200 molecules (30 zeptomoles). Moths*, like most insects, generally produce pheromones in nano- to picogram quantities (Van den berg *et al*, 1991). This makes a synthetic sample with a known configuration a handy tool or reference in assigning the configuration of the naturally occurring one. It is also vital that one compares the synthetic and the natural semiochemical sample by physical analysis and biological assay to ascertain the configuration of the natural semiochemical. In this case specific rotation becomes a useful and widely accepted technique especially when done in comparison to a reference sample. This may give an indication on the purity of the natural semiochemical.



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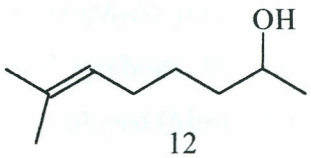
The observed optical rotation values vary widely; ranging from $[\alpha] = 0$ (for the cockroach, *Nauphoeta cinerea*, nymph recognition pheromone) to $[\alpha]_D = -547$ (for periplanone the American cockroach, *Periplanta americana*, pheromone) (Mori *et al,* 1994; Kuwahara *et*

* moths are widely studied and have been used as a model in the study of insect olfaction.

al, 1990). Where [α]=0 the specific rotation of the natural product does not provide useful clue to its absolute configuration. In this case enantioselective synthesis coupled with chiral GC analysis becomes quite useful. NMR spectroscopy is useful in determining the absolute configuration and estimation of the enantiomeric composition if sufficient amounts of the sample are available to measure the spectrum of chiral derivatives or in presence of chiral shift reagents (Mori, 1997). The former technique was successfully applied to study sulcatol (**12**) the aggregation pheromone of the ambrosia beetle, *Gnathotricus sulcatus* (Mori, 1997).



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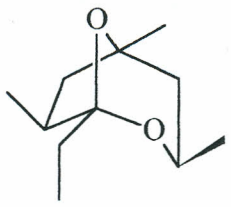
Alternatively, cellulose-based chiral stationary phase HPLC and cyclodextrin-based chiral stationary phase GC are popular methods for resolution of enantiomers and determination of absolute configuration (Miller *et al*, 1996; Konig, 1992). Even with achiral stationary phase chromatography, derivitazation with chiral reagents is still useful.

It has been observed that enantioselective synthesis can be executed by one, or a combination, of the following methods (Mori, 1997); derivatization from a known chiral and non-racemic building block(s), chemical and enzymatic assymetric synthesis, chemical and enzymatic enantiomer separation during synthesis.

The first approach was successfully applied to synthesis sordidin (**13**), a semiochemical from the banana weevil, *Cosmopolites sordidus* (Beauhaire *et al*, 1995; Mori *et al*, 1997b). However, it is inappropriate for simple lactones due to their labile nature and difficulty in protection. In this case, the other two methods become quite useful. Enzymatic synthesis is quite difficult to use in cases where antipodes are desired owing to the fact that enzymes have high enantiospecificity. Both chemical and enzymatic assymetric synthesis have been successfully applied by Mori's group in the synthesis of *endo*- and *exo*-isobrevicomin (**14b**) (Mori *et al*, 1997a) a volatile from the male mountain pine beetle, *Dendroctonus ponderosae* (Francke *et al*, 1996).



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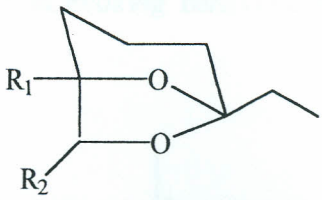
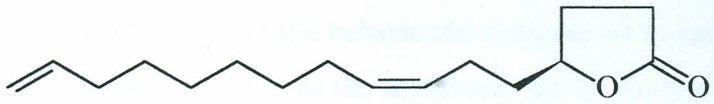


14a Multistriatin:R_1 =H,R_2 =Me
14b isobrevicomin: R_1=Me, R_2=H

Optical resolution has been successfully applied in the synthesis of both enantiomers of Z-7, 15-hexadecadien-4-olide (**15**) (Nakayama *et al*, 1997), a female-produced sex pheromone of the yellowish elongate chafer, *Heptophylla picea*. The configuration was assigned as R (Leal *et al*, 1996). In semiochemical synthesis, bio-transformations catalysed by enzymes and micro-organisms are frequently employed (Mori, 1995).



15

The recognition of the importance of chirality in semiochemistry is the most notable advancement in this field in the last two decades. Structure-activity relationships in semiochemistry can be grouped into ten broad classes based on the pheromone model (Mori, 1997).

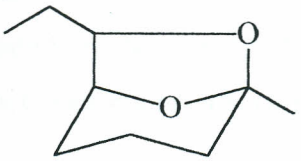
2.2 Stereo-structure-activity studies in semiochemistry

There are several different scenarios when only a particular isomer(s) is active or a combination of these at a given ratio offer maximum response. Alternatively, isomers may act antagonistically thus recording no stereo-structure-activity relationships in semiochemistry.

2.2.1 The antipode inactive and has no effect on activity

This holds the largest number of bio-regulators and semiochemicals. The class is exemplified by *exo*-brevicomin (**16**) where only the (1R, 5S, 7R)- isomer is bioactive (Wood *et al*, 1976; Kobayashi *et al*, 1980). (3S, 4R)-Faranal (**17**) is the only active isomer for the pharaoh's ant (Kobayashi *et al*, 1980). It has also been observed that *Scolytus multistriatus* (elm bark beetle) responds much more strongly to α- than to δ-multistriatin

(**14a**) but brevicomin replaces α-multistriatin in evoking behavioural responses (Angst *et al*, 1982).



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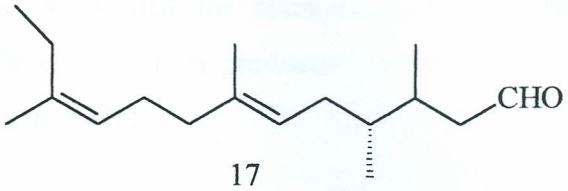

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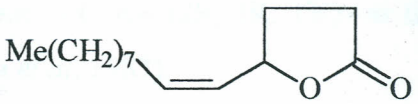
2.2.2 The antipode is inactive and inhibits activity

This class demands synthesis of absolute pure enantiomers in order to ensure useful exploitation of the semiochemicals. Examples include disparlure (**11**) where (7R, 8S)-isomer is the active isomer and the (7S, 8R)- inhibits responses by *Lymantria dispar*. However, the former has no effect on the behavioural response of *L. monacha* (Vite *et al*, 1976). Japonilure (**18**) has R-(+)-isomer as the active one; the S- strongly inhibits the action of the R- (Tumlison *et al*, 1977).



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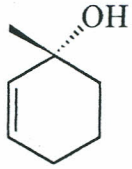
2.2.3 Both isomers required for bioactivity

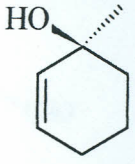
This class of semiochemicals calls for the test of the enantiomers over a wide range of ratios and observing the trend to establish the ratio eliciting maximum response. It is represented by the ambrosia beetle, *Gnathotrichus sulcatus*, which naturally produces sulcatol (**12**) in a ratio of 35:65 (R: S). The two isomers are inactive independently but the racemic (1:1) mixture is even more active than the naturally produced semiochemical (Haniotakis *et al*, 1986). The racemic mixture is active but the single enantiomers and a blend containing 1:3 of R:S was inactive (Kim *et al*, 1998). Although the ambrosia beetle responds to a broad range of mixtures of enantiomers of this molecule including the racemate, *G. retesus* produces only the (S)- isomer and does not respond to the racemate.

2.2.4 Enantiomeric mixture produced and both enantiomers active independently

In this case the combined effect of the enantiomers is usually additive rather than synergistic and both enantiomers may be needed for maximum response. This was demonstrated in the female Douglas fir beetle which produces a 55:45 mixture of it's aggregation pheromone (**19**) (Lindgren *et al*, 1992).



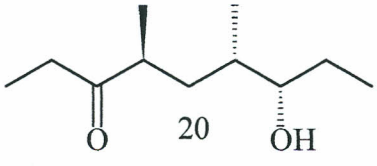
19R



19S

2.2.5 Diastereomer inactive but inhibits bioactivity

Like the earlier cases, this calls for synthesis of absolute pure compounds avoiding contamination with the inhibitory diastereomers. Examples in this class are; serricornin (**20**) which has (4S, 6S, 7S)- as the active one while (4S, 6S, 7R)-isomer has an inhibitory effect (Mori *et al*, 1986). Stegobinone (**21**) has (2S, 3R, 1'R)- as the active and (2S, 3R, 1'S)-isomer as an inhibitor (Kodama *et al*, 1987).



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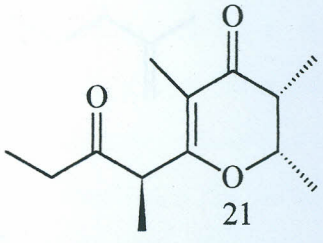

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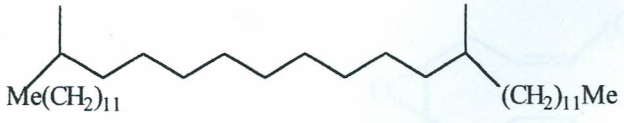
2.2.6 Only the *meso* compound is active

This has been reported for the tsetse fly sex pheromone where *meso*-alkanes seemed to be bioactive. (13R, 23S)-isomer (**22**) was shown to be the female-produced sex pheromone of *Glossina pallidipes*. Neither (13S, 23R)- or (13S, 23S)-isomer was active (McDowell *et al*, 1985).



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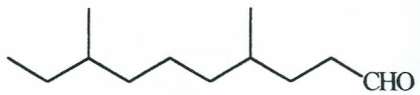
2.2.7 Diastereomeric mixture more active than the most active isomer

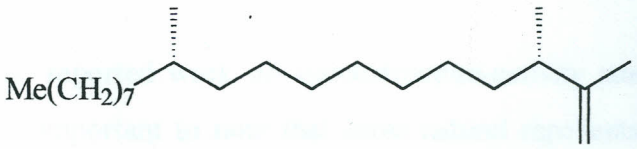
For application purposes, this class of semiochemicals does not need stereospecific synthesis since a mixture is more viable to evoke highest level of response from the insect. For the red flour beetle, *Tribolium castaneum*, it has been observed that a mixture of (4R, 8R)- and (4R, 8S)– isomers of tribolure (**23**) at a ratio of 8:2, respectively, was about ten times more active than the natural (4R, 8R)- isomer alone (Suzuki *et al*, 1984).



23

2.2.8 Pure natural isomer, antipode and diastereomers all active

This is usually observed as a result of failure by the organism to discriminate between the different isomers, giving a positive response to all. This is exhibited in the male German cockroach, *Blattella germanica*. The insect does not discriminate between the four stereoisomers of the female-produced sex pheromone although the natural product is (3S, 11S)-(**24**) (Nishida *et al*, 1983).



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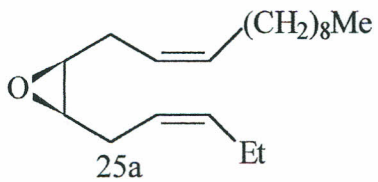
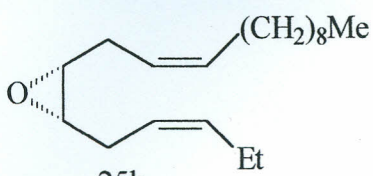
2.2.9 Species-specific enantiomers or diastereomers

This calls for a varied and well-organized bio-assay of the synthetic isomers. There has to be an extensive test of the stereoisomers against all species of the particular genus or family. It may also be used in species differentiation, as is the case with the winter-flying geometrid moths, *Colotois pennaria* and *Erannis defoliaria* responding to the (6R, 7S)-isomer (**25a**) and (6S, 7R)-isomer (**25b**), respectively (Szocs *et al*, 1993).



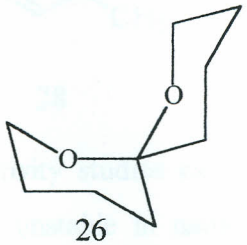
25a



25b

2.2.10 Enantiomer or diastereomer shows sex specificity

This group is common in pheromone work. The insect may produce a racemic mixture but each sex responds to one of the isomers. The olive fruit fly produces a racemic mixture of olean (**26**), but the (R)- isomer is active on the males and the (S)- on the females.



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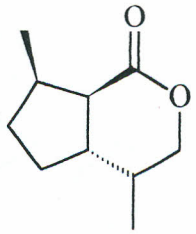
Chemists and biologists (semiochemists) have unveiled the remarkable diversity in the stereochemical aspects of semiochemics perception. It is thus evident that insects use chirality to enrich their communication systems. For application purposes, it is important to note that the stereoisomers of a semiochemical may act as an inhibitor of the bio-activity of the other. It is only after a detailed stereostructure-activity study that a user gets a clear indication as to what parameters should be ignored in the use of a particular bioactive compound (Mori, 1997).

Although there is little reported work on stereostructure-activity studies in synthetic and natural repellents, it is important to note that some natural repellents have chiral centres, which may influence their activity either positively or negatively. Examples include the newly identified mosquito repellent, nepetalactone (**27**), which has the *trans-cis* isomer more active than the best insect repellent in the market, DEET (**28**). The antipode (*cis-cis*) is ten times less active (Anonymous, 2001). However, non significance of stereochemistry has been reported in repellents like *p*-menthane-3, 8-diols (**29**) against the *Anopheles gambiae* mosquito (Barasa, 2000).



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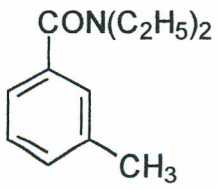

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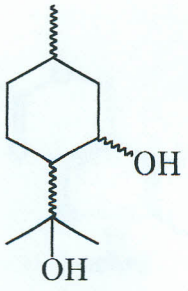
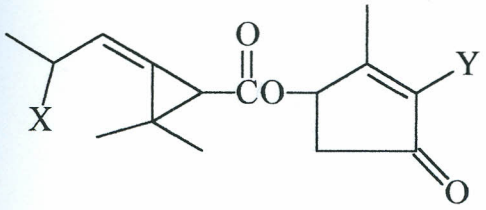


29

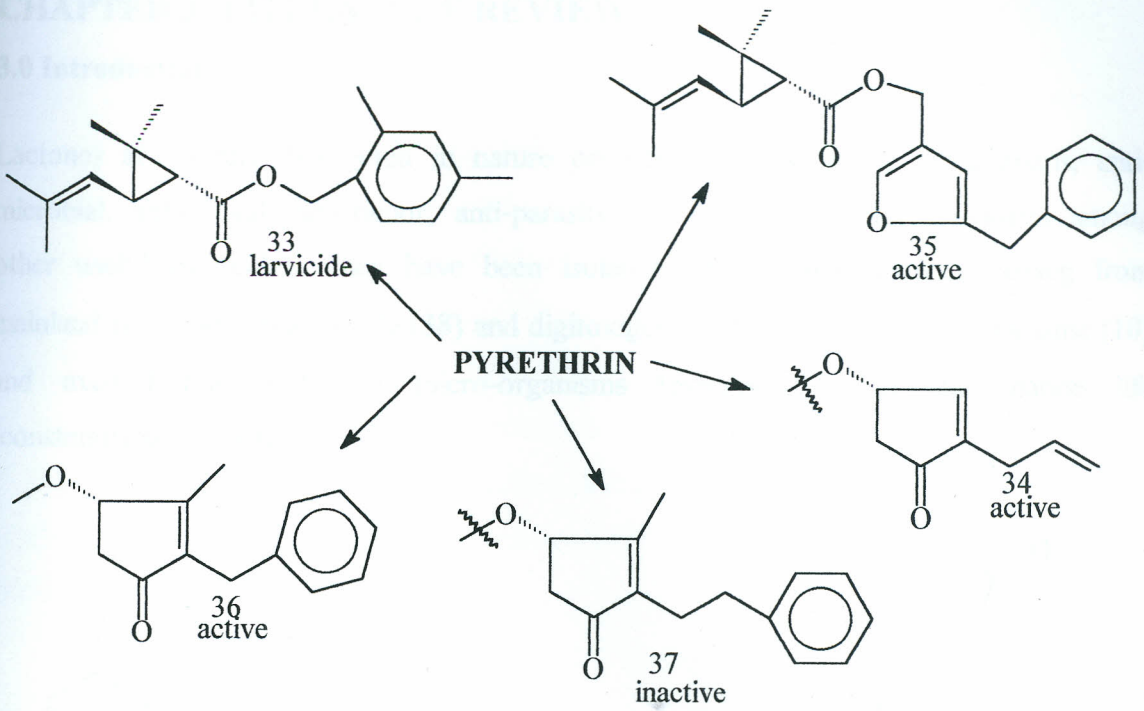
The benefits of stereo-structure-activity studies can be found in the pyrethrins. The six natural pyrethrins (**30-32**) are too unstable in nature but their structure has served as excellent prototypes for the development of a range of synthetic compounds with modified properties and activity.



X	Y	
CH_3	C_3H_7	**30a**
CH_3OCO	C_5H_7	**30b**
CH_3	C_4H_7	**31a**
CH_3OCO	C_4H_7	**31b**
CH_3	C_5H_9	**32a**
COOMe	C_5H_9	**32b**

Structural variation (Scheme 1) of the pyrethrins led to identification of a powerful range of repellents/insecticides that are stable in sunlight, rapidly degraded by mammals and soil micro-fauna. The structural analogues have found widespread use ranging from repellents, larvicides to insecticides and acting across a large number of insect species.

It has also been shown that the optical form at the chiral centres in the pyrethrins is important for activity (Elliots, 1983). From the above discussion, the stereostructure-activity relationship in semiochemicals plays such a crucial role that it should not be ignored for their successful application in pest and vector control. It is with this in mind that we decided to undertake the stereo-structure-activity studies of δ-octalactone, a potential tsetse fly allomone previously isolated from the waterbuck (*Kobus deffassa*) (Gikonyo, 1999).

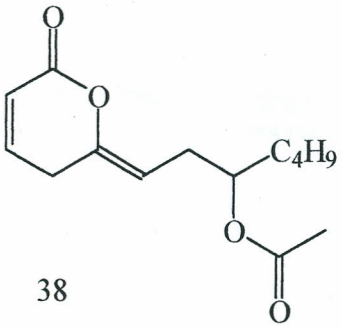

33
larvicide
35
active
PYRETHRIN
34
active
36
active
37
inactive

Scheme 1.

CHAPTER 3: LITERATURE REVIEW

3.0 Introduction

Lactones are widely distributed in nature occurring as phytotoxins, mycotoxins, anti-microbial, anti-fungal, anti-tumor, anti-parasitic, semiochemicals and antibiotics among other useful molecules. They have been isolated from diverse sources varying from mainland flora [umuravumbolide (**38**) and digitoxigenin (**39**)] and fauna [δ-octalactone (**10**) and massoilactone (**40**)] to micro-organisms [pyrenocine (**41**)] and marine life [constanolactone A (**42**)].



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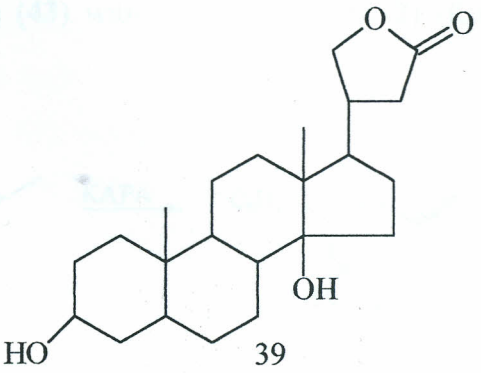

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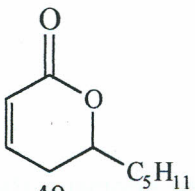

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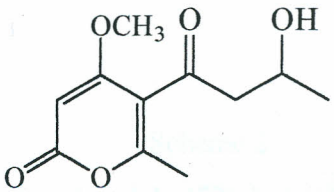

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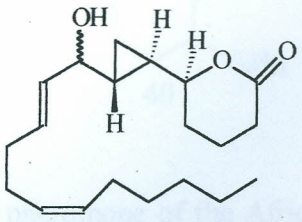
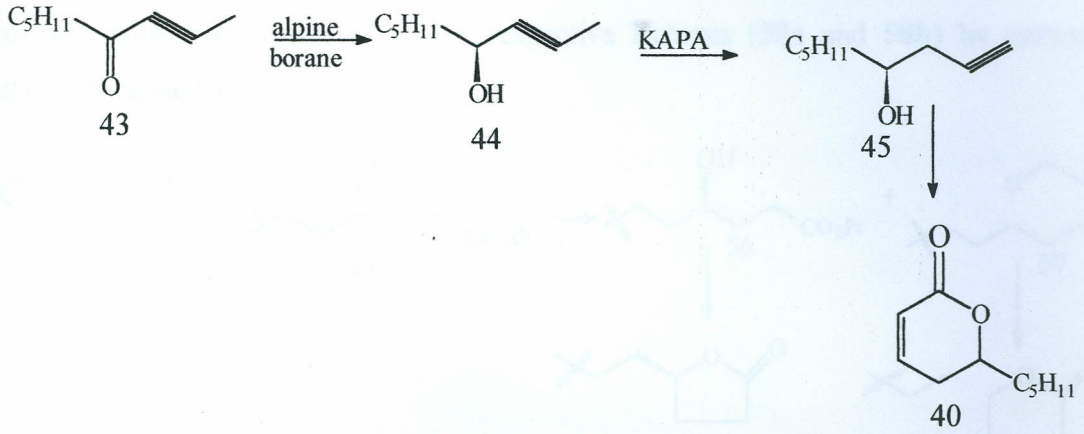


42

In synthesis, they are used as vital intermediates in the construction of macromolecules. However, their use is limited by the presence of the electron rich lactone moiety making them labile to acid or strong bases. The activated *exo*-cyclic C_6 limits their use in various addition reactions due to the possibility of the reaction taking place at this position in preference to any other carbon in the molecule.

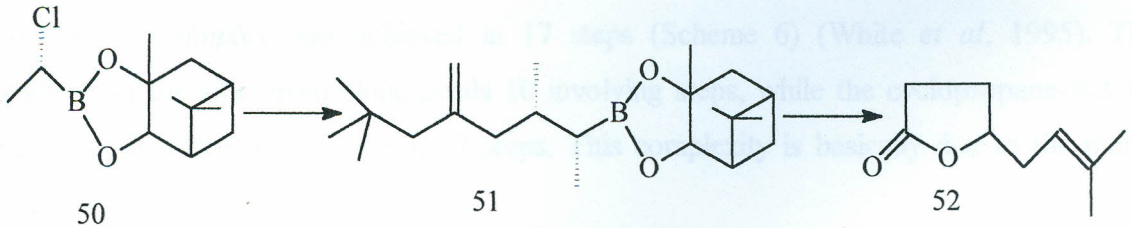
3.1 Lactone chemistry

Lactone synthesis may be simple or complex depending on synthon accessibility. Basically, they have been synthesised via Baeyer-Villiger oxidation of ketones, iodolactonization of oxygenated alkenes, degradation of macrocycles, biomimetric synthesis via cyclisation of hydroxy acids, aldol condensation, Claisen rearrangements, tandem cyclization (Diels-Alder reaction), Killiani-Fischer process (Morrison, *et al,* 1992; Solomons, 1997; Vogel, 1994; Carey *et al*, 1990; Carruthers, 1986) among many other abbreviated synthetic routes and/or methodologies, the list being inexhaustive. Massoilactone (**40**), a defense allomone of the formicine ant, has been synthesized using boranes as reducing agents of 1,2-dicarbonyl compounds and acetylinic ketones (**43**) with high ee (Scheme 2) (Koskinen, 1993).



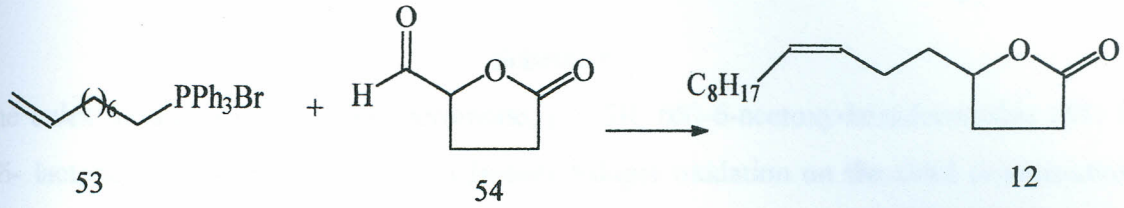
Scheme 2

Another lactone semiochemical, eldanolide (**52**) is a wing gland pheromone of the African sugarcane borer, *Eldana saccharina*. Matteson (1986) synthesized eldanolide via an organoborane intermediate (**50**) (Scheme 3).



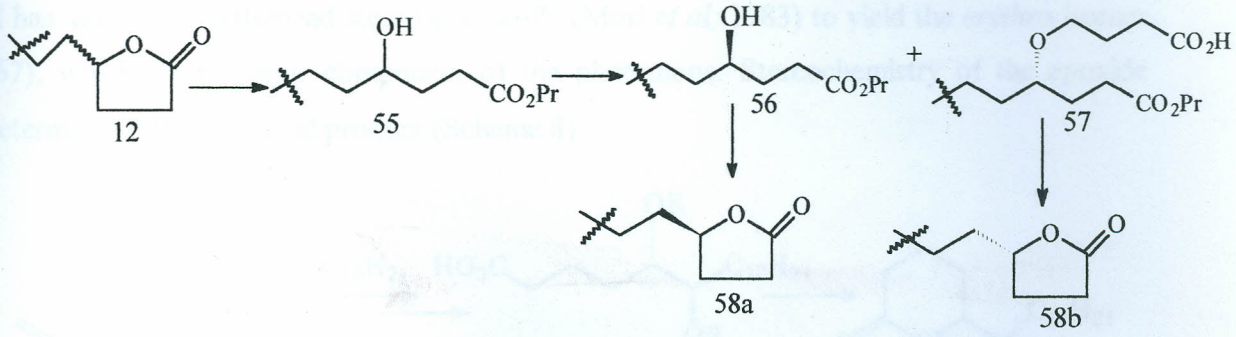
Scheme 3

A simple synthesis of racemic of hexadeca-7, 15-dien-4-olide (**12**), a female-produced sex pheromone of the yellowish elongate chaffer (*Heptophylla picea*) (Leal *et al*, 1996; Mori *et al*, 1997a) (Scheme 4), basically involved a Wittig reaction to fuse an aldofuranone to a ylide that carries the desired side chain under Bestmann conditions (Mori, 1997).



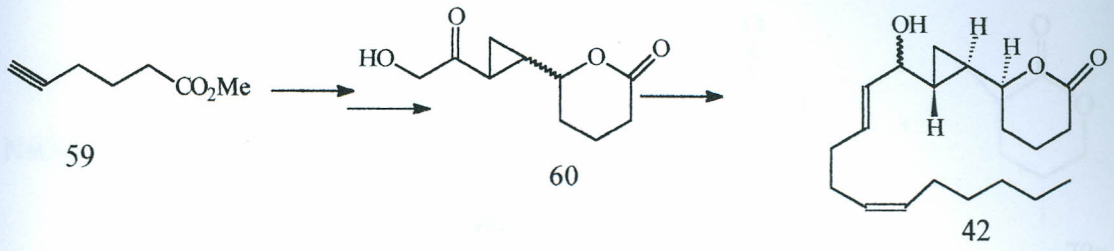
Scheme 4

However, the chiral synthesis was much involving requiring five steps to resolve the racemate. The lactone was first converted to a hydroxy ester (**55**), which was reacted with an enzyme to give R- (**56**) and S-isomer (**57**), selectively. These were separable by chromatography and converted to the respective lactones (**58a** and **58b**) by enzymatic methods (Scheme 5).



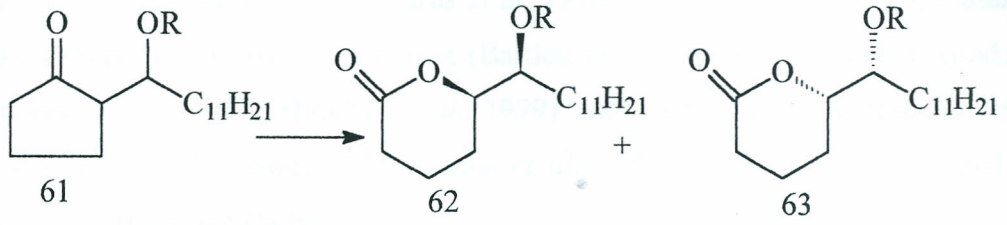
Scheme 5

The synthesis of lactones with various chiral centers coupled with presence of other functional groups is an example of a technically complex process. For instance, the cyclopropane containing eicosanoid lactone, constanolactone A (**42**), from the marine algae *Constantinea simplex* was achieved in 17 steps (Scheme 6) (White *et al*, 1995). The synthesis of the side chain alone needs 10 involving steps, while the cyclopropane-lactone fragment (**60**) involves yet another 7 steps. This complexity is basically due to the multi-functionality of the molecule.



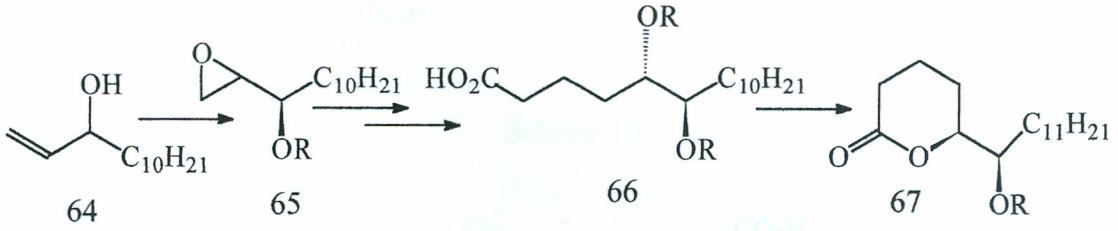
Scheme 6

The culex mosquito oviposition pheromone, (-) (5R, 6S)-6-acetoxy-hexadecanolide (**63**) is a δ- lactone. It was synthesized via a Baeyer-Villiger oxidation on the aldol condensation product (**61**) of cyclopentanone and undecanal (Scheme 7) (Otieno *et al*, 1988).



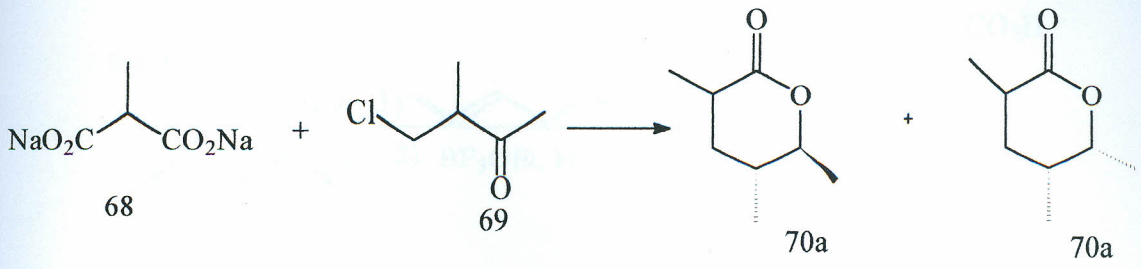
Scheme 7

It has also been synthesized stereospecifically (Mori *et al,* 1983) to yield the *erythro* isomer (**67**), which is the major component of the pheromone. Stereochemistry of the epoxide determines that of the final product (Scheme 8).



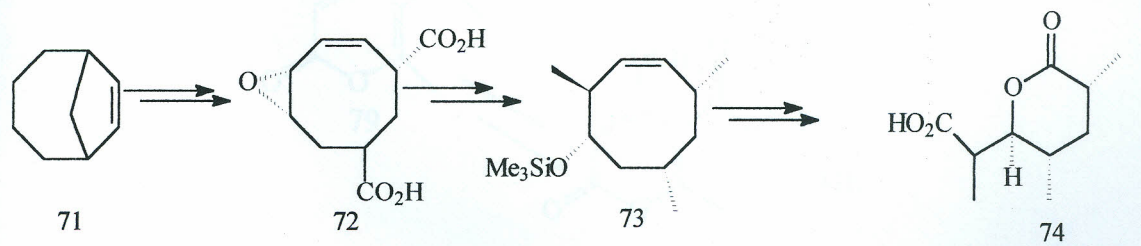
Scheme 8

The madibular gland secretion of *Calomyrmex* males, 3,5,6-trimethyltetrahydropyran-2-one (**70**), was synthesised from condensation of 1-chloro-2-methylbutan-3-one (**69**) and sodium methylmalonate (**68**) to give the racemic mixture (Scheme 9) (Pilli *et al*, 1999).

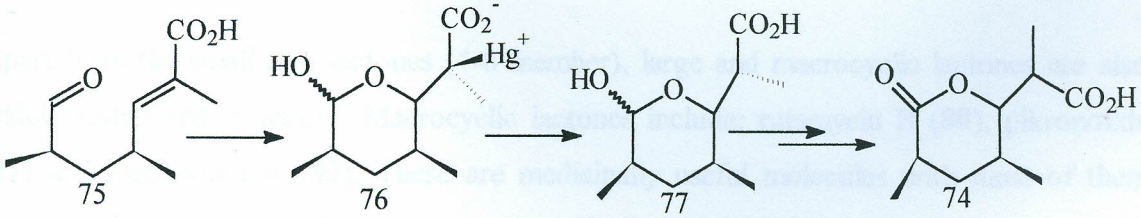


Scheme 9

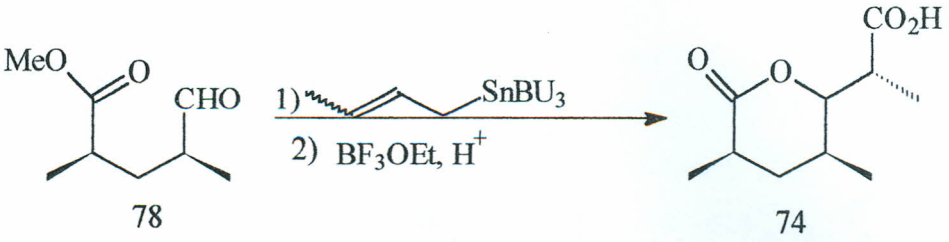
The Prelog-Djerassi lactone (**74**) occupies a vital position in the chemistry of antibiotics synthesis. This lactone has been the subject of extensive study and has been obtained by degradation of large molecules (Rickards *et al*, 1970; Manwaring *et al*, 1970; Masamune *et al*, 1975) (Scheme 10), oxy-mercuration (Bartlett *et al*, 1980) (Scheme 11), condensation (Masamune *et al*, 1981; Hirama *et al*, 1979) and chiral carbohydrate-based synthesis (Ireland *et al*, 1976). However, Maruyama *et al*, (1981) employing crotyltin and borane designed a shorter route (Scheme 12).



Scheme 10



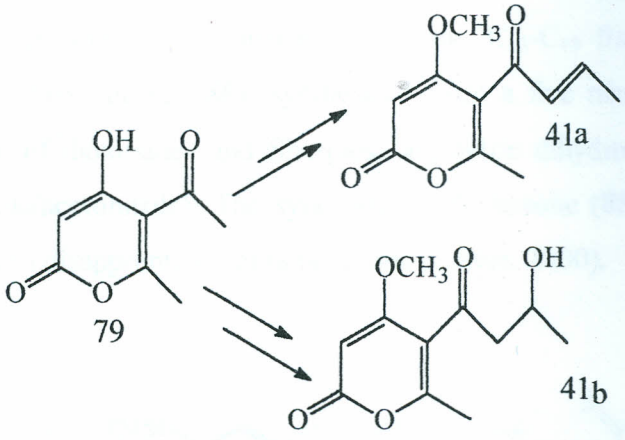
Scheme 11



78 74

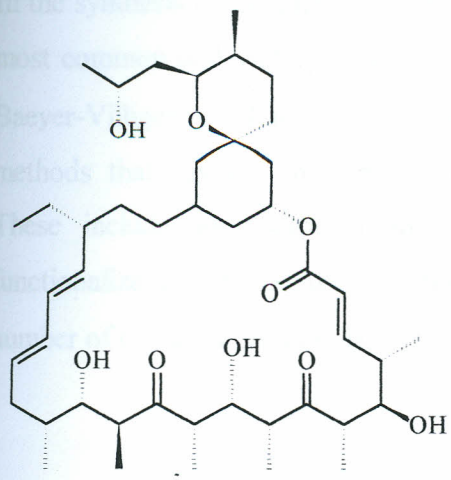
Scheme 12

Pyrenocine A and B (**41**) are phytotoxins isolated from the culture filtrates of *Pyrenochaeta terrestris* and have inhibitory effect on lettuce germination. The pair has been synthesized from 5-aceto-4-hydroxy-6-methyl-α-pyrone (**79**) (Scheme 13) (Koskinen, 1993).



Scheme 13

Apart from the small ring lactones (4-6 member), large and macrocyclic lactones are also widely distributed in nature. Macrocyclic lactones include; rutamycin B (**80**), pikronolide (**81**) and methylonolide (**82**). These are medicinally useful molecules with most of them being used as antibiotics. However, their use is limited with many remaining unexplored due to their occurrence in minute quantities in nature and the difficulties involved in their synthesis.



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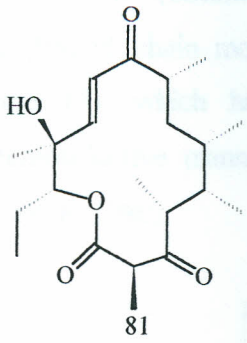

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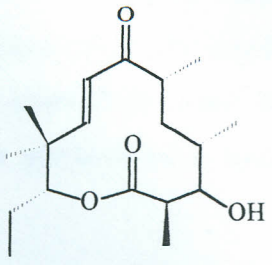
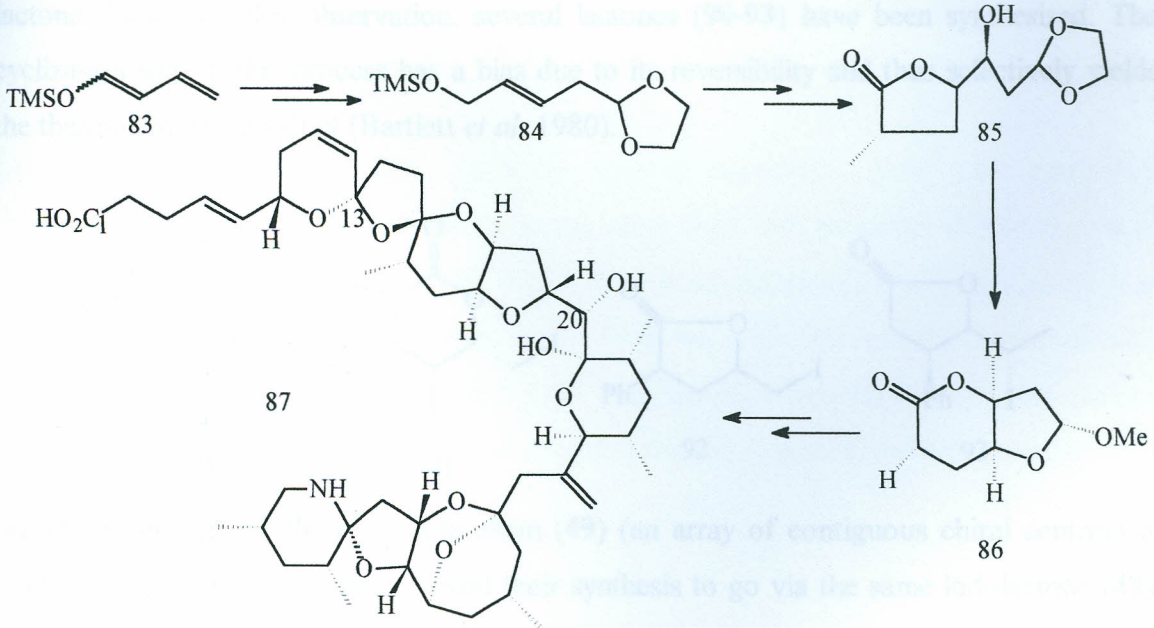


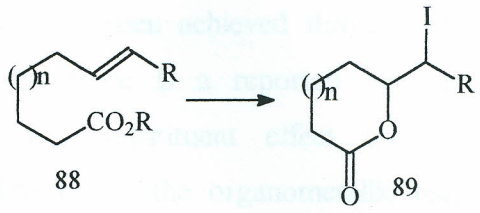
82

As vital synthetic intermediates, lactones are widely used in the synthesis of oxygenated macrocycles. For azaspiracid (**87**), construction of the C_{13}-C_{19} fragment (Scheme 14) involved a bicyclic methoxy acetal (**86**), synthesised from a five membered lactone (**85**). This involved the use of dioxolanes and Sharpless asymetric dihydroxylation followed by lactonization (imidazole/acetonitrile). The synthesis of the lactone (**85**) gave a "frustrating 36% yield" and still with disappointing contaminations (Carter, 2000).



Scheme 14

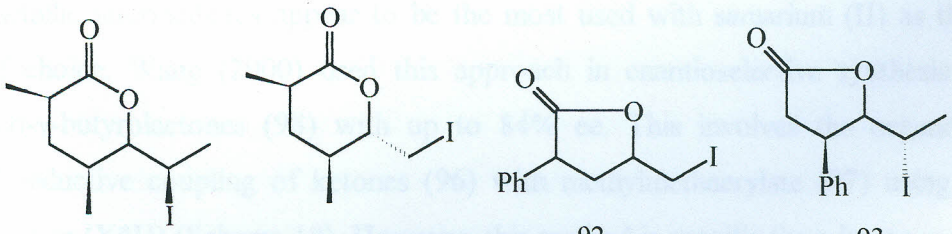
In the synthesis of lactones, the functional group may be obtained by various methods. The most common and widely used one is the insertion of oxygen into a cyclic system through Baeyer-Villiger oxidation of α-alkylated ketone (Solomons, 1997). However, other methods that involve the cyclization of straight chain molecules have been developed. These include iodolactonization (Scheme 15), which has been extensively used to functionalize double bonds in a regio/stereoselective manner in the synthesis of a large number of cyclic molecules (Bartlett *et al*, 1978; 1980).



88 89

Scheme 15

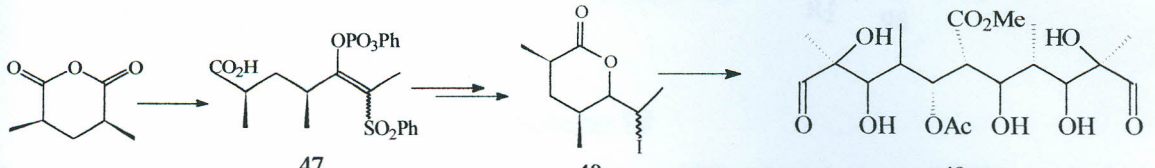
Equilibration of a cyclic intermediate and therefore thermodynamic control of the streochemistry can be achieved by reaction of an appropriate carboxylic acid with iodine in acetonitrile in the absence of a base. This leads to *cis* - *trans* equilibration via a protonated lactone. Based on this observation, several lactones (**90-93**) have been synthesised. The cyclization step in this process has a bias due to its reversibility and thus selectively yields the thermodynamic product (Bartlett *et al*, 1980).



90 91 92 93

Structural analogy of the *ansa* side chain (**49**) (an array of contiguous chiral centres) of rifamycins and streptovaricins, enabled their synthesis to go via the same iodolactone (**48**). The δ,∈-unsaturated acid (**47**) was synthesized from *meso*-2, 4-dimethyl glutaric anhydride

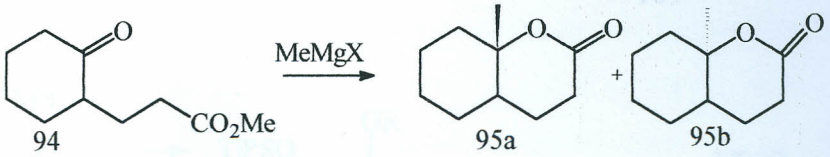
(**46**) via a β-ketosulfone (Allinger, 1959). This was iodolactonized to avail the iodolactones (**48**) (Scheme 16) (Bartlett, 1980).



46 47 48 49

Scheme 16

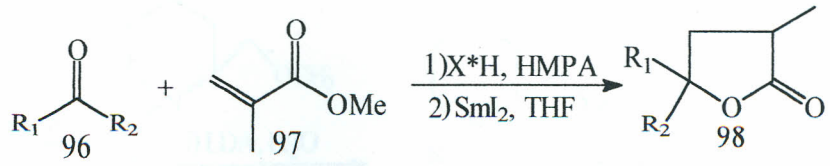
Synthesis of δ-lactones has also been achieved through alkylation of δ-keto esters via Grignard reaction. However, there is a reported mechanistic change depending on conformational dynamism and substituent effect on stereo-selection. A proposed mechanism involves coordination of the organometallic reagent and the substrate (**94**) before the alkyl transfer and lactonization (Di Maio *et al*, 1982). This is represented by the synthesis of the bicyclic δ-lactone (**95**) (Scheme 17).



94 95a 95b

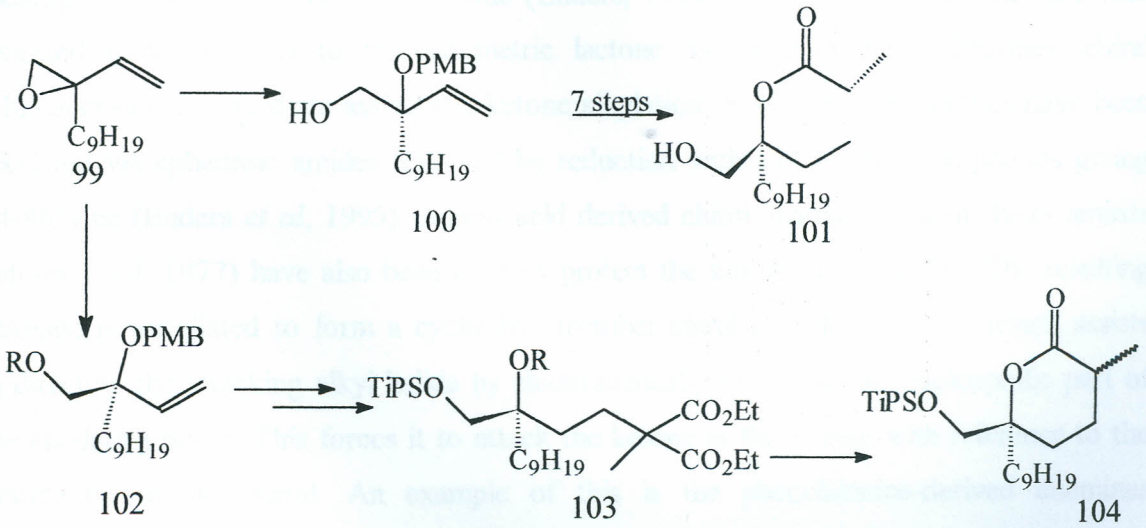
Scheme 17

For enantioselectivity in lactone synthesis, various approaches have been employed. Organometallic intermediates appear to be the most used with samarium (II) as the metal cation of choice. Wang (2000) used this approach in enantioselective synthesis of α,γ-substituted-γ-butyrolactones (**98**) with up to 84% ee. This involves the organometallic mediated reductive coupling of ketones (**96**) with methylmethacrylate (**97**) using various proton sources (X*H) (Scheme 18). However, this method is specific for γ-lactones.



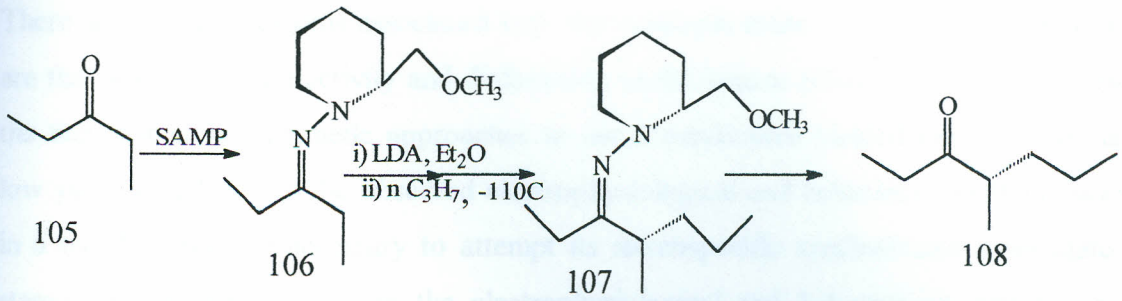
Scheme 18

In the synthesis of the antibiotic (-)-malyngolide (**104**), Trost *et al* (2000) applied the enantioselective alkylation of vinyl epoxides using palladium (0) catalysed assymetric allylic alkylation (AAA) to give chiral monoprotected vinylglycidol (**101**) with 99% ee (Scheme 19). Seven steps led to the conversion of this chiral building block to the target ester or lactone (Trost *et al*, 2000; 1999; 1998).



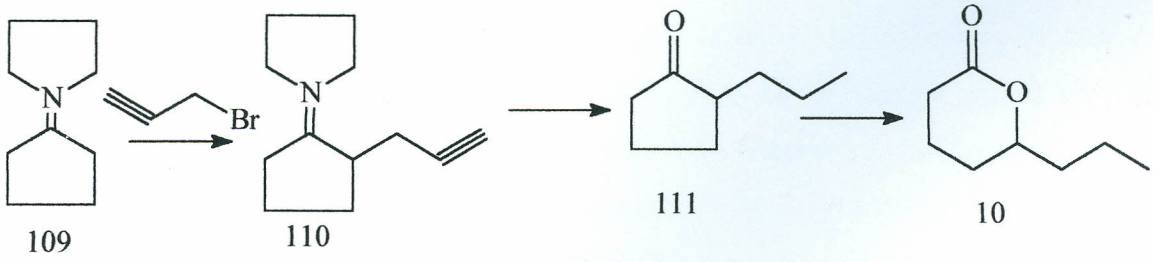
Scheme 19

α-Alkylation of ketones followed by Baeyer-Villiger reaction is also a viable route, but for enantioselectivity, it requires chiral alkylation of ketones. A more efficient way of chiral induction during alkylation of ketones is via nitrogen containing chiral auxiliaries (Meyers *et al*, 1981). The use of chiral hydrazones has been exploited as exemplified in the synthesis of (+)-(S)-4-methyl-3-heptanone (**108**) (Scheme 20), the principal alarm pheromone of the leaf cutting ant, *Atta texana* (Enders *et al*, 1979ab).



Scheme 20

These reactions have been proposed to occur through a 4-step mechanism, which involves the hooking of the ketone, metallation, electrophillic attack and finally nucleophillic cleavage to liberate the alkylated ketone (Enders, 1984). The generated ketones are then oxidized using peracids to the assymetric lactone. Other than the hydrazones, chiral lithioenamines also mediate assymetric ketone alkylation. Simple acyclic ketones have been alkylated via ephedrine amides followed by reduction with alkyllithium compounds giving 44-88% ee (Enders *et al*, 1995). Amino acid derived chiral enamines and methoxy amines (Meyer *et al*, 1977) have also been used to protect the ketone functionality. The resulting enamine is metallated to form a cyclic five-member chiral metallo-complex, which assists in directing the attacking alkyl halide by electrostatically attracting the nucleophilic part of the alkylating agent. This forces it to attack the ketone at the *si* face with reference to the orientation of the metal. An example of this is the phenylalanine-derived enamines proposed by Meyer *et al* (1977). Disregarding chiral induction, this approach is useful in synthesis of racemic lactones. This is exemplified in the synthesis of our target molecule, δ-octalactone (**10**) (Gikonyo, 1999).



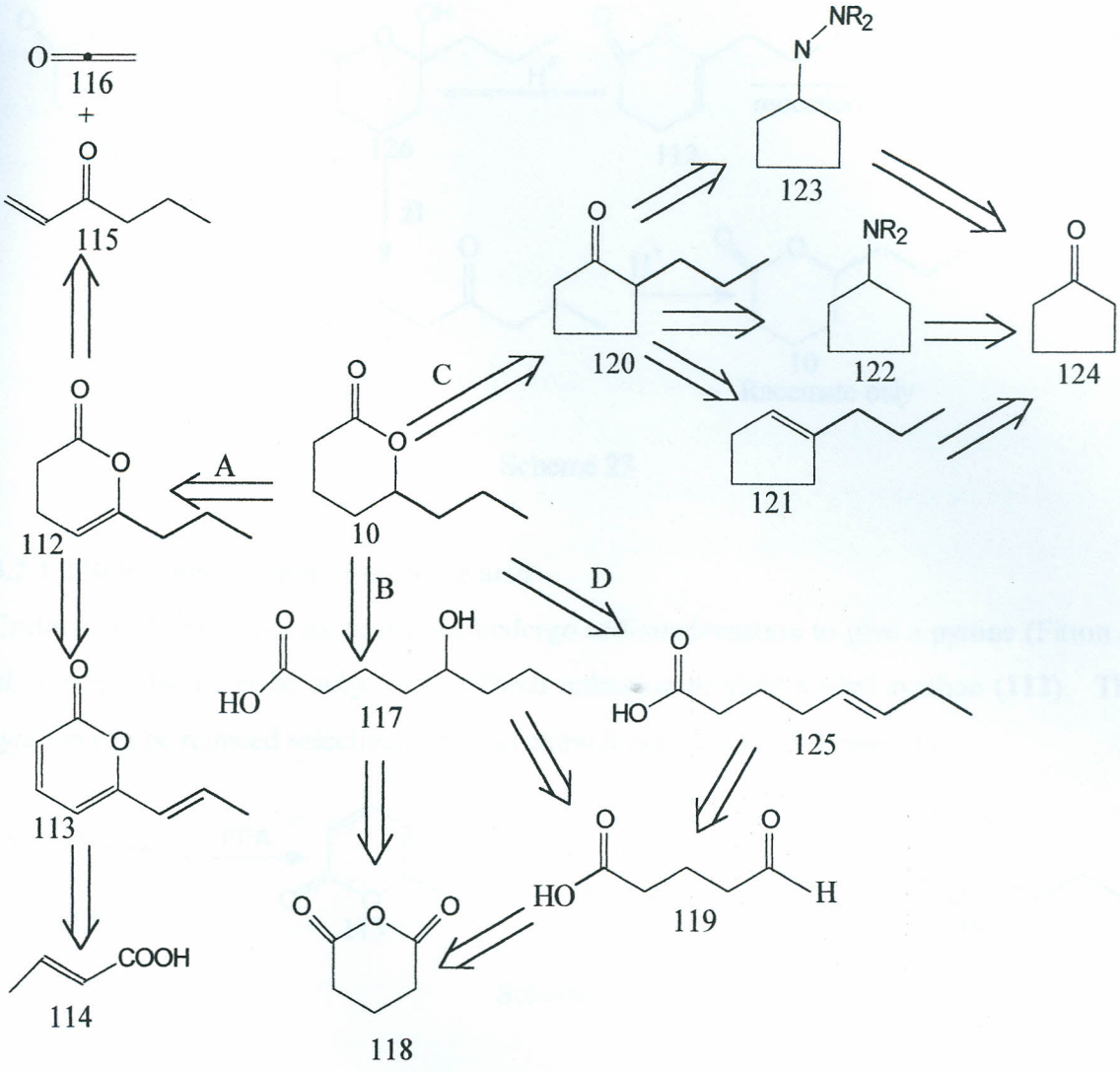
Scheme 21

There are several problems associated with this synthetic route. Two serious shortcomings are the lack of stereoselectivity and dialkylation of the ketone (Gikonyo, 1999). In view of the fact that earlier synthetic approaches to the δ-octalactone yielded racemic material in low yields coupled with the observed electrophysiological and behaviour modifying activity in a blend, it became necessary to attempt its stereospecific synthesis and investigate the stereochemical implications on the electrophysiological and behaviourial activity on the tsetse fly. Due to the several available approaches to lactone synthesis, it became necessary to examine all these approaches through retrosynthetic analysis and select ones that could be efficiently and economically used for the synthesis of the racemic (R)- and (S)- δ-octalactone.

3.2 Retrosynthetic analysis of δ-octalactone

This is a logical design of how the target molecule can be obtained from readily available and easily accessible, simple molecules (synthons). Several retrosynthetic routes were proposed for δ-octalactone (Scheme 22).

The retrosynthetic scheme proposes 4 pivotal molecules in the synthesis of δ-octalactone. From cyclic intermediates the strategic roles of 3,4-dihydropyranone (**112**) (route A) and 2-propylcyclopentanone (**120**) (route C) in the synthesis of δ-octalactone was envisaged. From acyclic systems, the intermediacy of 5-hydroxyoctanoic acid (**117**) (route B) and 5-octenoic acid (**125**) (route D) were recognised as vital in this endeavour.

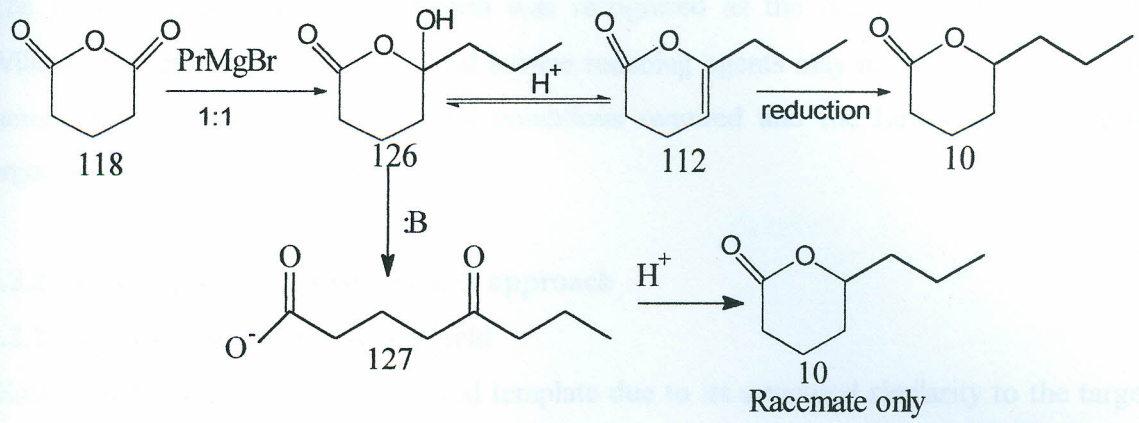


Scheme 22

3.2.1 Reduction of alkyldihydropyrone approach

3.2.1.1 Grignard alkylation of glutaric anhydride.

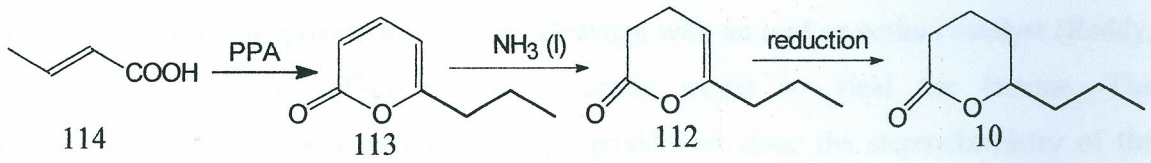
The stoichiometric 1:1 reaction of glutaric anhydride (**118**) and propylmagnesium halide is a viable route (scheme 23) to a hemiacetal (**126**), which can be readily converted to the lactone through the dihydropyrone (**112**). For nonchiral synthesis the hemiacetal can also be converted to a δ-oxo acid (**127**) by a strong base followed by acid catalysed lactonization of the enol. Reduction of the intermediate pyrone (**112**) would afford the required product (Scheme 23). However, great care needs to be taken to ensure that the reaction occurs at only one carbonyl of the glutaric anhydride (**118**).



Scheme 23

3.2.1.2 Self condensation of crotonic acid

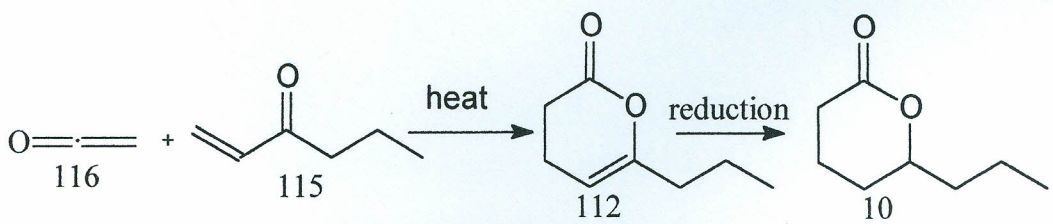
Crotonic acid (**114**) like its analogues undergo self-condensation to give a pyrone (Fitton *et al,* 1965), which can be subjected to Birch reduction to yield a vital synthon (**112**). The synthon can be reduced selectively to yield chiral δ-octalactone (Scheme 24).



Scheme 24

3.2.1.3 Diels-Alder reaction between an enone and a ketene

The dihydropyranone (**112**) may be obtained via a Diels-Alder reaction using chiral chloroboronate or vanadium catalyst (Carruthers, 1986) (Scheme 25). However, the generation of ketene (**116**) was anticipated to be difficult due to the toxicity and high reactivity of the involved reagents.



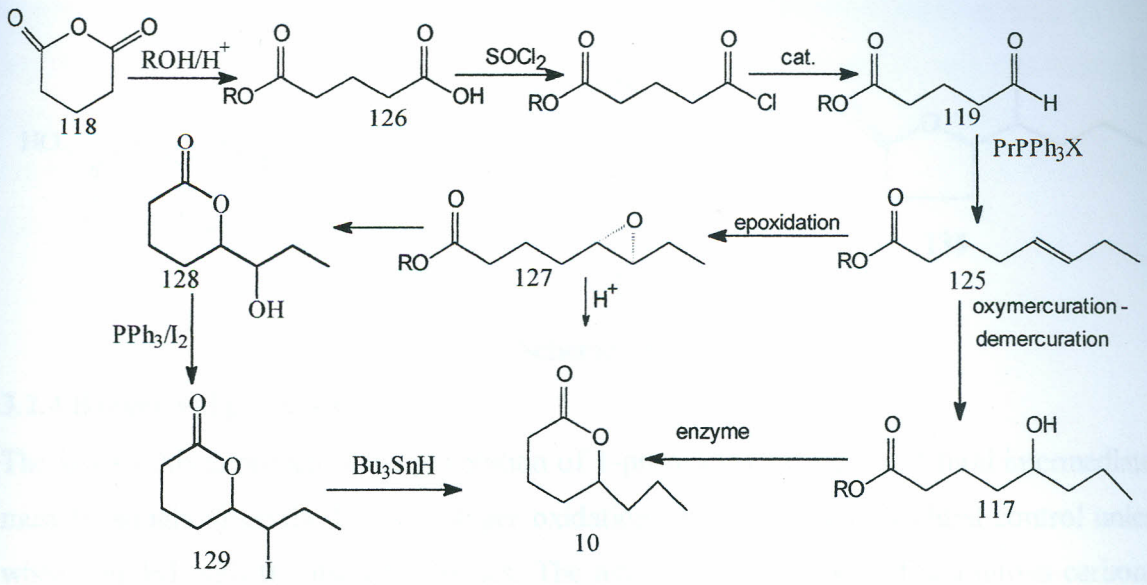
Scheme 25

The main bottleneck in this approach was recognised as the final reductive step using Wilkinson's catalyst. The use of chiral borane reducing agents may result in the opening of lactone ring due to the experimental conditions required and the Lewis acid nature of organoboranes.

3.2.2 Lactonisation of 5-hydroxyacid approach

3.2.2.1 Cyclization of the hydroxyacid

Glutaric anhydride (**118**) offers a good template due to its structural similarity to the target molecule. The conversion of this molecule to a 5-oxoacid/ester requires ring opening to an ester acid (**126**) followed by halogenation-dehalogenation to give an aldehyde (**119**). Acid catalysed alcoholysis (nucleophilic) followed by treatment with thionyl chloride can yield an acyl chloride-ester. Rosenmund reduction is an efficient dehalogenation method that could give the corresponding aldehyde (Manwaring *et al*, 1970). Alternatively, Sorrel's Cu (I) catalyst or DIBALH may be used (Sorrel *et al*, 1978). A Wittig reaction with the appropriate phosphonium salt would yield the unsaturated ester (**125**) that can be cyclised by converting it to an epoxide followed by cleavage with an acid or cerium catalyst (Reddy, 2001). This leads to self-cyclization in acidic media to yield the lactone. The stereochemistry would be controlled during epoxidation since the stereochemistry of the epoxide determines the orientation of the hydroxyl group upon cleavage. Alternatively, oxymercuration-demercuration would also generate the hydroxyl, but the regio- and stereoselectivity would be low. Enzyme catalysed cyclization would give the two isomers. The 5-hydroxyacid/ester can also be accessed through the reduction of the corresponding 5-ketoacid/ester. Enantioselectivity of the reduction can be induced using enzyme catalysts (Scilimati *et al*, 1988) to give δ-octalactone (Scheme 26).

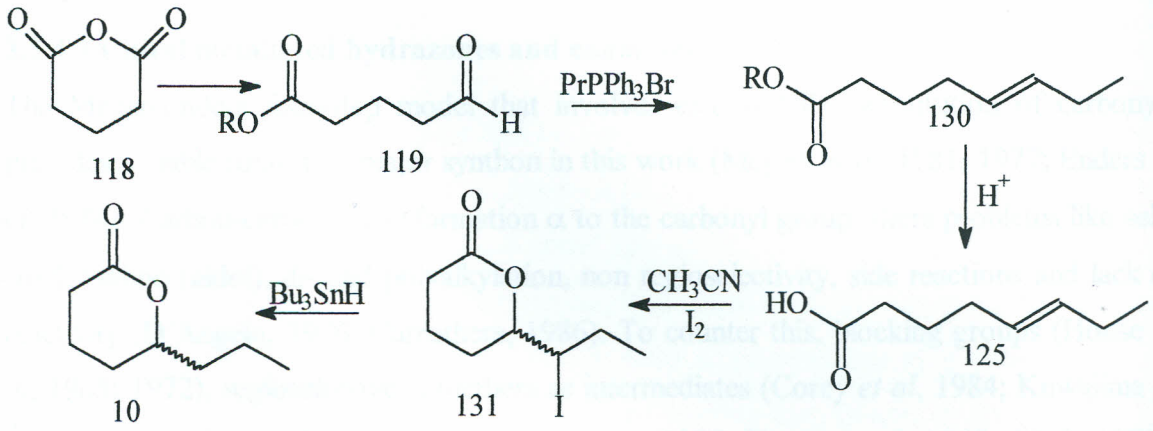


Scheme 26

3.2.3: Iodolactonisation

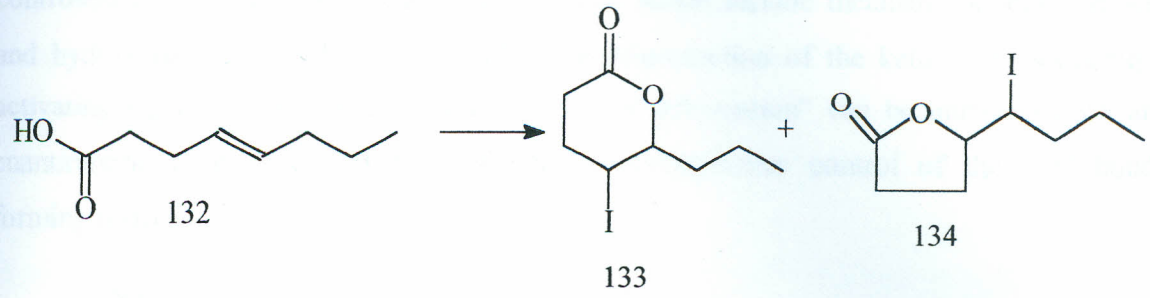
3.2.3.1 Iodolactonisation of 5-octenoic acid (125)

Glutaric anhydride (**118**) can give 5-octenoic acid (**125**) as shown in scheme 26 above. The iodolactonization of this acid using acetonitrile and iodine yields the iodolactone (**129**), which yields the lactone through tributyltin-induced reduction (Bartlett, 1980) (Scheme 27).



Scheme 27

The same procedure can be used with 4-octenoic acid (**132**) (Scheme 28). The presence of iodine in the ring may induce stereochemical control. However, the formation of a γ-lactone (**134**) cannot be ruled out.



Scheme 28

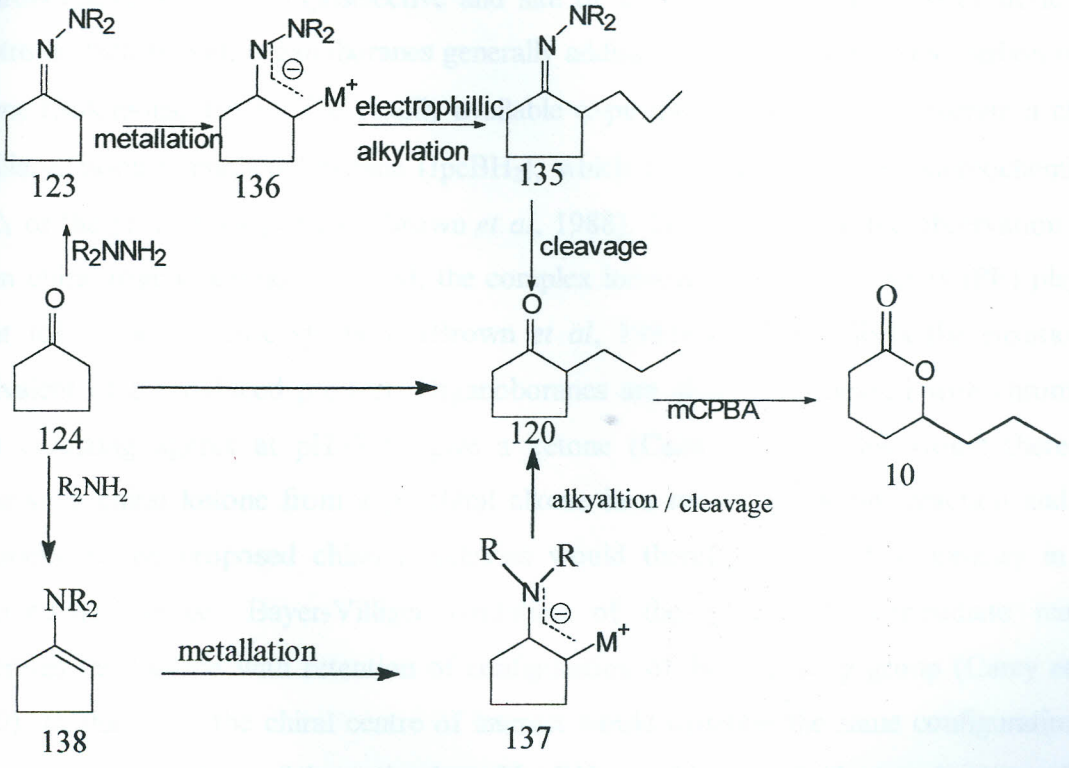
3.2.4 Baeyer-villiger approach

The key to this approach is the generation of 1-propylcyclopentanone. Chiral intermediates must be sought since the Baeyer-villiger oxidation offers no room for chiral control unless when coupled with the use of enzymes. The asymmetric reduction of ubiquitous carbonyl groups occupies a position of prime importance in asymmetric synthesis. The reductive alkylation of cyclopentanone involves a Grignard reagent adding to the carbonyl yielding an alcohol. Acid catalyzed dehydration, hydroboration and oxidative cleavage yield the alkylated cyclopentanone. Steric factors around the intermediate alkene assists in stereocontrol due to the preferences of organoboranes to bond at disubstituted carbon as opposed to trisubstituted one. However, to achieve the chiral alkylation various methods can be applied and these generate several routes to the target lactone.

3.2.4.1 Chiral metallated hydrazones and enamines

The Meyer-Enders four-step model that involves electrophillic substitution of carbonyls provides a viable route to a major synthon in this work (Meyers *et al*, 1981, 1977; Enders *et al*, 1984). Carbon-carbon bond formation α to the carbonyl group offers problems like self-condensation (aldol), di- and polyalkylation, non regioselectivity, side reactions and lack of reactivity (D'Angelo, 1976; Carruthers, 1986). To counter this, blocking groups (House *et al*, 1965; 1972), regioselective enolethers as intermediates (Corey *et al*, 1984; Kuwajima *et al*, 1982; Negishi *et al* 1979; 1983; Rasmunsen, 1977; House *et al*, 1965; 1969; 1971), temporary activating groups (Stowell, 1979; Krapcho, 1982; House, 1972; Kurth *et al*, 1985), phosphonate activating group (Hong *et al*, 1996; Lee *et al*, 1997; 1999; 2000) and nitrogen derivatives of ketones (Kuehne *et al*, 1970., Whitesell *et al*, 1983; Meyers *et al*, 1981., Hickmott, 1982; Gikonyo, 1999., Smith *et al*, 1983) have all been tried. These can be

controlled by use of lithioenolate type reagents, which include metallated imines, oximes and hydrazones (aza enolates). It involves an N-protection of the ketone, consequently, activating C_2 for electrophillic alkylation. "Chiral information" can be introduced via an enantiomeric pure N-reagent thus enabling enantioselective control of the C-C bond forming process.



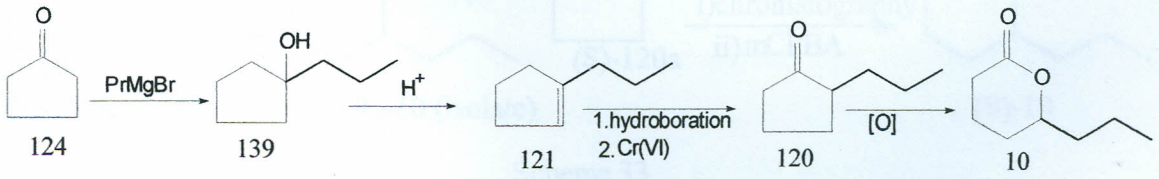
Scheme 29

Borrowing from the work of Meyers and Enders, RAMP, SAMP and phenylalanine derived chiral methoxy amines can be used as good chiral auxilliaries. In absence of the chiral N-auxilliaries, the electrophile attacks from either side of the symmetric prochiral π-system at C_2 and/or C_5 leading to mono and dialkylation. The chiral N-auxilliaries can be complexed with cyclopentanone, and then metallated to yield a five-member ring, which bears a specific orientation due to the chirality of the C_1 of the ring that, in turn, is governed by the source of the reagent. This helps control the orientation of the face of attack of the electrophillic alkylating reagent which is electrostatically attracted and coordinated to the metal before attacking the π-system thus effecting a bias. This mechanism has been shown to be similar for lithiated methoxyamines, oximes and hydrazones, though they slightly

differ in experimental conditions (Scheme 29). The eventual cleavage may be via transition metals (Kamal *et al*, 2000) or by mineral acid/water (Gikonyo, 1999; Meyers *et al*, 1981; Enders *et al*, 1984)

3.2.4.2. Chiral hydroboration-oxidation

Hydroboration is highly regioselective and site of addition is dictated by both steric and electronic factors with organoboranes generally adding to the least substituted carbon of an alkene (Solomons, 1997). The readily available α-pinene can be used to generate a chiral auxiliary, isopinocamphenyl borane ($IpcBH_2$), which can help control the stereochemistry at C_1 of the propylcyclopentene (Brown *et al*, 1988). This is based on the observation that when chiral organoboranes are used, the complex induced proximity effect (CIPE) plays a great role in asymmetric synthesis (Brown *et al*, 1982abc). This affects the enantiomer equivalent of the reduced product. Organoboranes are also easily cleaved with chromium (VI) oxidizing agents at pH>3 to give a ketone (Caine, 1991). This would therefore generate a chiral ketone from a prochiral alkene in a two-step one-pot reaction and the bulkiness of the proposed chiral auxilliaries would therefore induced asymmetry in the intermediate ketone. Bayer-Villiger oxidation of the generated intermediate readily generates the lactone with retention of configuration of the migrating group (Carey *et al*, 1990). In this case, the chiral centre of interest would maintain the same configuration as that of the ketone. Use of the antipodes of $IpcBH_2$ would generate the two isomers of the target molecule (Scheme 30).



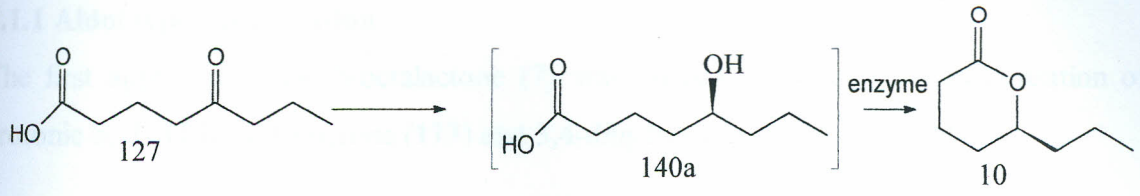
124 139 121 120 10

Scheme 30

2.2.5. Resolution

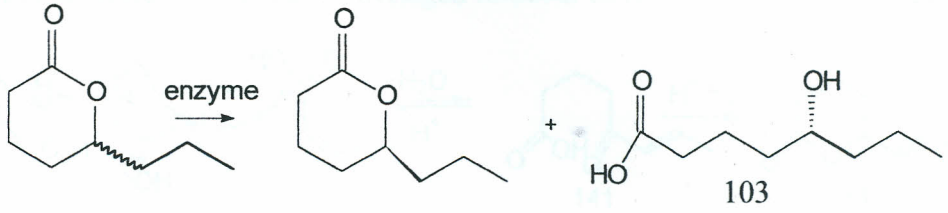
Enzymatic reduction using esterases (PLE and HLE) and lipase (PPL) can yield the (R)-isomer in 100% ee starting from a 5-ketoacid (**127**) (Blanco *et al*, 1988) (Scheme 31). Yeast

can also be used to selectively reduce 5-keto-acid to the lactone with a bias to one enantiomer (Seebach *et al*, 1984).



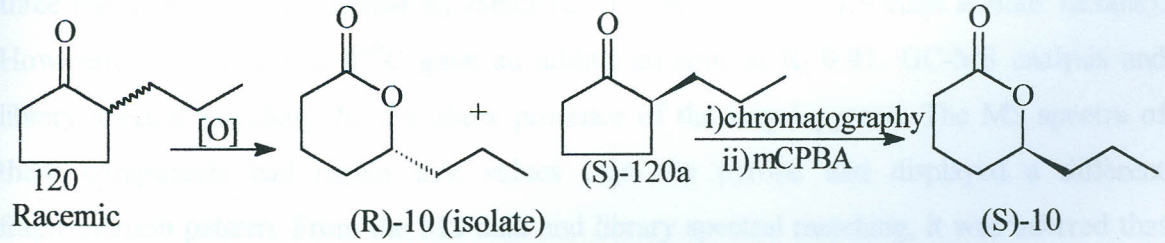
Scheme 31.

Lipase (PPL) (Scilimati *et al*, 1988) and bakers yeast (Utaka *et al*, 1987) converts the (S)-isomer into the hydroxy acid (**140a**) leaving the R- isomer intact at a PH>7. The (S)- isomer can be re-cyclised by lowering PH after separation (Scheme 32).



Scheme 32

The use of monooxygenase-based oxidation of racemic 1-propylcyclopentanone (**120**) gives the (R)-lactone (Scheme 33) (Schwarz-Linek *et al*, 2001). The pure (S)-ketone (**120a**) can be separated out and oxidized using peracids to give the (S)-lactone.



Scheme 33

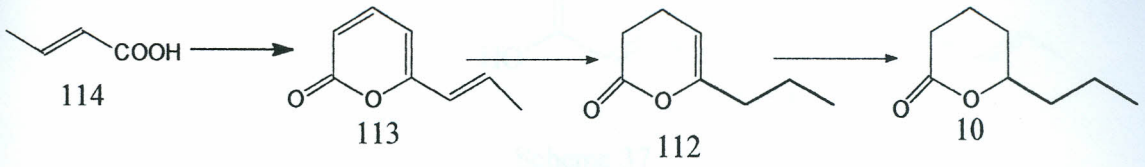
With the various synthetic strategies for the δ-octalactone, we set out to attempt the synthesis in both achiral and chiral ways using the proposed routes.

CHAPTER 4: SYNTHESIS OF δ- OCTALACTONE

4.1 Racemic synthesis

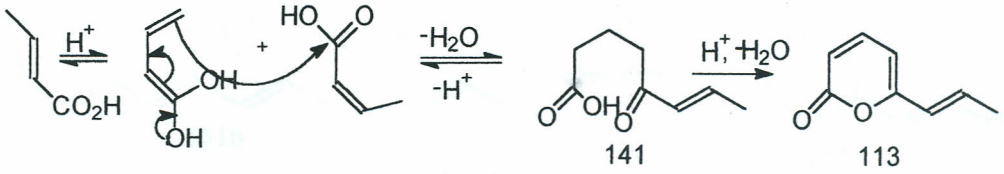
4.1.1 Aldol type condensation

The first approach to the δ-octalactone (**7**) was envisaged through the condensation of crotonic acid (**114**) to the pyrone (**113**) and 3,4-dihydropyranone (Scheme 34)



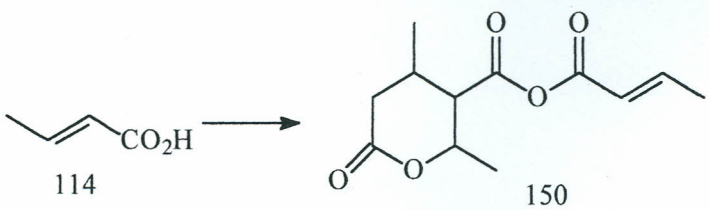
Scheme 34

The mechanism of this reaction was envisaged to occur as represented in scheme 35.

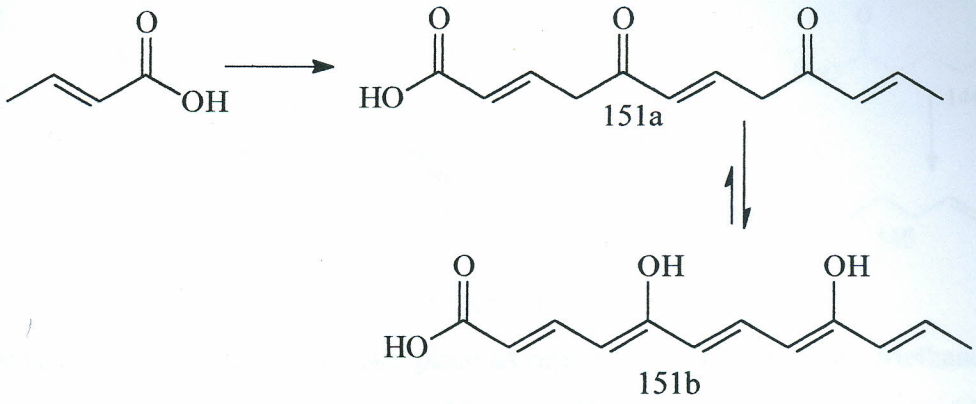


Scheme 35

Attempts on the condensation of crotonic acid were performed at 110, 80 and 65^0 C. TLC analysis indicated three similar spots. The reaction that ensued was shown to be temperature dependent due to variation in the number of TLC spots. At 110^0 and 65^{0}C, all three spots observed had similar R_f values (0.33, 0.46 and 0.57; 1:9 ethyl acetate: hexane). However, the reaction at 80^{0}C gave an additional spot at R_f 0.91. GC-MS analysis and library spectral matching did not show presence of the target pyrone. The MS spectra of these compounds had higher m/z values than the pyrone and displayed a different fragmentation pattern. From the MS data and library spectral matching, it was inferred that compounds **150** (from Diels-Alder reaction, Scheme 36) **151** and **152** (from Claisen type condensation and lactonization, (Scheme 37 and 38) were formed.

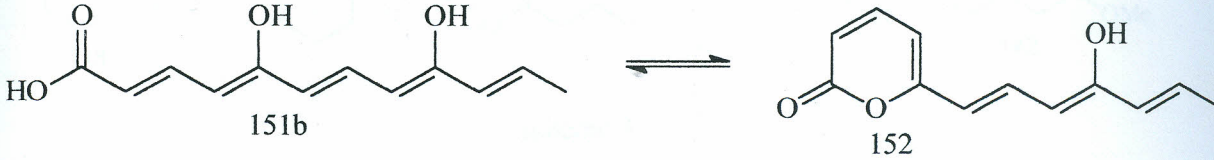


Scheme 36



Scheme 37

The presence of a δ-hydroxyl group in the acid (**151b**) could lead to cyclization in acidic media to give a pyrone as presented in scheme 38.

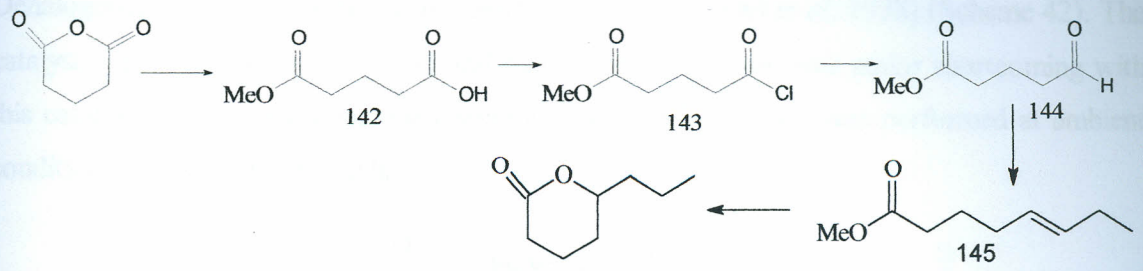


Scheme 38

However, the proposed structures are not conclusive since NMR and/or other spectroscopic analysis besides MS were not used to authenticate them. More work is recommended. Attempts to change reaction conditions to give the required pyrone failed. This necessitated the second approach to δ-octalactone synthesis.

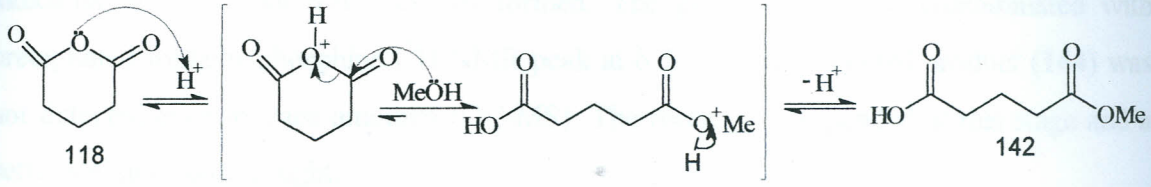
4.1.2 Glutaric anhydride alcoholysis

This approach to δ-octalactone involves trans-esterification of glutaric anhydride leading to ring opening to give the acid ester (methyl glutarate (**142**) (Scheme 39) followed by the transformation to the ester- acyl halide (**143**), the aldehyde (**144**) and finally the Wittig reaction to give the alkene ester (**145**).



Scheme 39

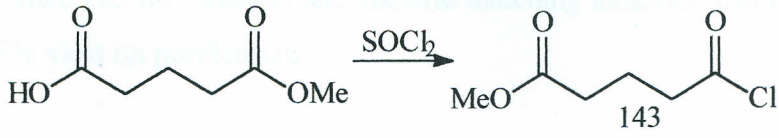
The methanolysis is envisaged to take place as represented in scheme 40. Methanolysis of glutaric anhydride was performed at ambient conditions using HCl and quenching with $NaHCO_3$.



Scheme 40

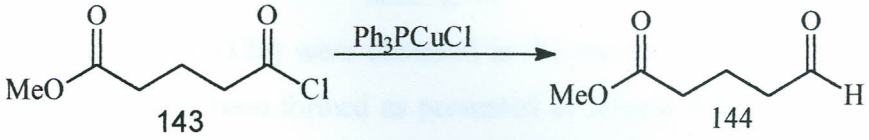
Concentration of the organic layer gave white crystals. GC analysis showed a product at 98.3% purity and 65% yield. GC-MS analysis, spectral matching suggested this to be the target molecule (**142**) with characteristic MS peaks at m/z value (M-31) and (M-44) while ^{1}H NMR gave a characteristic peak at δ 3.66 (3H), 2.44 (4H) and 1.99 (2H).

The formation of the acyl halide (**143**) was achieved by use of thionyl chloride in dichloromethane (Scheme 41) (Vogel, 1994). TLC analysis of the organic fraction gave a major spot at R_f 0.38. MS analysis indicated this to be the target compound characterised by m/z (M^+-31), (M^+-35) and (M^+-63) (due to the loss of Cl and CO) peaks. Absence of M^+-44 and presence of M^+-63 peak confirmed that the cid had been converted to the acyl halide. ^{1}H NMR peak at δ 3.66 also indicated that the ester group was still intact.



Scheme 41

Dehalogenation was performed using Sorrel's catalyst (Sorrel *et al*, 1978) (Scheme 42). The catalyst was prepared in 79% yield and high purity. However, one major shortcoming with this catalyst was its high equivalent weight. The dehalogenation was performed at ambient conditions to give white crystals.

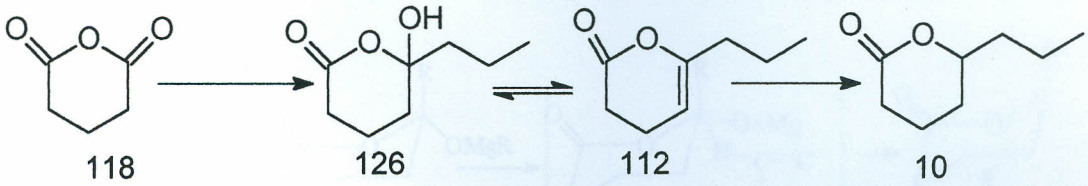


Scheme 42

Analysis of the obtained product by ^1H NMR did not show a peak between δ 9-10 indicating that the aldehyde was not formed. The product was also contaminated with precipitated triphenylphosphine (^1H NMR peak at δ 7.76). The expected product (**144**) was not detected even in trace amounts (GC-MS). The route was suspended at this stage and a better yielding route sought.

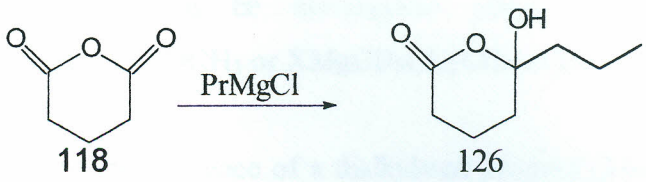
4.1.3. Glutaric anhydride alkylation

The next approach was envisaged through monoalkylation of glutaric anhydride to give a hemiacetal (**126**), dehydration and reduction would follow (Scheme 43)



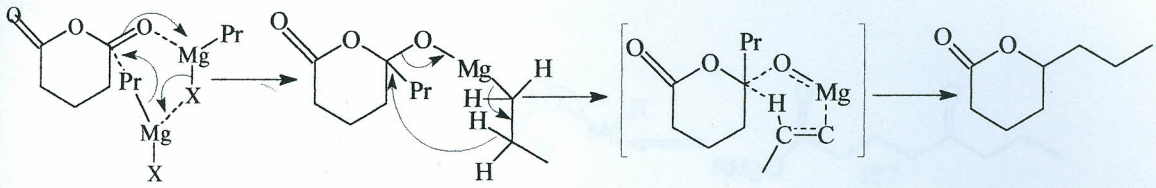
Scheme 43

The stoichiometric (1:1) alkylation of glutaric anhydride (Scheme 44) was performed under nitrogen in ether and worked up in acidic medium. TLC analysis of the organic extract gave 3 spots at R_f 0.73, 0.69 and 0.59 (2:3 ethyl acetate: hexane) while GC analysis indicated a peak at R_t 24.51 min. GC-MS analysis and spectral matching indicated this to be the target lactone (**7**) at 3.5% yield on purification.



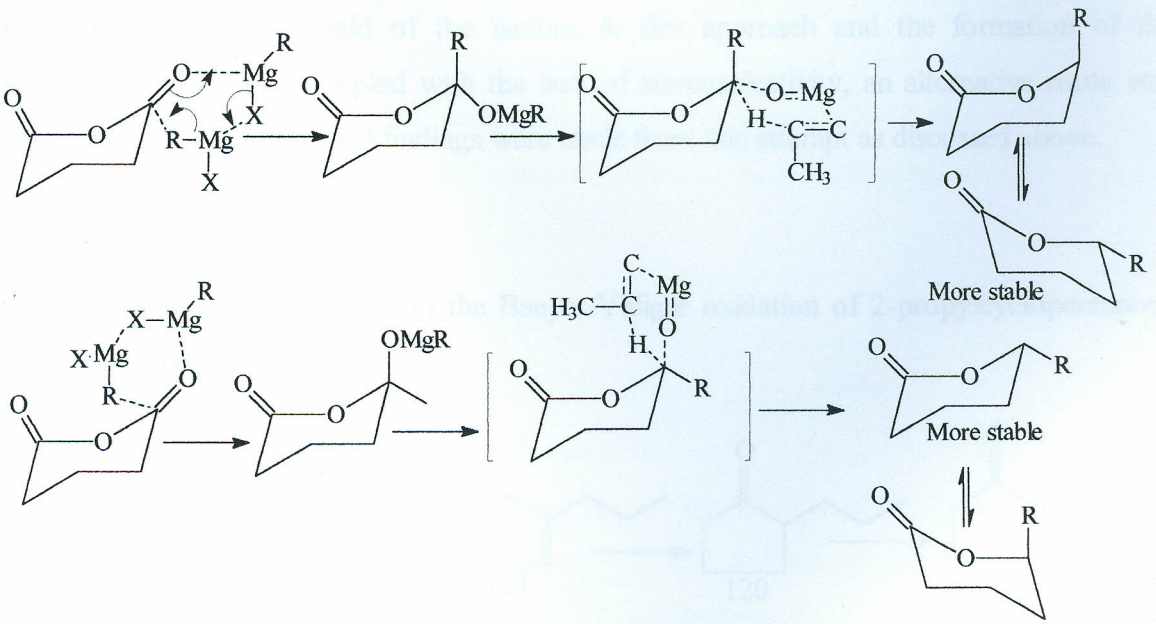
Scheme 44

No traces of the hemiacetal (**126**) were observed in the reaction mixture. We hypothesised that the lactone could have been formed as presented in scheme 45. This is comparable to the mechanism in the Merwein-Pondorf-Varley reduction (Carey *et al*, 1990) but with a hydride transfer from the organomagnesium reagent occurring to give δ-octalactone.



Scheme 45

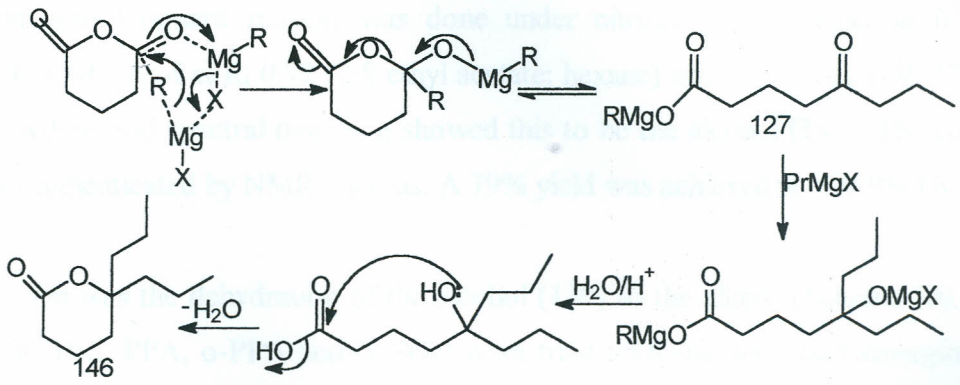
The tandem process that ensues offers no room for stereo control and/or yield improvement. The equal probability of the propyl group being transferred from any side of the prochiral carbonyl ensures that the alkylation is not stereoselective; similarly addition of the hydride is non-stereospecific (Scheme 46). Further studies with labelled compounds are needed.



Scheme 46

The proposed mechanism can be investigated using labelled compounds like $XMgCH_2CD_2CH_3$, $XMgCH_2CHDCH_3$ or $XMgCD_2CH_2CH_3$ as the Grignard reagents.

GC-MS analysis indicated the presence of a dialkylated product (**146**) (30.17%, R_t 31.75 min) suggesting a possible intermediacy of δ-keto acid (**127**) from the organomagnesium intermediate (Scheme 47). We propose that the structure is **146** as opposed to a 1,5-dialkylation, since the MS spectra gave two prominent peaks at m/z 141 and 71. The 1,5-dialkylated product could have given a major peak at m/z 127 that was not present even in trace amounts. This strongly supports the proposed mechanism but further mechanistic studies are recommended.

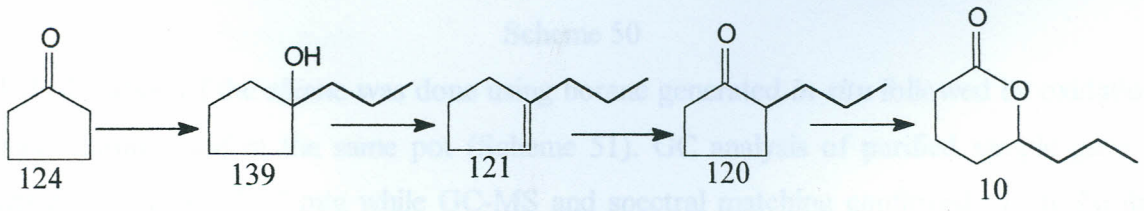


Scheme 47

Due to the miserable yield of the lactone in this approach and the formation of the dialkylated by-product coupled with the lack of stereoselectivity, an alternative route was investigated. However, novel findings were made from this attempt as discussed above.

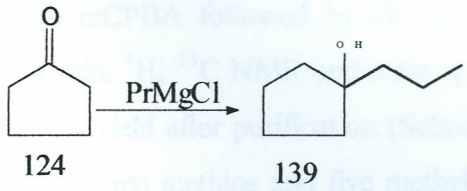
4.1.4 Bayer-Villiger oxidation

The final approach was found in the Baeyer-Villiger oxidation of 2-propylcyclopentanone (**120**) (Scheme 48).



Scheme 48

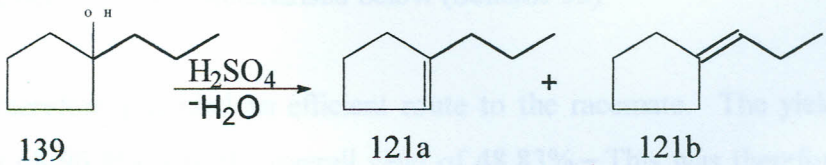
However, previous attempts to δ-lactone synthesis using this approach presented serious problems in the alkylation of the cyclopentanone giving dialkylated products and the lactone being produced non-stereoselectively (Gikonyo, 1999). Consequently, an alternative regioselective route had to be found. An indirect route via Grignard alkylation was envisaged (Scheme 49).



Scheme 49

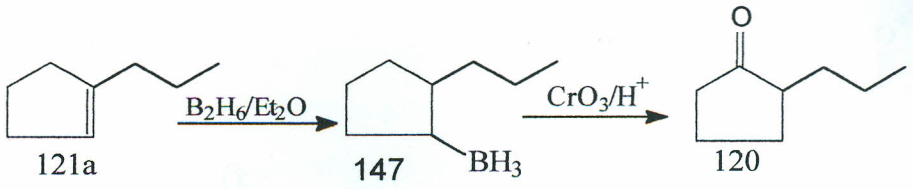
Alkylation with Grignard reagent was done under nitrogen in dry ether at 0°C. TLC analysis showed a spot at R_f 0.72 (2:8 ethyl acetate: hexane) and a GC peak at R_t 17.38 min. GC-MS analysis and spectral matching showed this to be the alcohol (**139**). The compound (**139**) was authenticated by NMR analysis. A 79% yield was achieved at >99.9% GC purity.

The next step was the dehydration of the alcohol (**139**) to the alkene (Scheme 50). Several acids (HBr, HCl, PPA, o-PPA and H_2SO_4) were tried with sulphuric acid emerging as the most suitable and was therefore adopted. GC analysis of the organic extract gave two closely eluting peaks at R_t 13.399 and 13.915 min in the ratio of 22:1. GC-MS analysis showed these to be the *endo*- (**121a**) and *exo*-cyclic (**121b**) alkenes respectively. PTSA was used to epimerize the *exo*- to the *endo*-isomer (Iura *et al*, 2000). Completion of the reaction was monitored by disappearance of the minor GC peak to give 93.9% yield of **121a**.



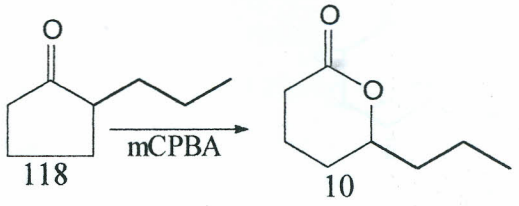
Scheme 50

Hydroboration of the alkene was done using borane generated *in situ* followed by oxidation using chromic acid in the same pot (Scheme 51). GC analysis of purified sample gave a major peak at R_t 22.17 min while GC-MS and spectral matching confirmed this to be the target ketone (**120**) in 68% yield after purification.



Scheme 51

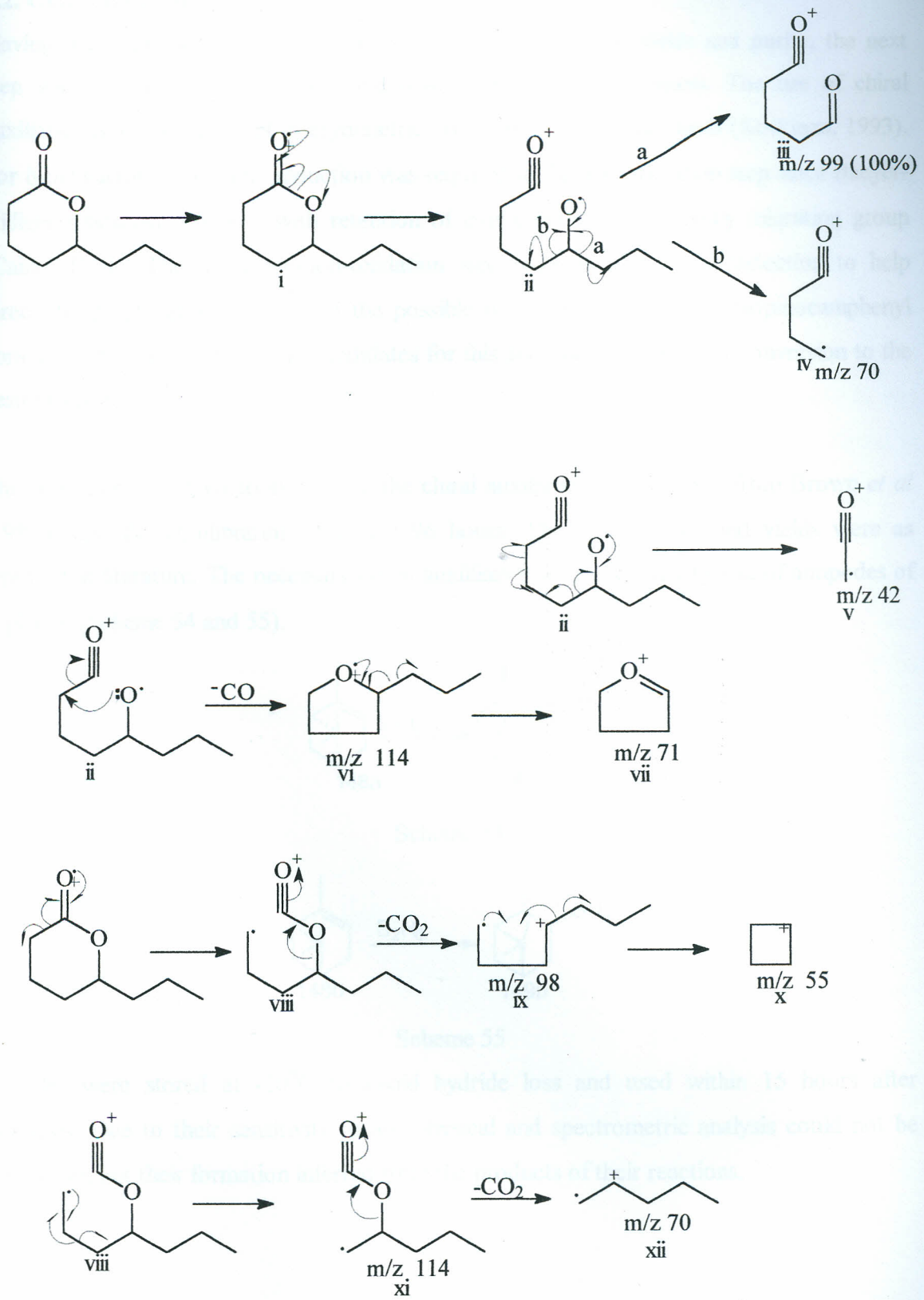
Baeyer-Villiger oxidation with mCPBA followed by GC analysis of the organic extract gave a GC peak at R_t 24.484 min. ^1H, ^{13}C NMR and mass spectral analysis confirmed the presence of the lactone in 96.8% yield after purification (Scheme 52). ^{13}C NMR analysis of the lactone indicated one methyl, one methine and five methylene carbons from the DEPT spectrum. A diagnostic peak at δ 80.36 for the CH carbon on the lactone moeity (C-5) confirmed that the oxidation occurred. Similarly, all other carbons were justified.



Scheme 52

The ^1H spectrum had a characteristic unresolved peak at δ 4.29 (m, 1H) indicating the proton on C-5 and a triplet at δ 2.39 coupled to a proton up field characteristic of the C-2 protons. Another triplet at δ 0.87 coupled to a proton downfield was assigned the C-8 protons. The rest of the proton signals were unresolved at around δ 1.309-1.568. The ^{13}C spectrum gave a characteristic peak at δ 80.36 for the C-5, 18.49 (C-3) and 13.85 (C-8). The MS fragmentations are summarised below (Scheme 53)

This route therefore provided an efficient route to the racemate. The yields in each step ranged from 68-96.8% with the overall yield of 48.83%. This was therefore envisaged as an economically viable route.

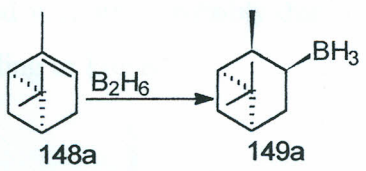

i
ii
a
b
iii
m/z 99 (100%)
iv
m/z 70
v
m/z 42
-CO
vi
m/z 114
vii
m/z 71
viii
-CO2
ix
m/z 98
x
m/z 55
xi
m/z 114
xii
m/z 70

Scheme 53

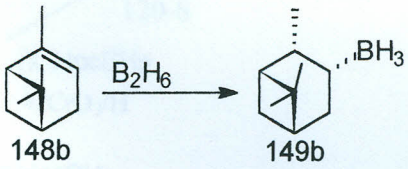
4.2. Chiral synthesis

Having achieved the synthesis of the racemate in satisfactory yields and purity, the next step was to make the route selective towards the possible isomers. The use of chiral auxiliaries is a powerful tool in asymmetric synthesis of natural products (Koskinen, 1993). For δ-octalactone (7), chiral induction was required in the hydroboration step since Baeyer-Villiger oxidation proceeds with retention of configuration of the bulky migrating group (Caine, 1991). The hydroboration-oxidation step requires high chiral induction to help direct the propyl group to each of the possible orientations selectively. Isopinocamphenyl boranes were envisaged as ideal candidates for this and due to the ease of conversion to the desired ketones.

The procedure followed to synthesize the chiral auxiliaries was adopted from Brown *et al* (1982a) and the equilibration done for 96 hours. The observations and yields were as reported in literature. The necessary chiral auxilliaries were prepared by use of antipodes of α-pinene (Scheme 54 and 55).



Scheme 54

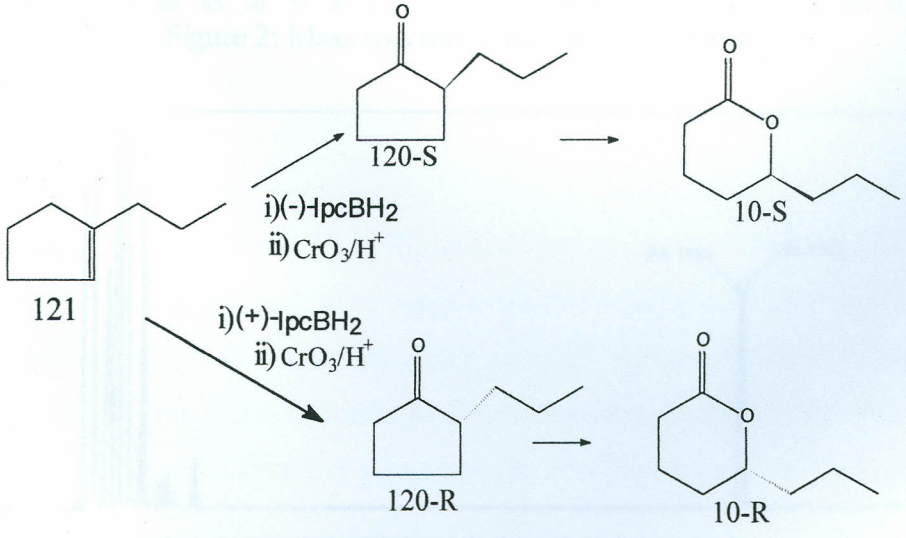


Scheme 55

Samples were stored at -10^{0}C to avoid hydride loss and used within 15 hours after synthesis. Due to their sensitivity to air, physical and spectrometric analysis could not be carried out but their formation inferred from the products of their reactions.

4.2.2 Synthesis of (R)- and (S)-δ-octalactone

Hydroboration of the 1-propylcyclopentene (**121a**) with the chiral auxilliaries was done as already detailed in the achiral hydroboration. However, attempts to use the earlier prepared chromic acid in the oxidation gave a secondary alcohol and other contaminants. Chromium trioxide in sulphuric acid at PH<3 was a better option since no 2^0 alcohol was detected and the purity of the product was higher. The target ketone (**120**) was isolated in satisfactory yields (38 for R-, 46% for S-) and lactonization done with mCPBA (Scheme 56). Use of antipodes of the chiral auxilliaries gave the two isomers independently. GC, GC-MS (Figure 2) and mass spectral matching confirmed the authencity of the products. Chiral GC analysis indicated that the antipodes of chiral auxiliaries gave different isomers of the product. Chiral GC peaks at R_t 26.6 and 26.8 min without baseline resolution (Figure 3) were detected at different ratios for the antipodes of chiral auxiliaries. (+)-$IpcBH_2$ gave a lower ee (60%) while the antipode gave >97%. This could be due to the lower ee of the starting materials. (-)-α-Pinene used was of 87% ee as one of >99% ee was not commercially available. From the purity of the starting materials an ee of 71.8% was expected, however the observed was 60% probably due to the increased production of the diisopinocamphenyl borane leading to low ee.



Scheme 56:

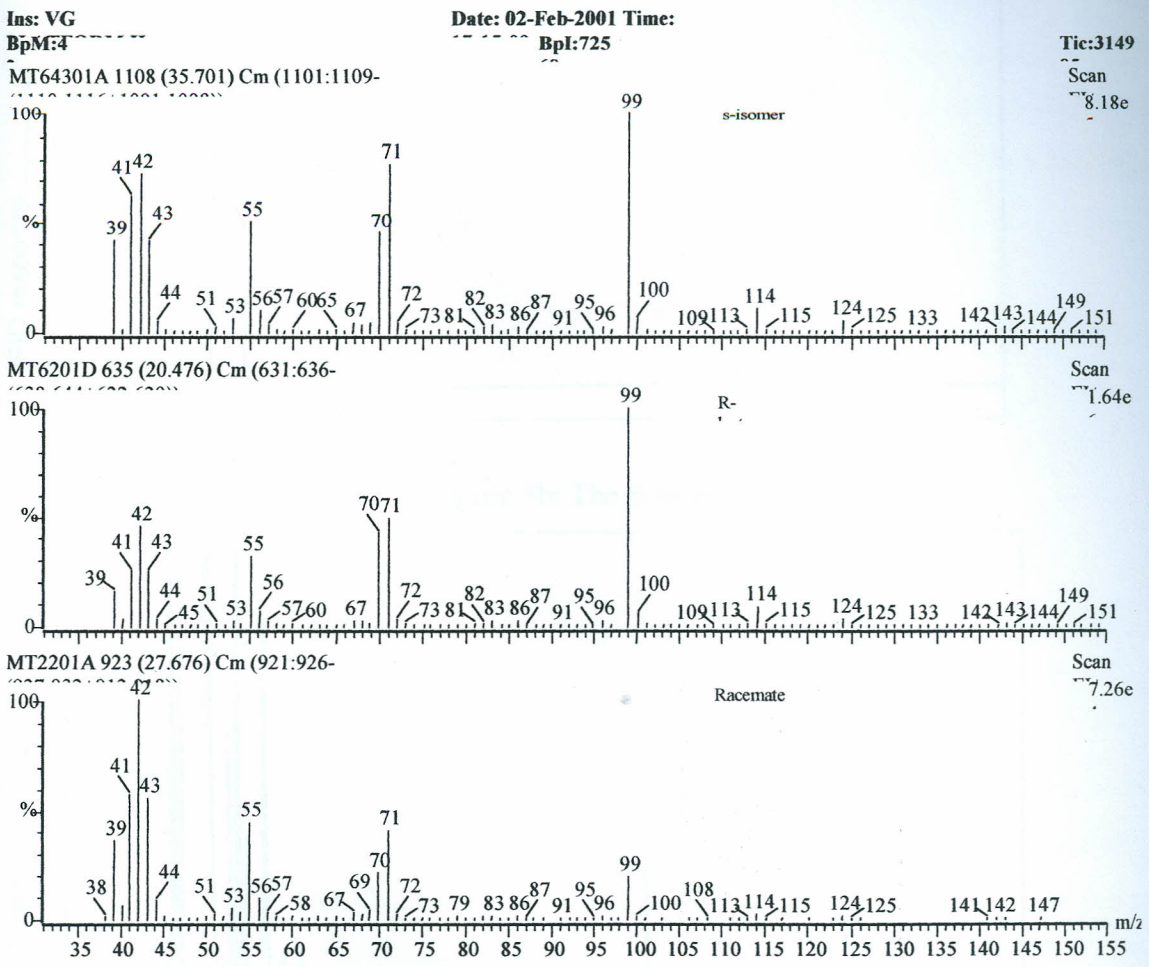


Figure 2: Mass spectra of the synthetic lactone

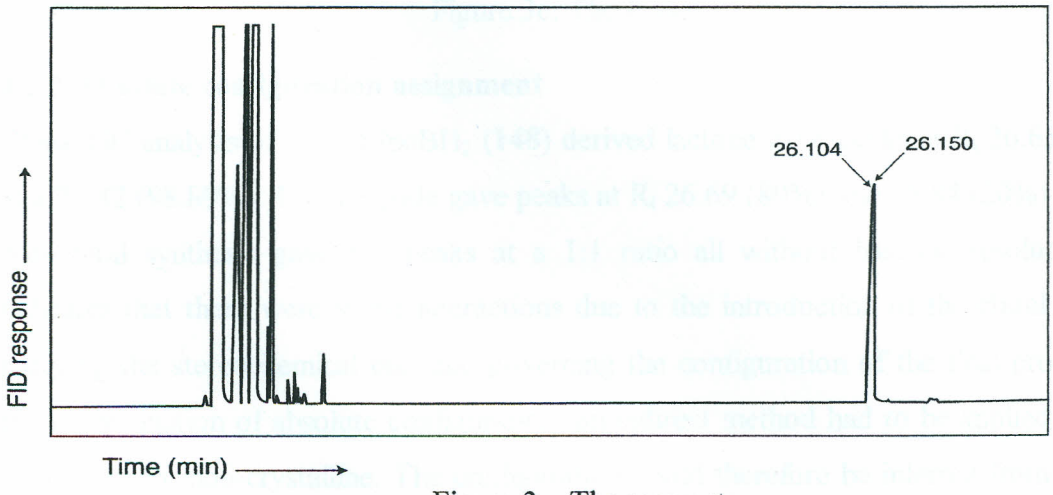


Figure 3a: The racemate

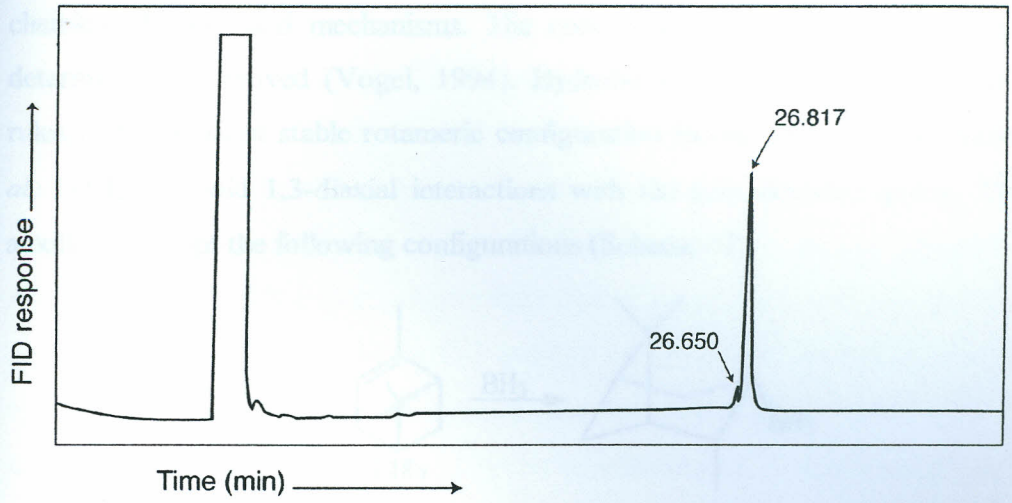


Figure 3b: The S-isomer

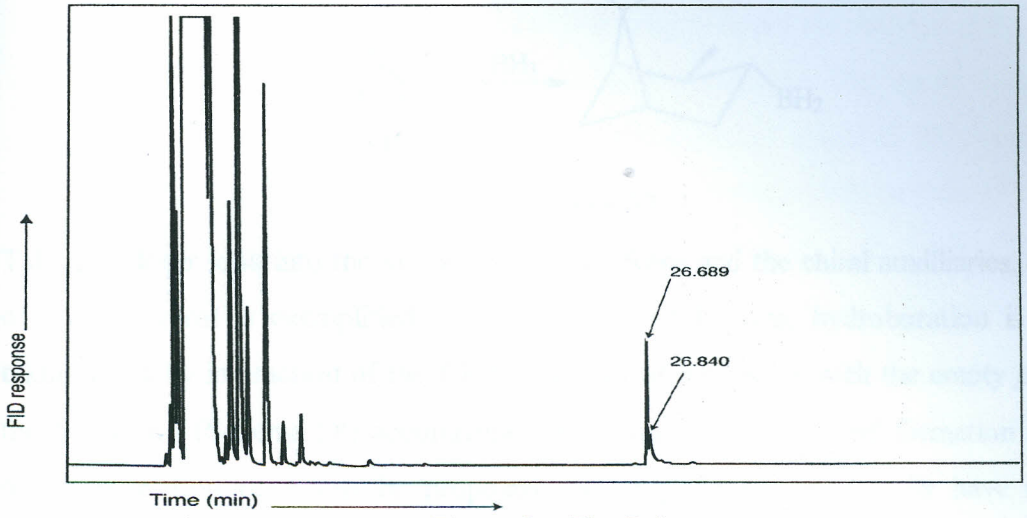
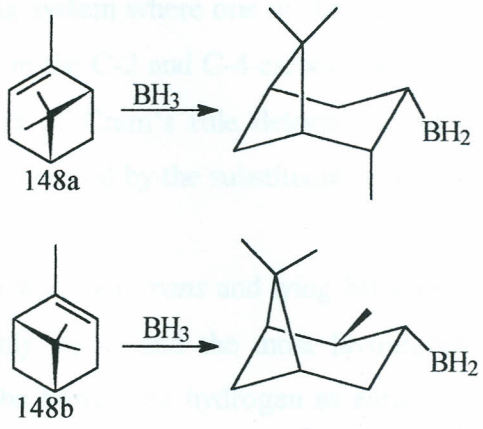


Figure 3c: The R-isomer

4.2.3 Absolute configuration assignment

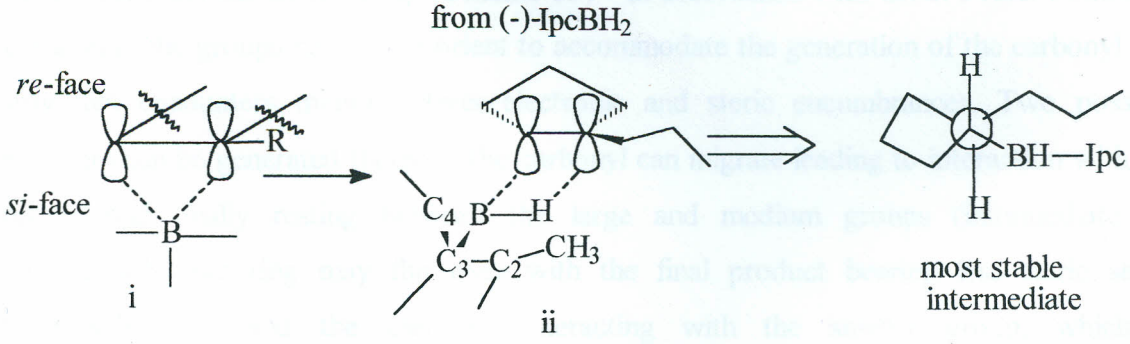
Chiral GC analysis of the (-)-IpcBH$_2$ (**148**) derived lactone gave peaks at R_t 26.65 (1.11%) and 26.82 (98.89%). The antipode gave peaks at R_t 26.69 (80%) and 26.84 (20%) while the non-chiral synthesis gave the peaks at a 1:1 ratio all without baseline resolution. This indicates that there were steric interactions due to the introduction of the chiral auxilliary inducing the stereochemical bias and governing the configuration of the final product. For the determination of absolute configuration, an indirect method had to be applied since the lactones were non-crystalline. The configuration could therefore be inferred from reactions using the same auxilliaries in the synthesis of 2-alkylated cyclopentanols (Brown *et al*, 1982b, 1984ab). Otherwise, the same configuration can be obtained by considering molecular and conformational studies of the reaction intermediates and applying accredited

chemical theories and mechanisms. The conformation of the chiral auxilliaries has been determined and proved (Vogel, 1994). Hydroboration products conform to Auwer-Skita rules, with the most stable rotameric configuration having an equatorial borane (Brown *et al*, 1964) to avoid 1,3-diaxial interactions with the *gem* dimethyl group. Thus the chiral auxilliaries adopt the following configurations (Scheme 57).



Scheme 57

Taking a closer look into the interaction of the alkene and the chiral auxilliaries, the essence of chiral control is exemplified. In molecular orbital terms, hydroboration is viewed as taking place by interaction of the filled π-orbitals of the alkene with the empty *p*-orbitals of the boron in i (Scheme 58) accompanied by a concerted C-H bond formation in ii. Thus some correlation rules can be proposed, but they do not necessarily have mechanistic implications. However, since they serve to correlate the observed configuration in a considerable number of cases in both alcohol and chiral olefin synthesis, they may be quite valuable in predicting the structure of the products. Taking the (-)-IpcBH$_2$ the following transition state can be envisaged (Brown *et al*, 1964; Jurczak *et al*, 1986) (Scheme 58).



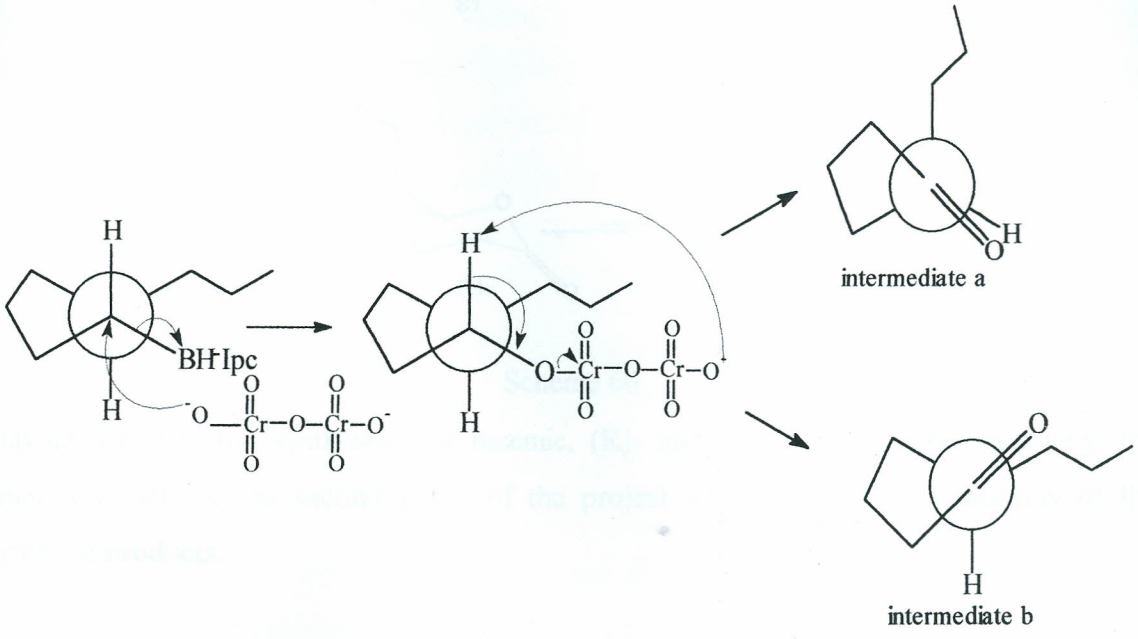
Scheme 58

The addition of the B-H bond to a C=C bond can be interpreted in terms of a 4 centre transition state (Brown *et al*, 1982b). This leads to the formation of a rigid intermediate, which should be strongly influenced by the steric factors of the chiral auxiliary and the olefin (ii) above. Therefore, the most stable intermediate is one in which the methylene groups are away from the C-2 and C-4 because Z-alkenes encounter less steric encumbrance. Thus in a ring system where one of the carbons is dialkylated, the most bulky group has to be furthest from the C-2 and C-4 carbons, which correspond to ring carbons in cyclopentanone. Application of Cram's rule determines the orientation of the remaining alkyl group that is largely influenced by the substituent on C-3 as shown in scheme 58.

With the large group on each carbon *trans* and lying between the medium and small group, this transition state is highly stable and the most favourable. The cyclic transition state implies a *syn* addition of the boron and hydrogen as earlier observed (Brown *et al*, 1964, 1982ab) with bond breaking and bond formation occurring simultaneously (SN_2 reaction) and the complex induced proximity effect (CIPE) coming into great effect during bond formation (Brown *et al*, 1988). For the sake of this discussion, the two faces of the alkene can be labelled *re-* and *si-* as suggested below (Scheme 58). For the (-)-IpcBH$_2$, the only thermodynamically feasible attack is from the *si* –face since the α-methyl group (C-2) is avoided and the *gem*-dimethyl is far from the reacting site. An attack from the *re*-face leaves the α-methyl and the ring carbons to interact generating a lot of steric strain to the intermediate. Having oriented the propyl group, oxidation follows to generate the ketone.

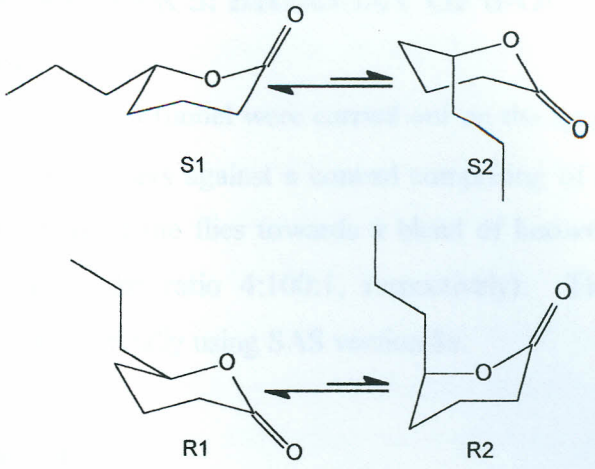
The Cr (VI) oxidant attacks the C-B bond away from the propyl and ring carbons between the medium and the small group (Scheme 59) - in accordance with Cram's rule. Following the attack, the groups have to re-orient to accommodate the generation of the carbonyl in a way that encounters minimal stereo-electronic and steric encumbrances. Two possible rotamers can be generated thereof. The carbonyl can migrate leading to interaction with the propyl and finally resting between the large and medium groups (intermediate b). Alternatively, the ring may flip over with the final product bearing less steric strain (intermediate a) and the carbonyl interacting with the smaller group, which is thermodynamically more favourable. Thus, the oxidation occurs with retention of

configuration at the chiral centre. This implies that the stereochemistry and structure of the oxygenated compound can be predicted with confidence. Thus hydroboration is the only step that governs the observed bias in the ratio of the isomers.



Scheme 59

Baeyer-Villiger oxidation is known to occur with retention of configuration (Carey *et al*, 1990) therefore the generated lactone will bear the stereochemistry inherited from the intermediate ketone. From this argument it can be inferred that the (-)-$IpcBH_2$ yields the (S)- isomer while a similar argument indicates that the (+)-$IpcBH_2$ gives the (R)-isomer. Therefore, the chiral GC analysis peaks at 26.69 and 26.82 min are for the (S)- and the (R)-isomers, respectively. To obtain the absolute conformation of the derived isomers, the cyclohexane model can be adopted. From conformational analysis, the isomers with an equatorial propyl group are the most stable. Therefore the structures of the synthesized isomers are proposed to be R_1 and S_1 based on the above argument and borrowing from the substituted cyclohexanone model (Kagan, 1979) (Scheme 60).



Scheme 60

Having achieved the synthesised of racemic, (R)- and (S)- δ-octalactone separately, the stage was set for the second phase of the project which involved the bioassay of the synthetic products.

CHAPTER 5: BIOASSAY OF δ-OCTALACTONE

5.1 Behavioural bioassay

Behavioural bioassays in the wind tunnel were carried out on the synthetic δ-octalactone as the racemate, (R)- and (S)- isomers against a control comprising of paraffin oil. This was compared with the behaviour of the flies towards a blend of known attractants (*p*-cresol, acetone and 1-octen-3-ol in the ratio 4:100:1, respectively). The data was analysed parametrically and non-parametrically using SAS version 8e.

5.1.1 Racemic δ-octalactone

The direction of flight varied from flying just out of the release cage to the 200 mm wide midsection, treated or control arm. A proportion flew either to the control or the treated arm and remained there during the observation period. Some flies moved upwind and then back to the mid-section where they remained throughout the experiment. Out of the insects that did not make any directional choice, some showed un-directional movements within the midsection by changing resting sites, while a few remained in the release cage inactivated.

Analysis of the initial direction of flight indicated that for 0 (blank), 0.1, 1.0, and 2.5 mg/200 µl, of the treatment with the racemic lactone, no statistically significant differences in fly distribution between the control and treated arms were observed. For the attractant, significantly more flies opted for the treated (72.41%, $p<0.05$) as compared to the control. For the racemic lactone at 0.5 mg/200 µl, more flies initially opted for the control (69.23%, $p< 0.05$). The variation of the initial direction of flight showed no defined trend with increase in dosage (Figure 4), therefore the behaviour is not dose dependent. Since only one dose (0.5 mg/200 µl) showed significant difference and the behaviour was not consistence it can be inferred that the initial direction of flight is not important in the analysis of repellent properties of an odour in the wind tunnel. At the dispensed doses, the initial direction of flight was dependent on the chemical dispensed (χ^2_2 26.11, $p<0.05$) suggesting that preference was influenced by the odour. However, the occurrence of a maximum in the number of flies keeping off the treated at 0.5 mg/200 µl suggests that there

could be several factors influencing the initial choice. These may be acting antagonistically with a maximum bias to one when a dose of 0.5 mg/200 µl of the racemic lactone is dispensed. Otherwise, it is possible that a dose of 0.5 mg/200 µl of the racemate represents a dose at which maximum repellence response is observed for the *G. m. morsitans* However, the initial behaviour may be explained by the fact that flies will always show positive anemotaxis due to activation by the wind. The dispensed odours only help differentiate the two arms of the tunnel and therefore inducing arm preference for the flies. Otherwise the generated wind is enough to elicit unidirectional flight. This is supported by the proportion unpreferentially leaving midsection when the blank is dispensed (64.29%) (Table 1).

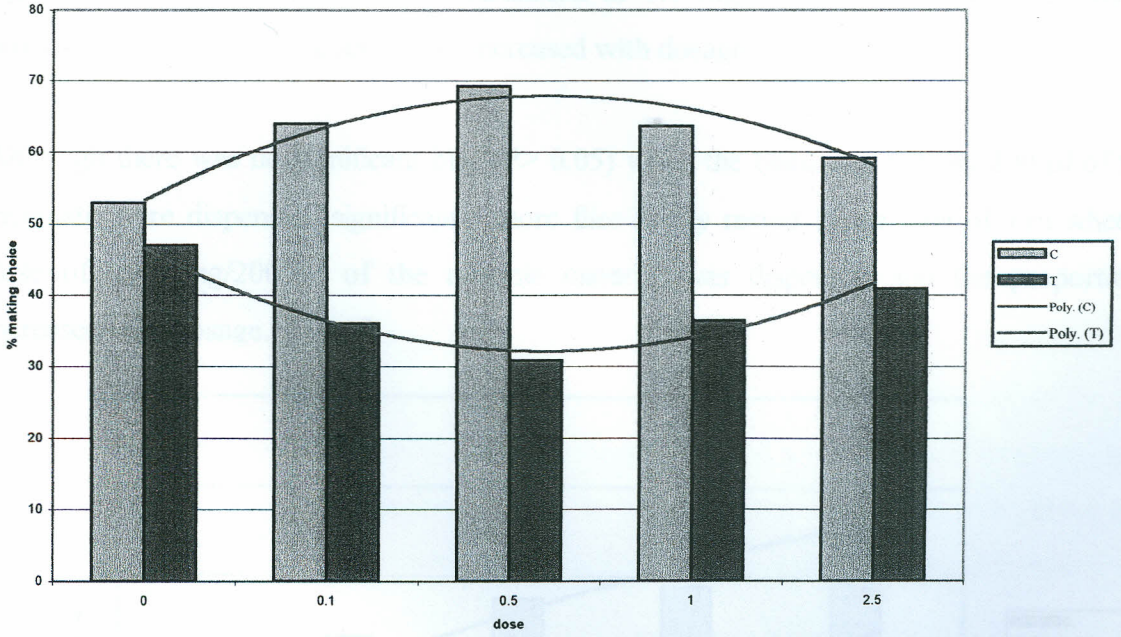


Figure 4: % Initial fly choice when the racemic mixture of δ-octalactone was dispensed

The final distribution of the flies on exposure to the racemate was found to be dose dependent as confirmed by a gradual variation in the proportion of flies showing preference to either of the two arms with a higher proportion increasingly going to the control arm indicating an increase in the repellent nature of dispensed odours (Figure 5). It was concluded that repellence increases with dosage. The final distribution of the flies was also dependent on the chemical dispensed (χ^2_2 20.054, $p<0.05$). However, the second and third directions of flight were not dependent on the odour dispensed (χ^2_2 6.64, $p>0.05$; χ^2_2 3.373,

p>0.05, respectively). For the attractant, the final distribution between the control and the treated was not significantly different (p>0.05) and this may be explained by the absence of a host or an arrestant, prompting the flies to redistribute themselves randomly regardless of the odours emanating from either arms. Notably, 41±1% of the flies used in the blank initially remained in the midsection after leaving the release cage and a similar proportion finally rested in the midsection. This confirmed that for the blank there was no motivation for the flies to leave the midsection since there was no odour, and this confirms the expected final random distribution. Fewer flies were observed to finally rest in the treated arm when the lactone was dispensed and this behaviour was found to be dependent on the chemical dispensed (χ^2_2 21.73, p<0.05). The racemic lactone showed no statistically significant differences in final fly distribution at 0.1 mg/200 µl. However, the bias in proportions resting in the control arm increased with dosage (Figure 5).

Although there was no significant bias (P> 0.05) when the blank and 0.1 mg/200 µl of the racemate were dispensed, significantly more flies finally rested in the control arm when a dose of ≥0.5 mg/200 µl of the racemic material was dispensed and the proportions increased with dosage.

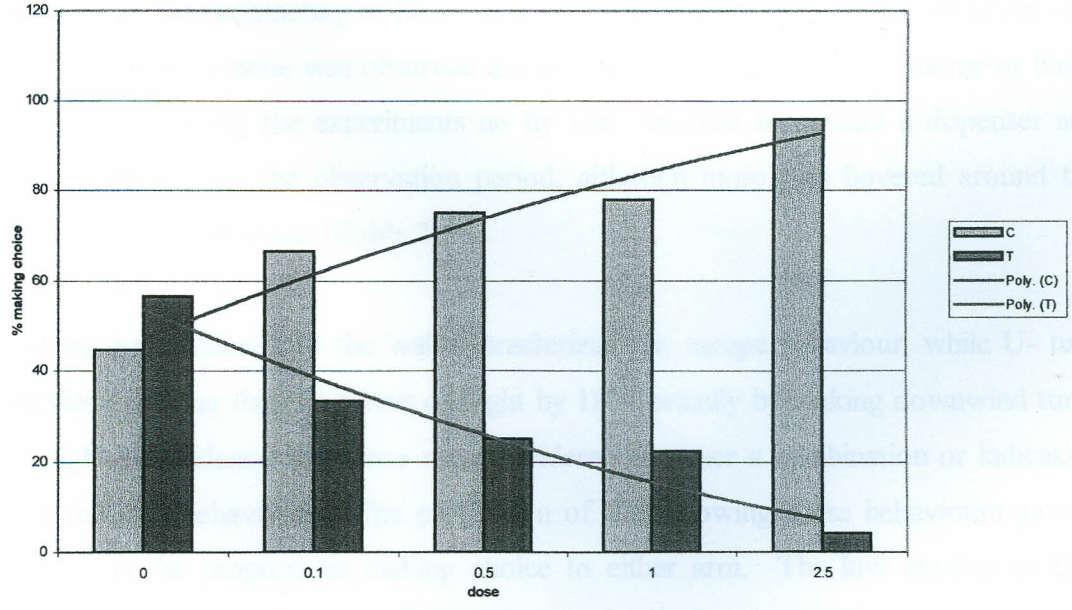


Figure 5: % Final fly distribution when the racemate was dispensed

Parametric analysis of the maximum upwind distances covered by the flies using t-test (SAS version 8e, 2000) showed no significant differences between the control and treated arms irrespective of the odour dispensed (Table 1).

					%Fly distribution					
Odour dispensed	Dose (mg)[#]	N	% Flies activated	% Flies leaving midsection	Initial flight direction		Final resting position		Average maximum upwind flight (mm) (± s.e)	
					C	T	C	T	C	T
Attractant	1	72	83.33	70.83	27.59	72.41*	58.82	41.18	321.1±13.0	294.1±15.8
Blank	0	84	71.43	64.29	52.94	47.06	55.47	44.53	299.6±12.5	312.8±11.6
Lactone	0.1	65	75.38	60	64	36	66.67	33.33	312.1±14.2	309±15.6
Lactone	0.5	62	82.26	66.13	69.23	30.77*	75	25**	307.1±13.1	307.9±15.5
Lactone	1	61	77.05	50.82	63.64	36.36	77.78	22.22**	314.6±11.7	307.3±13.3
Lactone	2.5	69	73.91	65.22	59.09	40.91	95.83	4.17***	321.8±13.1	295.3±17.0

[#]in 200µl of paraffin oil. * P<0.05 ** P<0.01 *** P<0.001 (χ^2 test), C=control, T= treatment

Table 1: Behaviour of *G.m.m.* in a choice wind tunnel in which the racemic δ-octalactone and a blend of known attractant were dispensed

There were more flies contacting or hovering around the dispensers when the attractant was dispensed but fewer or none was observed around the dispensers when the lactone or blank were dispensed. During the experiments no fly was observed to contact a dispenser and remain on it throughout the observation period, although more flies hovered around the attractant-containing dispenser (Table 2).

Insects flying perpendicular to the wall characterized the escape behaviour, while U- turn involved flies changing their direction of flight by 180^0, usually by making downwind turns while in flight. Avoidance behaviour was considered as either a combination or indication of any of the two behaviours. The proportion of flies showing these behaviours gave a similar trend as the proportions making choice to either arm. The low number of flies showing avoidance behaviour coupled with the observed non significance in the final resting position led to the deduction that a dose of 0.1 mg/200 µl of the racemate does not show any significant repellent properties. The attractant recorded the lowest number of

flies showing avoidance behaviour followed by the blank and 0.1 while 2.5 mg/200 µl of the racemic material showed the highest (Table 2).

%Flies showing characteristic behaviours.

Odour dispensed	Dose (mg)#	N	% Flies activated	Escape behaviour		U turn behaviour		Avoidance behaviour		Contacting dispenser	
				C	T	C	T	C	T	C	T
Attractant	1	72	83.33	4.17	1.39	1.39	4.17	5.56	5.56	1.39	12.50
Blank	0	84	71.43	9.52	10.71	7.14	7.14	15.47	17.85	0.00	0.00
Lactone	0.1	65	75.38	3.08	10.77	3.08	7.69	6.16	15.38	4.62	1.54
Lactone	0.5	62	82.26	11.29	22.58	11.29	9.68	19.35	27.42	0.00	0.00
Lactone	1	61	77.05	6.56	16.39	3.28	21.31	6.56	27.86	3.28	1.64
Lactone	2.5	69	73.91	7.25	23.19	7.25	24.64	14.5	37.69	1.45	0.00

#in 200µl of paraffin oil. C=control, T= treatment

Table 2: Behaviour of G.m.m. in a choice wind tunnel in which the racemic δ-octalactone and a blend of known attractant were dispensed

From the analysis of the initial direction of flight and final fly resting positions coupled with escape, U-turn and avoidance behaviour the repellent activity of the racemic δ-octalactone has been demonstrated in a wind tunnel. The final fly distribution was found to be dependent on the chemical dispensed ($p<0.05$) and therefore the observed fly distribution was due to the dispensed odours. It can thus be inferred that the racemic lactone has an offensive property towards the flies making them stay away from the odour in significantly large proportions. The racemic lactone has therefore been shown to be a tsetse (*G .m. morsitans*) repellent at ≥0.5 mg/200 µl. However, the flight distance was not significant since there are other cues like heat gradient, colour and body movements that are necessary for the fly to locate a host and thus determine how far the insect has to fly during positive anemotaxis.

5.2 Bioassay of the (R)- and (S)-δ-octalactone

Having concluded the bioassay of the racemic mixture and shown that it is a repellent the assay of the two enantiomers was conducted. 3 day starved teneral *G. m. morsitans* flies were used. The dose of choice for the isomers was 1 mg/200 µl based on the fact that at a

comparable dose (2.5 mg/200 µl), the racemate showed repellence at >99% confidence limit. The results were compared to those of the attractant and blank.

5.2.1 The (S)-isomer

The proportion of flies activated averaged 74.60% while 60.32% left the mid-section. The initial direction of flight was biased to the treated (68.97%, $p<0.05$). Finally, 55.56% rested in the control arm compared to 44.44% ($p>0.05$) that rested in the treated arm (Figure 6).

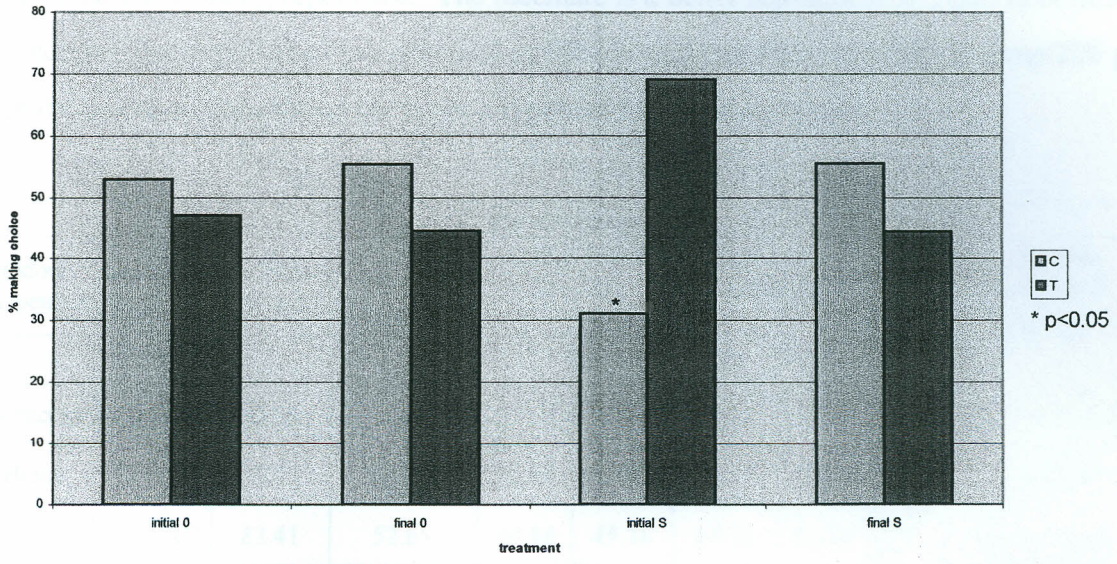


Figure 6: % Fly choice when the (S)-isomers was dispensed

The average maximum distance covered upwind by the flies in the control arm (297.1±14.00 mm) was not statistically different from that of the treated arm (277.9±11.71 mm) (Table 3).

The proportion of flies showing avoidance behaviour was found to be 13.33% in the control and 28.00% in the treated arm, while those that showed escape behaviour were 9.33% and 8.0% for the control and treated arms respectively. For those showing U-turn behaviour, 5.33% and 21.33% were observed in the control and treated arms, respectively (Table 4). No flies were observed to hover or contact the dispensers.

The proportion of flies making choice in the initial direction of flight showed attraction at the dispensed dose. However, the proportions showing characteristic behaviours did not support this observation. However, for the (S)- isomer based on the initial direction of flight, it shows attractancy although this could be due to the hunger status of the flies making them follow any host derived odour. However, a more detailed behavioural study is recommended. The final resting position results compared closely with those of the blank. However, it is clear that the activity of the (S)-isomer is not comparable to that of the racemate in evoking repellence. The racemate is a better repellent at an equivalent dose. Considering that repellence was expected at 99% confidence limit or better at 1 mg/200 µl as in the racemate, the (S)-isomer is not a repellent for *G. m. morsitans*.

				Fly distribution (%)						
Odour dispensed	Dose (mg)#	N	% Flies activated	% Flies leaving midsection	Initial flight direction		Final resting position		Average maximum upwind flight (mm) (± s.e)	
					C	T	C	T	C	T
Attractant	1	72	83.33	70.83	27.59	72.41*	58.82	41.18	321.1±13.0	294.1±15.8
Blank	0	84	71.43	64.29	52.94	47.06	55.47	44.53	299.6±12.5	312.8±11.6
R isomer	1	67	82.41	52.65	54.84	45.16	67.74	32.26*	276.8±13.8	306±14.3
S isomer	1	75	74.6	60.32	31.03	68.97*	55.56	44.44	297.1±14.0	277.9±11.7

#in 200µl of paraffin oil. * P<0.05 **P<0.01 ***P<0.001 (χ^2 test), C=control, T= treatment

Table 3: Behaviour of *G. m. m.* in a choice wind tunnel in which the isomers of δ-octalactone and a blend of known attractant were dispensed.

				Flies showing characteristic behaviours (%).							
Odour dispensed	Dose (mg)#	N	% flies activated	Escape behaviour		U turn behaviour		Avoidance behaviour		Contacting dispenser	
				C	T	C	T	C	T	C	T
Attractant	1	72	83.33	4.17	1.39	1.39	4.17	5.56	5.56	1.39	12.50
Blank	0	84	71.43	9.52	10.71	7.14	7.14	15.47	17.85	0.00	0.00
R isomer	1	77	82.41	11.94	10.45	10.45	19.40	19.40	25.37	0.00	1.49
S isomer	1	75	74.6	9.33	8.00	5.33	21.33	13.33	28.00	0.00	0.00

#in 200µl of paraffin oil. C=control, T= treatment

Table 4: Behaviour of *G. m. m.* in a choice wind tunnel in which the isomers of δ-octalactone and a blend of known attractant were dispensed.

5.2.2 The (R)- isomer

The proportion of flies activated was 82.41% while 52.65% left the mid section. The initial direction of flight was biased to the control (54.84%, $p>0.05$) while 45.16% flew to the treated though not statistically different. Finally, significantly more flies (67.74%, $p<0.05$) rested in the control arm compared to the treated arm (Figure 7).

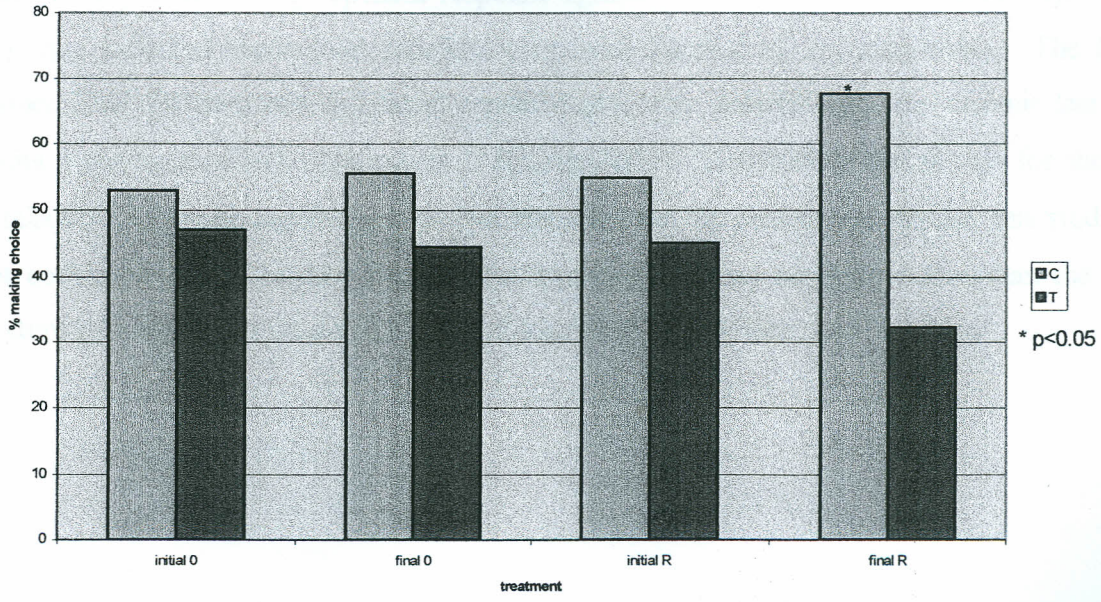


Figure 7: % Fly choice when the (R)- isomer was dispensed

The average maximum upwind distance covered by the flies in the control arm (276.8±13.78 mm) was not significantly different from that on the treated arm (306.0±14.26 mm). The proportion of flies showing avoidance behaviour in the treated arm was 25.37% compared to 19.40% in the control arm. Flies showing escape behaviour were 11.94% and 10.45% for the control and treated arms respectively. For U-turn behaviour, 10.45% and 19.40% were observed in the control and treated arms respectively. No flies were observed to hover around the treated dispenser (Table 4).

Since the isomer was synthesized at 60% ee, the observed mild repellency of the (R)-isomer could be linked to the presence of the antipode (1:4 S:R) as in the racemate. There

is need to synthesise this isomer in >98% ee and test it for repellence. From this work it can be deduced that the racemate is a better repellent than the (R)- isomer.

Conclusion.

From the obtained results it can be inferred that neither of the two isomers [(R)- and (S)- δ-octalactone] elicits strong repellent response against *G .m. morsitans*, independently. The (R)- has indicated some mild repellent properties (against *G. m. morsitans*). The (S)-isomer does not have any significant repellent property. Interestingly, the racemic lactone elicits a strong repellent response at ≥ 0.5 mg/200µl. It therefore seems that for the δ-octalactone to evoke repellence, the two isomers must be present. However, this study is not conclusive since another blend of the two isomers may be more active than the 1:1 mixture that has been tested in this work. More work is therefore recommended.

CHAPTER 6: CONCLUSION

From this study, an abbreviated synthetic route to the lactone (racemic and enantiomers) has been developed. The high yields coupled with the ability to control stereochemistry, make this route superior to the one reported earlier by Gikonyo *et al* 2002. The route can also be utilized in the synthesis of other analogues in good yields, purity and enantioselectively.

A new route to lactones from an anhydride has been abbreviated for the first time by use of Grignard reagent in a one-pot domino/tandem reaction to mono- or dialkylated lactones with the latter occurring in higher yields. The reaction mechanism for the formation of the δ-lactone from the anhydride needs further investigations.

The racemic δ-octalactone has shown potency as a tsetse (*G. m. morsitan*) repellent with the activity being dose dependent. It has been proved that both enantiomers of the lactone are required for the repellent activity.

The initial direction of flight is not useful in deducing repellence in the wind tunnel bioassay. However, the final resting position clearly indicates repellent activity especially when supported by avoidance, escape or U-turn behaviours. It is noted that the initial direction of flight is useful in determining the attractive property of an odour especially when supported by the flies hovering or contacting the dispensers. This is consistent with earlier observations by Gikonyo (1999), when allomonal blends were dispensed in a choice wind tunnel and compared with kairomones and synthetic attractants.

From this work, stereochemistry has been shown not to play a significant role in the potency of δ-octalactone as a tsetse fly repellent with the (R)- showing mild repellence (probably due to the presence of the antipode) and the (S)- giving no significant activity. The racemate showed a strong repellent property and therefore there is no need for stereoselective synthesis of the lactone for its exploitation as a means of controlling tsetse fly bites.

The repellent property of the racemic δ-octalactone has shown some dose dependence as evident in the increase in proportions and bias in the number of flies finally resting away from dispensers containing δ-octalactone with increase in dosage.

A dose of 0.5 mg/200µl of the racemate is an ideal repellent for *G. m. m* as it keeps them away throughout. This has been indicated by significantly different fly distribution in initial and final fly preferences, which was not observed in other doses of the lactone.

6.1 Recommendations for further work

Detailed behavioural study is recommended for the isomers (separately and as blends) to establish the blend(s) with the highest activity.

A study of the chirality of the natural allomone from the waterbuck volatiles in comparison with the synthetic ones is recommended in order to determine the natural enantiomeric composition of the allomone. This may be achieved by chiral GC analysis of the natural allomone.

Structure-activity studies on the lactone, to establish whether other forms of isomerism (diastereomerism) can improve the observed repellent activity. Along the same lines, analogues of the allomone should be synthesized and their activities investigated with the aim of improving the potency of the octalactone as a tsetse repellent.

The repellent should be formulated and tested in the field to determine the potentials of using it in the control of trypanosomosis.

CHAPTER 7.0: EXPERIMENTAL.

7.1 General Procedures

Glassware were soaked in chromic acid for at least 12 hours, washed thoroughly with water and finally rinsed with acetone before drying in an oven at 110 ^{0}C for at least 12 hours.

All solvents used were either Analar or HPLC grade and were used as supplied unless otherwise stated.

Reagents used had purities ranging from 95-100% and were used as supplied unless otherwise stated.

THF was dried before use by first passing through a column of alumina and then distilling over lithium aluminium hydride and stored under molecular sieve (5-8 mesh) in an amber bottle.

Diethyl ether was first passed through a column of alumina, then fractionally distilled under nitrogen and stored under molecular sieves (5-8 mesh) in a dark Winchester bottle.

Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} aluminium plates (5 x 10 cm, 0.2 mm film thickness). The TLC plates were visualised by spraying with 25% sulphuric acid in methanol and oven dried at 110 ^{0}C. Alternatively, plates were also observed under UV light (254 and 365 nm) before spraying.

Column chromatography (CC) was done using Merck silica gel 60 (0.04-0.063 mm, 230-400 mesh, Merck) and where necessary the silica gel was activated to grade III. Elution was done with solvents of varying polarities. Silica gel was used in the ratio of 1:30, sample: silica. Samples were loaded neat, in solution or adsorbed on a small amount of silica. Eluents were analysed by TLC and/or GC and those having similar R_f or R_t values pooled,

concentrated and stored or used as one sample. Several columns were used depending on sample size. Columns A (1 cm i.d); B (1.7 cm i.d) and C (2.4 cm i.d).

Melting point for pure solid samples were determined on Sanyo Gallenkamp apparatus and are uncorrected.

Gas chromatographys (GC) was performed on a Hewlett Packard (HP) 5890 series A GC (splitless injector mode) with a flame ionization detector (FID) linked to a HP 3393A integrator. Routine GC analysis was done using a HP-pona, medium polarity fused silica capillary column (50 m x 0.2 mm i.d.), coated with cross linked methylsilicone gum (0.33 μm film thickness) (column D) or DBM-5S non-polar column (9 m x 0.25 mm i.d.) coated with bonded and cross linked 5% phenylmethylpolysiloxane (0.25 μm film thickness) (column E). White spot nitrogen was used as the carrier gas with a flow rate of 0.42 ml/min. Temperature program was 50 °C (5 min) – 280 °C (5 min) @ 6 °C /min unless otherwise stated. Injection port temperature and detector temperatures were maintained at 270 °C. Chiral GC analysis was recorded on a similar instrument but using a J &W scientific β-dex 225 cyclodex column (30 m x 0.324 mm, 0.25 μm film thickness) containing 2,3-di-O-acetyl-6-O-TBDMS-β-cyclodextrin embedded in an intermediate polarity phase (column F). White spot nitrogen was used as the carrier gas at a flow rate of 0.55 ml/min. oven temperature programming was as in routine analysis but the final, injection port and detector temperature were maintained at 240 °C.

Mass spectrometry (MS) was performed on pure solid samples using the direct insertion probe (DIP) on a Fission Platform Mass Spectrometer operated at 70 eV and mass range set at 38-400 a.m.u.

Gas chromatography mass spectrometry (GC-MS) was performed on Fission platform mass spectrometer coupled to a Fission 8000 series gas chromatograph (GC). The GC was programmed as described earlier and helium used as carrier gas with source temperature at 180 ^{0}C and multiplier voltage at 1400V. The spectrometer was operated in the electron impact mode at 70 eV and spectra recorded as mass to charge ratio (m/z).

Nuclear magnetic resonance (NMR) spectra were recorded on Varian Mercury Gemini – 200 MHz spectrometer. ^{1}H and ^{13}C spectra were recorded at 200 and 50 MHz, respectively. Samples were dissolved in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as the internal standard. Chemical shifts were reported as δ values or parts per million (ppm) relative to TMS. The multiplicities of the NMR peaks are referred to as singlet (s), doublet (d), triplet (t), quartet (q), quintet (qin), sextet (sex) and multiplet (m). ^{13}C NMR spectra were recorded in the proton noise decoupled (PND) mode and the multiplicities determined from the distortionless enhancement polarization transfer (DEPT) experiments.

Insects;

Glossina morsitans morsitans pupae were collected from ILRI one week after oviposition and maintained at the ICIPE insectary at 12 hours light per day, 26±1^{0}C, 70±5% relative humidity until emergence. Newly hatched flies were sexed and the experimental groups starved while the rest were fed on pig blood through feeding membranes. Flies were hatched in a 20 x 15 x 15 cm cages and immediately after emergence, the experimental group was transferred to a cylindrical cage (25 cm diameter, 4 cm height) and kept at 26±1 ^{0}C and 70±5% relative humidity for three days before being used in bioassays. The rest were kept on pig blood through artificial feeding membranes.

6.2. Racemic synthesis

Condensation of crotonic acid (114)

Polyphosphoric acid (2.6 g) and crotonic acid (0.2 g, 2.35 mmol) were dissolved in 20 ml of xylene in a 50 ml 3-neck flask equipped with a magnetic stirrer bar, reflux condenser and a thermometer. The mixture was stirred and refluxed at 110 °C for 2 hours. The resulting yellowish-brown oily substance was poured into ice-cold water and shaken vigorously for 20 minutes before the reaction was quenched with $NaHCO_3$ until effervescence stopped. The mixture was extracted with ether (3 x 10 ml), the organic extracts combined, dried ($MgSO_4$) and concentrated *in vacuo*. TLC analysis showed 3 spots (R_f 0.33, 0.46, 0.57; 2:8 EtOAc: Hexane) with the major compound occurring at R_f 0.46. GC analysis (column E, 50 (5 min)-280 (2 min) @ 6 °C/min) showed 3 peaks (R_t 10.78, 12.23 and 12.36 min) with the major peak occurring at 10.78 min (84.22%). Column chromatography (column C) gave

0.094 g of the pure compound as confirmed by GC analysis (R_t 10.78). GC-MS analysis of sample revelead that the compound was the Diels-Alder product **150** Found: MS m/z 327 (13%), 281 (7%), 175 (5%), 174 (40%), 159 (100%), 146 (15%), 145 (15%), 131 (45%), 128 (10%), 115 (62%), 106 (15%), 103 (18%), 91 (10%), 78 (20%), 77 (22%), 69 (28%).

Similar results were obtained when the reaction was done at 65 °C. However, at 80 °C, another compound (R_f 0.91, GC R_t 15.28 min, column A, 60-180 (5 min) @15 ^{0}C/min-290 (5 min) @ 20 °C/min) was detected in the reaction mixture. Chromatography on column B gave 12 x 7 ml fractions, with 2-6 (R_f 0.9, R_t 10.77 min) giving the Diels-Alder product **150** while 8-12 (R_f 0.46, R_t 15.3 min) gave compound **151b** from condensation of the crotonic acid. Found: MS m/z 221 (5%), 220 (18%), 205 (100%), 189 (5%), 177 (20%), 161 (10%), 145 (25%), 141 (10%), 133(10%), 128 (15%), 115 (18%), 105 (22%), 91 (25%), 77 (22%), 57 (68%). GC-MS analysis also revealed the presence of compound **152** in smaller quantities. Found: MS m/z 220 (3%), 205 (9%), 174 (89%), 159 (100%), 146 (18%), 131 (35%), 115 (31%), 91 (29%), 77 (17%). The structures were not authenticated by other means since they were not the targeted. The structures are derived from MS data only.

Methyl glutarate (142)

Glutaric anhydride (13.68 g, 120 mmol) and methanol (5 ml) were placed in a 100 ml round bottom flask equipped with a magnetic stirrer bar and a reflux condenser. The mixture was stirred for 5 minutes after which 2 drops of 36% HCl solution were added. The reaction was left for 10 hours with vigorous stirring. To the mixture, water (40 ml) was added followed by brine (30 ml) and then vigorously shaken. The mixture was extracted with CH_2Cl_2 (3 x 20 ml), organic extracts combined and reaction quenched with sodium bircarbonate. The sample was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 11.38 g (65%) of methyl glutarate (**118**) as confirmed by GC (column B, R_t 32.36, 98.3%). Found: MS m/z 133 (M-CH_3), 115 (M-OCH_3, 13%), 101(M-CO_2H, 3%) 86 (M-CH_3CO_2H, 90%), 73 (M-$CH_2CO_2CH_3$, 20%), 60 (M-$C_3H_6CO_2H$, 58%), 58 (CO_2CH_3, M-$C_2H_4CO_2CH_3$, 52%), 45 (COOH, 88%), 42 (M- OMe –$C_2H_4CO_2H$). ^{1}H NMR ($CDCl_3$) δ 3.6 (3H, s, OCH_3), 2.4 (4H, t, H-2, H-4), 1.9 (2H, q, H-3).

Acyl halide (143) synthesis

The acid-ester (**142**) (11.3 g, 76.4 mmol) was dissolved in CH_2Cl_2 (5 ml) in a 25 ml round-bottom flask equipped with a magnetic stirrer bar, and a water bubbler. The solution was cooled in an ice bath and thionyl chloride (9.2 g, 78 mmol) added dropwise with vigorous stirring. The reaction mixture was further stirred for 3 hours at room temperature, water (50 ml) added and extracted with $CHCl_3$ (3 x 15 ml). The organic extracts were combined and washed with $NaHCO_3$ until effervescence stopped. TLC analysis of the organic layer showed a major spot at R_f 0.381 (2:1:1 n-C_6H_{14}:EtOAc:CH_2Cl_2). Concentration *in vacuo* and further by blowing with a gentle stream of nitrogen gave a white crystalline material that was recrystalized (acetone-water) to give 6.05 g (53%) of the acid chloride (**143**). Found: m.p. 78.5-81^{0}C; MS m/z 133 (M-OCH_3), 128 (M-HCl), 114 (M-50), 100 (M-COCl) and 87 (M-CH_2COCl); ^{1}H NMR δ. 1.24 (2H, t, H-3), 1.98 (2H, q, H-4), 2.44 (4H, t, H-2), 3.66 (3H, s, OCH_3).

Bis (triphenylphosphine)tetrahydroborato cuprous(1) (Sorrel's catalyst)

This was prepared according to Sorrel *et al,* (1978) in 76% yield. Found m.p. 164.5-166 °C.

The aldehyde (144)

To a stirred solution of the acyl halide (**144**) (0.645 g, 41 mmol) in 5 ml of acetone, 2.74 g (45 mmol, 10% excess) of Sorrel's catalyst was added in one portion followed by 15 ml of acetone. The suspension was then stirred for 1.75 hours, filtered and the residue washed with dry ether (6 x 5 ml). The solvent was removed *in vacuo* to give 0.584 g (12%)of the crude aldehyde as white crystals. Recrystalization (acetone-water) afforded the pure white crystals with m.p. 189-191^{0}C. Found: MS m/z 129 (M-H), 115 (M-CH_3, 100%), 100 (M-30), 87 (115-28), 86 (115-29), 101 (M-29), 74 (M-56); ^{1}H NMR δppm 1.24 (s, impurity), 2.03 (q,C3) 2.17 (s, impurity), 2.45 (q,C2 &C4), 2.75 (t, impurity), 3.68 (s, OMe), no peak was observed between 9-10 indicating no aldehyde was formed.

Alkylation of glutaric anhydride (118)

A clean 50 ml 3-neck flask was fitted with a pressure equalizing dropping funnel, magnetic stirrer bar and all outlets covered with suba seals. The set up was flamed to dryness under a

gentle steam of white spot nitrogen, allowed to cool and the gas stream replaced with a balloon filled with nitrogen. Propylmagnesium chloride (4.4 ml, 2.0 M solution in ether, 8.8 mmol) was placed in the flask. Glutaric anhydride (1.0 g, 8.8 mmol) in 10 ml of dry ether was placed in the dropping funnel and added dropwise (10 minutes) while cooling the flask in an ice-water bath. The reaction mixture was stirred at room temperature for a further 30 minutes then poured into crushed ice made from 150 ml of distilled de-ionised water. A solution of 30% HCl (10 ml) was added and the mixture vigorously shaken for 5 minutes. The solution was extracted with ether (3 x 30 ml) and the combined organic extracts quenched with $NaHCO_3$ until effervescence stopped. The organic extract was washed with water (2 x 5 ml), separated and dried over $NaSO_4$. TLC analysis showed traces of the starting material alongside three other spots (R_f 0.75, 0.69, 0.59). GC analysis (column E) showed 3 peaks (R_t 7.56, 10.31 and 16.32 min). The mixture was concentrated *in vacuo* and subjected to fractionation by column chromatography (column B) to give 67 x 3 ml fractions. Fractions 35-40 contained the δ-octalactone alongside an impurity. The lactone (43.7 mg, 3.5%) was obtained by further fractionation (column A) to give a 98% GC pure sample. The identity of the lactone was confirmed by GC, (R_t 24.51 min, column D), GC co-injection, GC-MS and MS library spectral matching. Found: MS m/z 142 (M^+, 2%), 114 (M-CO, 10%), 99 (M-C_3H_7, 100%), 86 (5%), 71 (M-1-C_3H_7CO, 50%), 70 (M-C_3H_7CO, 47%).

1-Propylcyclopentan-1-ol (139)

A clean oven dried 50 ml three-neck flask was fitted with a pressure equalizing dropping funnel, a magnetic stirrer bar and all outlets covered with suba seals. Using needle inlets and outlets the flask was flamed under dry nitrogen. The flask was allowed to cool and the nitrogen stream replaced with a N_2 filled balloon. Propylmagnesium chloride (5 ml, 2 M in ether, 10 mmol) was transferred into the flask using a dry syringe. This was allowed to stir for 10 minutes after which cyclopentanone (**124**) (0.9 ml, 10 mmol) in 10 ml of ether was added dropwise from the funnel for 10 minutes while cooling the flask in an ice-water bath. The reaction mixture was allowed to stir for a further 30 minutes at room temperature after which it was poured into crushed ice (from 200 ml of distilled de-ionised water). A 30% solution of HCl (10 ml) was added and mixture extracted with dry ether (3 x 20 ml). To the

combined organic extracts, $NaHCO_3$ was added until effervescence stopped. The organic extract was washed with water (10 ml), organic layer separated and dried over anhydrous sodium carbonate. TLC analysis showed a major spot at R_f 0.72 (2:8 EtOAc: n-C_6H_{14}) and a major GC peak at R_t 17.38 min (Column E). The sample was subjected to purification by column chromatography on column A while eluting with n-hexane. Fractions (34 x 3 ml) were collected with 18-24 showing a single spot at R_f 0.72 (2:8 EtOAc: n-C_6H_{14}) and corresponding GC peak at R_t 17.6 min. GC-MS, ^1H and ^{13}C NMR analysis indicated this to be the alcohol of interest. These were pooled and solvent blown off with nitrogen while cooling the sample in ice to give 1.012 g (79%) of the alcohol (**139**). Found: MS m/z 128 (M^+, 2%), 127 (M-H, 5%), 111 (M-OH, 8%), 110 (M-H_2O, 11%), 95 (M-H_2O-CH_3), 85 (M-C_3H_7, 100%), 86 (M-H- C_3H_7, 42%); ^1H NMR ($CDCl_3$) δ 0.91 (3H, t, H-3') and 1.4-1.5 (10H, m, all CH_2);. ^{13}C NMR ($CDCl_3$). δ 15 (C-3'), 18.2 (C-2'), 24.0 (C-4, C-3), 39.9 (C-2, C-5), 44.1 (C-1'), 82.9 (C-1).

Propyl-1-cyclopentene (121)

The alcohol (**139**) (1.0 g, 8 mmol) and 2 drops of sulphuric acid (98%) were mixed in a pear-shaped flask (25 ml). The mixture was shaken vigorously (10 minutes), water (10 ml) added portion wise and the organic layer separated. The aqueous layer was extracted with ether (3 x 5 ml), organic extracts combined and reaction quenched with $NaHCO_3$ until effervescence stopped. The mixture was filtered, dried ($MgSO_4$) and solvent removed by a gently flowing nitrogen stream. The concentrate was subjected to column chromatography (column B) to give 0.95g (94%) of the alkene. Purity (97%) was determined by GC (R_t 13.4 min, column E) analysis. A GC peak at R_t 13.92 min (3%) was attributed to the *exo*-cyclic isomer (**121b**) that was epimerized to the *endo*- by refluxing the reaction mixture in presence of catalytic amounts of *p*-toluene sulphonic acid (5 mg) for 6 hours (Iura, 2000). The reaction was monitored by GC analysis. Completion was confirmed from the complete disappearence of the GC peak at R_t 13.92 min. The reaction was subsequently quenched by addition of 5ml of water and $NaHCO_3$ until effervescence stopped. The organic layer was separated, dried ($MgSO_4$), filtered and solvent removed to give 0.95 g (93.9%) of the desired alkene (**121a**). Found; MS m/z 110(M^+, 43%), 95 (M-CH_3, 15%), 82 (McLafferty rearrangement, 10%), 81(M- C_2H_5, 60%), 67 (M- C_3H_7, 100%).

Hydrobromic acid, *o*-polyphosphoric acid, polyphosphoric acid (PPA, mixture of isomers) and HCl were also tried using the same procedures.

2-Propylcyclopentanone (120)

In a dry (flamed) 3-neck flask (100 ml) equipped with a pressure equalizing dropping funnel (100 ml) and a condenser coupled to a $CaCl_2$ guard under a gentle stream of nitrogen, dry ether (15 ml), the alkene (**121a**) (2.75 g, 25 mmol) and 0.21 g (14 mmol) of lithium borohydride were mixed. A solution of BF_3.OEt (0.4 ml, 1.2 equiv.) in dry ether (5 ml) was placed in the funnel. The BF_3.OEt solution was added dropwise (15 min) to the mixture while cooling in ice bath and maintaining the temperature at 25-30 °C. Distilled de-ionised water (5 ml) was added slowly followed by dropwise addition (10 min) of 9 ml of chromic acid (prepared from 11 g $Na_2Cr_2O_4$, 8 ml 98% H_2SO_4 and diluted to 45 ml with water) while the temperature was maintained at 25-30 °C by cooling in ice-water bath. The resulting dark brown mixture was refluxed for 10 hours and the ether layer separated. The aqueous layer was extracted with ether (2 x 10 ml), the organic extracts combined, washed with brine and quenched with $NaHCO_3$ until effervescence stopped. The organic layer was separated, dried ($MgSO_4$) and the ketone purified by fractional distillation *in vacuo* (~ 11 mm Hg) to give 3 fractions (b.p. 23°, 28° and 35°C). The third fraction was examined by GC-MS and found to contain 88% of the ketone and 11% of the starting material. The residual straw-coloured paste was washed with (2 x 5 ml) pentane and washings examined by GC-MS. The first washing contained the pure ketone (100%) while the second contained 58%. The third fraction and the second washing were separately subjected to column chromatography (column A) to give the pure ketone (GC, R_t 22.17 min, 100%, column D). The combined product was availed in 68% yield (2.15 g, 17.08 mmol). Found: MS m/z 126 (M^+, 5%), 111(M- CH_3, 5%), 97 (M- CH_2CH_3, 5%), 84 (M+1- $CH_2CH_2CH_3$, 100%), 83 (M- $CH_2CH_2CH_3$, 30%), 69 (M- $CHCH_2CH_2CH_3$, 15%), 55 (M- $CH_2CHCH_2CH_2CH_3$, 60%).

δ-Octalactone (10)

To a solution of mCPBA (2.6 g, 15 mmol, 1.25 equiv) in 10 ml of dry $CHCl_3$ in a 3-neck round-bottomed flask (250 ml) fitted with a thermometer, magnetic stirrer bar, reflux condenser and pressure equalizing dropping funnel, 1.5 g (12 mmol) of the ketone (**120**) in

5 ml $CHCl_3$ was added drop wise (10 min) and the reaction mixture refluxed at 56 °C for 48 hours with gentle stirring. The reaction mixture was cooled in an ice bath and the white precipitate obtained filtered off, the filtrate concentrated *in vacuo* and dissolved in ether (50 ml). The ether solution was washed with 10% $NaHCO_3$ (4 x 10 ml). The organic extract was separated, washed with brine (10 ml), dried ($MgSO_4$), filtered and concentrated to give quantitative yield of the crude product. GC (Column E) analysis showed a minor (R_t 22.31) and a major (R_t 24.48 min) peak. The sample was chromatographed on column A to give 43 x 3 ml fractions. Fractions 16-22 were found to contain the target lactone (GC, R_t 24.48 min, column D). These were pooled and solvent removed to avail 1.65 g (11.62 mmol, 96.8% yield) of the δ-octalactone (10). The sample was authenticated by GC co-injection. The product was analysed by chiral GC (R_t 26.10 and 26.15 min, column F) and shown to have two close peaks in the ratio of 1:1 but without baseline resolution. Found: MS m/z 142 (M^+, 5%), 114 (M-CO, 10%), 99 (M-$CH_2CH_2CH_3$, 100%), 71 (M+1-CH_2CH_2- CO_2, 50%), 70 (M-$CH_2CH_2CH_3$-CO_2, 47%). ^1H NMR ($CDCl_3$) δ 0.9 (3H, t), 1.4 (8H, m), 2.4 (2H, t), 4.2 (1H, m). ^{13}C NMR ($CDCl_3$) δ 13.85 (C-8), 18.17 (C-7), 18.49 (C-3) 27.77 (C-6), 29.46 (C-4), 37.86 (C-2), 80.36 (C-5).

Chiral synthesis

(-)-Isopinocampenyl borane (149b)

This was prepared according to Brown *et al* (1982a). Briefly, in a 100 ml flask equipped with a magnetic stirrer bar, was placed 22.0 ml of 2.27 M of borane-THF complex (50 mmol) and 41.4 ml of dry THF to give of 0.7 M solution with respect to borane. (+)-(α)-Pinene (8.0 ml) was added and the reaction mixture stirred for 96 hours. GC analysis of the product indicated >90% of (-)-$IpcBH_2$ (**149b**) [R_t 14.65 min, column D, 50 (2 min)-290 (20 min) @ 10 °C/min], 5% Ipc_2BH (R_t 27.23 min) and traces of pinene (R_t 7.30 min).

(+)-Isopinocamphenyl borane (149a)

To prepare (+)-$IPCBH_2$, a similar procedure was followed as for the antipode but (+)-(α)-pinene was substituted with (-)-(α)-pinene. GC analysis gave a major peak at R_t 14.55 min (column D, 50 (2 min)-290 (20 min) @ 10 °C/min).

S-Propylcyclopentanone (S-120)

In a 250 ml 3-neck flask equipped with septum inlet, magnetic stirrer bar, dropping funnel and reflux condenser coupled to a tube leading to an oil bubbler, 2 ml (10 mmol) of (-)-$IpcBH_2$ (95%) in THF was added. The mixture was cooled to -25^{0}C (dry ice-methanol) and 1.5 ml (10 mmol) of the 1-propylcylopentene (**121a**) in THF (5 ml) added dropwise (10 minutes) from the dropping funnel while stirring. The mixture was stirred for 9 hours at that temperature after which ether (20 ml) was added and the reaction mixture allowed to warm to room temperature. Chromium trioxide-sulphuric acid solution (10 ml) (1g of chromium trioxide in 5 ml of 98% sulphuric acid and 5 ml of water, PH<3) was added dropwise (15 minutes) via the dropping funnel while maintaining the temperature at 25-30 ^{0}C by cooling in an ice-water bath. The acidic aqueous layer was washed with ether (2 x 15 ml) and the organic extracts combined, washed with 5 ml brine solution and $NaHCO_3$ added until effervescence stopped. The sample was then dried over anhydrous $MgSO_4$, filtered and purified by fractional distillation *in vacuo* to give 3 fractions. Fraction 2 was found to contain a major peak at R_t 22.12 minutes (56.61%) on GC analysis (Column D) and was subjected to column chromatography (SiO_2 grade III, column A). Fractions (35 x 2 ml) were collected and 13-17 found to contain the pure ketone (**S-120**) from GC analysis (R_t 22.12 min). These were pooled and solvent removed to give 0.577 g (46%) of the ketone. The sample was authenticated by GC co-injection. The spectral data was same as the racemic material.

(R)-Propylcyclopentanone

This was prepared as the antipode but using the (+)-$IpcBH_2$ as the hydroborating agent. The reaction was carried out at 10 mmol ratio and gave the (R)- ketone (**R-120**) at 38% yield (0.47 g). The sample was authenticated by GC co-injection. Spectral data was same as the racemic material.

(S)-δ-Octalactone (S-10)

The (S)- lactone was prepared in exactly the same way as the racemate except that for the (S)- ketone (0.5 g, 4 mmol), mCPBA (0.92 g, 5 mmol, 1.2 equiv.) was used to give 0.54 g (95%) of the product. Chiral GC analysis (column F) revealed two non-baseline resolved

peaks at R_t 26.65 (1.11%) and 26.82 (98.89%) min. The spectral properties were similar to those of the racemic material.

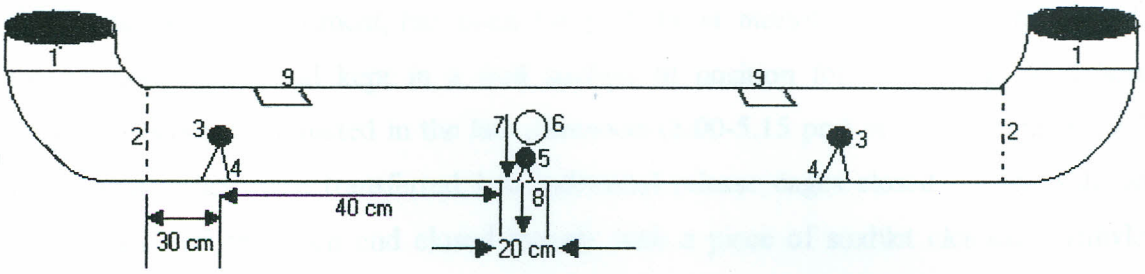
R-δ-Octalactone (R-10)

This was prepared in the same way as the S-isomer but using the (R)- isomer of the ketone and the antipode of the chiral auxiliary at 3.4 mmol ratio to give 0.464 g (96.2%) yield. Chiral GC analysis revealed two non-baseline resolved peaks at R_t 26.69 (80%) and 26.84 (20%) min. The spectral data was similar to that of the racemic lactone.

6.5. Bioassays

Behavioural bioassay

Behavioural bio-assays were carried out in a cylindrical plexiglass tunnel (180 cm long, 24 cm internal diameter), connected on both ends to charcoal (mesh 8-20) polyvinyl chloride (PVC) air filters (Figure 8). At the centre was a vent connected to an extracting fan via a PVC pipe, this divided the tunnel into two equal parts with a 20 cm wide central zone. The vent was covered with a wire mesh on which a short metallic stand was placed – for supporting the fly release cage. At the mid-section, air from both arms mixed. The upwind ends of the tunnel were sealed off with a PVC mesh, which acted as the barrier for the flies going upwind past the dispensers. This defined the length of the tunnel. On each arm, a window (15 x 10 cm) was made for sample introduction while at the mid-section a circular hole (4.7 cm diameter) was made for introduction of flies in release cages (3 cm diameter, 4 cm long). Two metallic racks were placed 400 mm upwind for holding sample dispensers. The tunnel was lit using fluorescent bulbs connected to an intensity regulator and light passed through fibreglass filter sheet placed 35 cm above the tunnel. The tunnel was placed on metallic stands approximately 80 cm above the ground, on which was laid a white sheet of paper with black stripes marked about 200 mm apart to offer contrast to the flies during flight. On the edge, away from the observer, the distances were marked out from the end of the mid-section. The wind speed in the tunnel was adjusted to 10 cm/sec, while the bio-assay room was maintained at 26±1^{0}C and 65±5% relative humidity.



1. activated charcoal
2. PVC gauze
3. odour dispenser
4. mettalic rack
5. insect release cage
6. window for introducing insect release cage
7. mettalic wire mesh
8. air flow
9. window for introducing odour dispenser

Figure 8: Diagram of the wind tunnel

The test odour consisted of the synthesised compounds (racemic mixture, the (R)- and the (S)- isomers, or an attractant) suspended in neat paraffin oil. Various doses were made from a stock solution of 10 mg/200 µl by serial dilution. In each experiment doses of 2.5, 1, 0.5 or 0.1 mg/200 µl were tested against a control containing an equivalent volume of the paraffin oil. For the blank, 200 µl of the neat oil was dispensed from both arms of the tunnel. The attractant was made from commercially available compounds (*p*-cresol, acetone and 1-octen-3-ol, which are known tsetse attractants, in the ratio 4:100:1 respectively). The attractant was dispensed at a dose of 1 mg/200 µl against a control.

Odour dispensers were made of clean black pieces of cloth tied on the open end of a plexiglass tube (5 cm long and 4.5 cm diameter) using methylene chloride soxhlet-cleaned elastic rubber bands. Through the windows on each arm of the tunnel, the dispensers were placed onto a metallic rack positioned 400 mm from the mid-section (upwind) such that they were mid-height of the wind tunnel with the cloth facing downwind. The test odour in paraffin oil was pipetted onto the centre of the cloth on one dispenser while onto a second dispenser, an equal volume of paraffin oil was pipetted to act as a control. With all windows tightly closed and the extracting fan on, the tunnel was allowed to equilibrate for one minute before the test fly was introduced.

On the day of the experiment, the unfed 3-day old *G. m. morsitans* flies were transferred to the bio-assay room and kept in a well sunlight-lit position for a minimum of 6 hours. Experiments were conducted in the late afternoon (3.00-5.15 pm) or early mornings (9.00-11.00 am). Flies were transferred into individual release cages closed on one side with PVC gauze and the open end closed loosely with a piece of soxhlet cleaned (methylene chloride) cotton wool. Using a forceps, the release cage containing a calm fly was placed cautiously in the middle of the tunnel (on a metallic rack) with the cotton wool plugged end facing the observer. The cotton wool was gently removed without disturbing the fly and the window tightly closed to avoid any air inflow. The behaviour of each fly was observed and recorded for 3 minutes after which the fly was aspirated using a vacuum pump. Behaviours recorded included; activation (scored when the fly showed any movement) initial direction of flight, escape behaviour (characterised by flying perpendicular to the tunnel wall), hovering around or contacting (touching or landing) the dispensers, U-turn (turning at 180° while in flight), distance covered during anemotaxis, walking and grooming.

Data analysis

Statistical analysis for differences in observed behaviours, were performed non-parametrically (chi square) and where applicable parametrically (t-test). Comparisons of proportions of tsetse fly distribution between the treated and the control arms of the tunnel were done using chi-square (SAS version 8e, 2000). Average maximum upwind distance covered by each fly was analysed parametrically while the direction of flight and final fly distribution were analysed non-parametrically.

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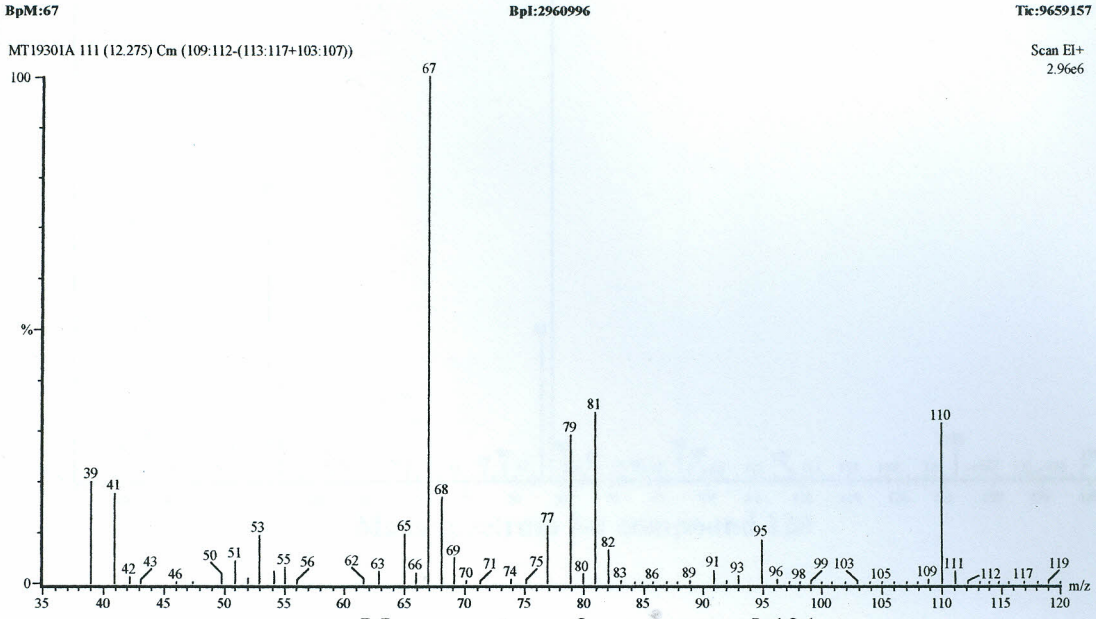
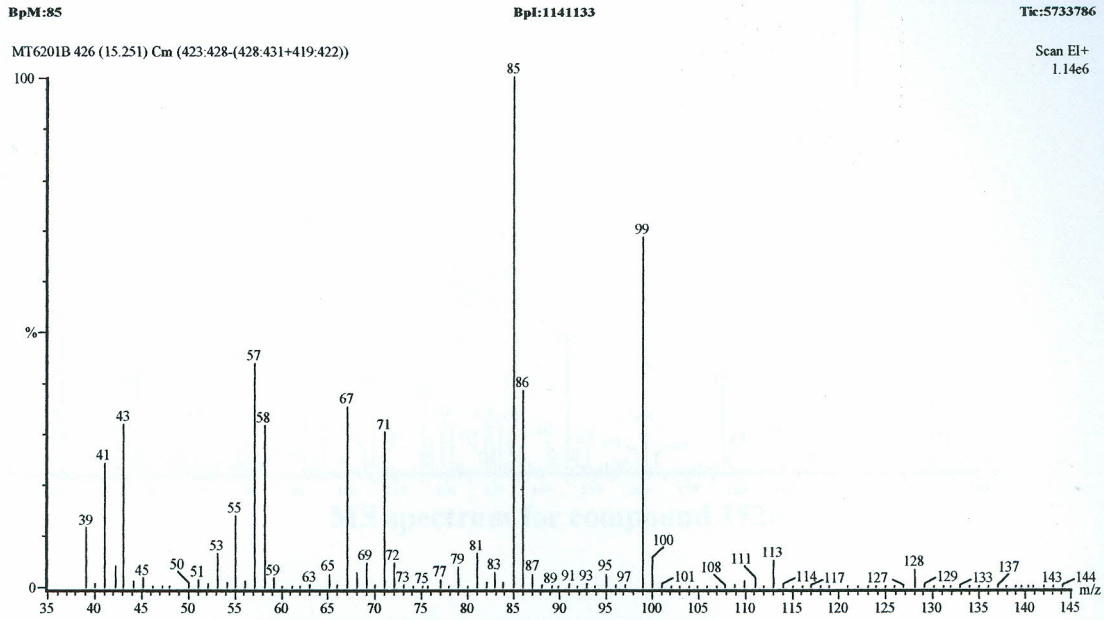
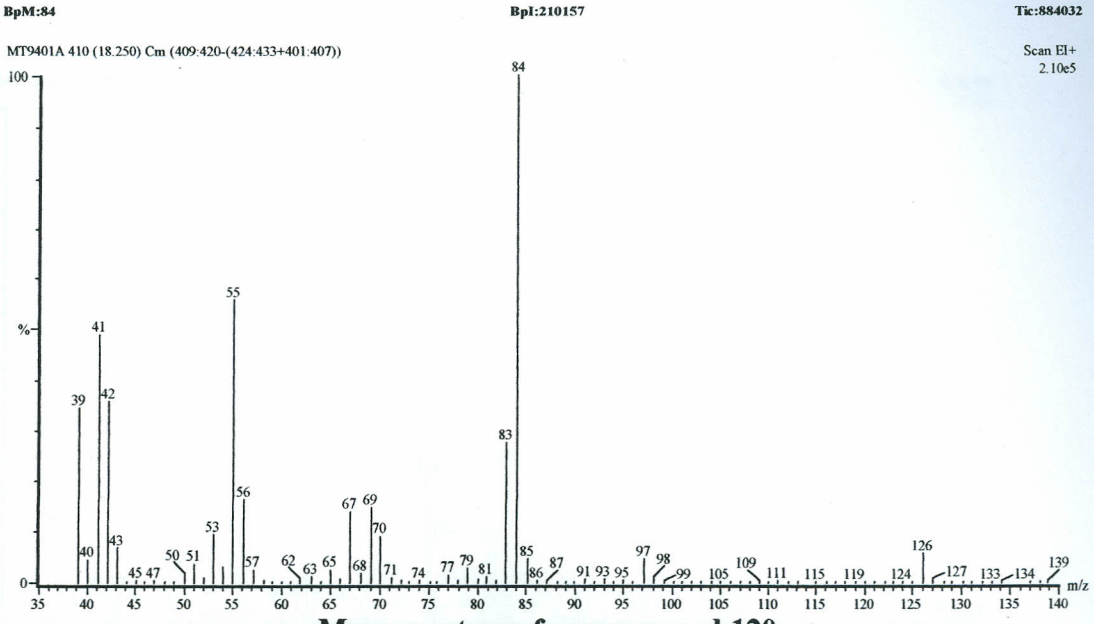
APPENDICES



Mass spectrum for compound 121

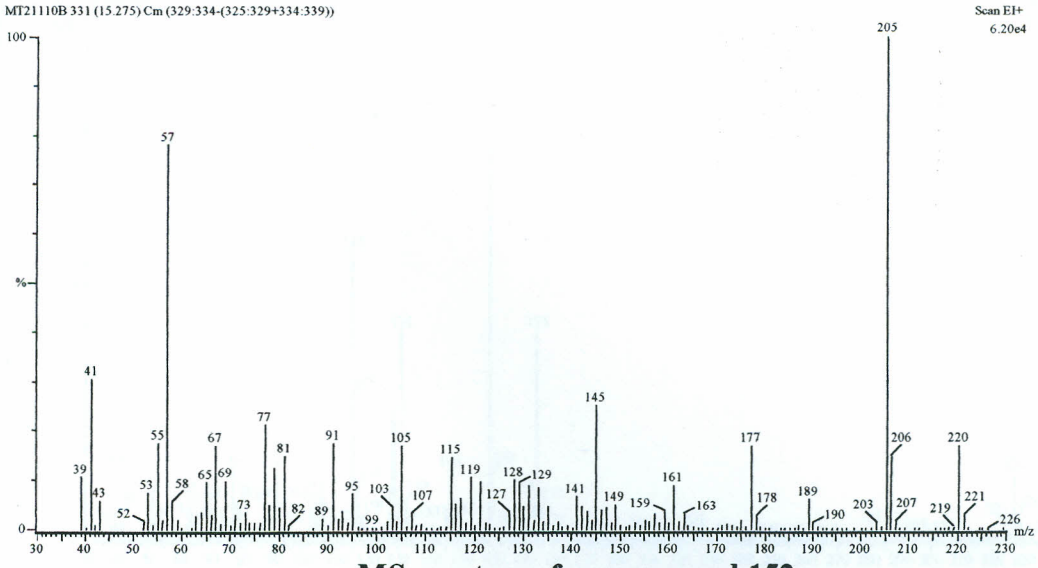


Mass spectrum for compound 139



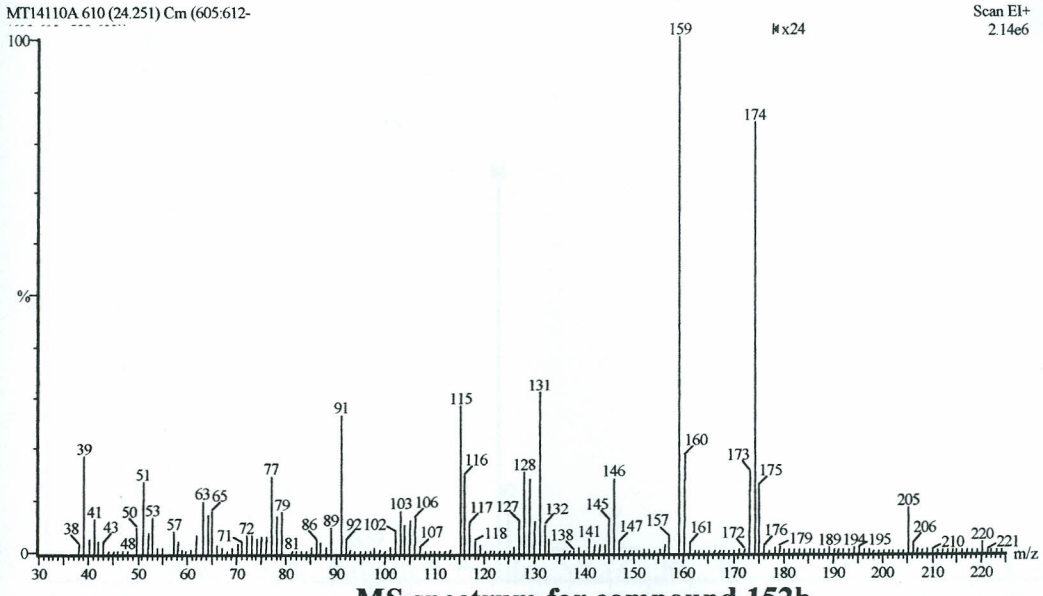
Mass spectrum for compound 120

B

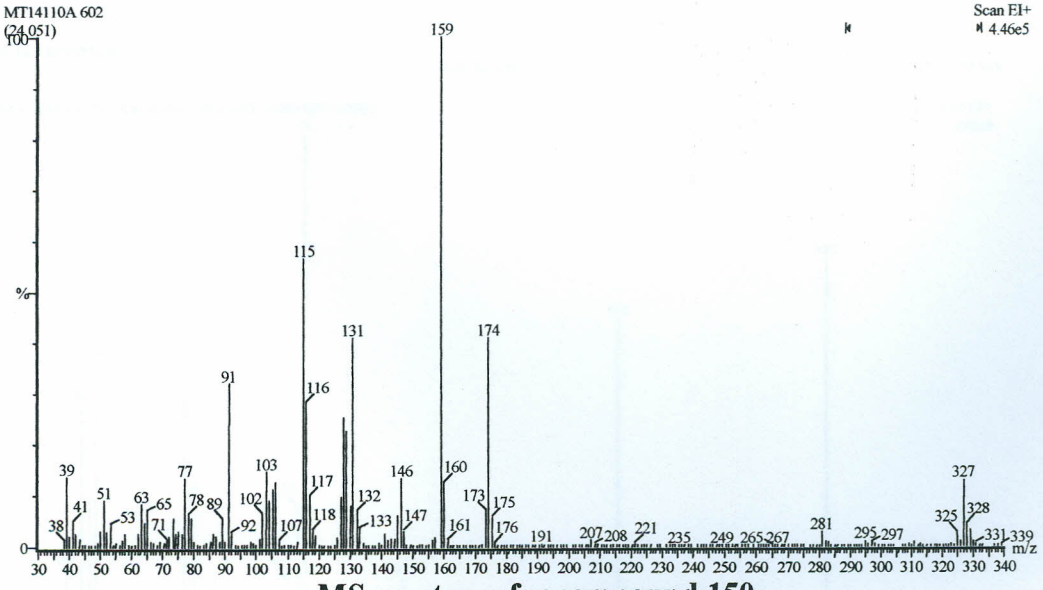


MS spectrum for compound 152a

I

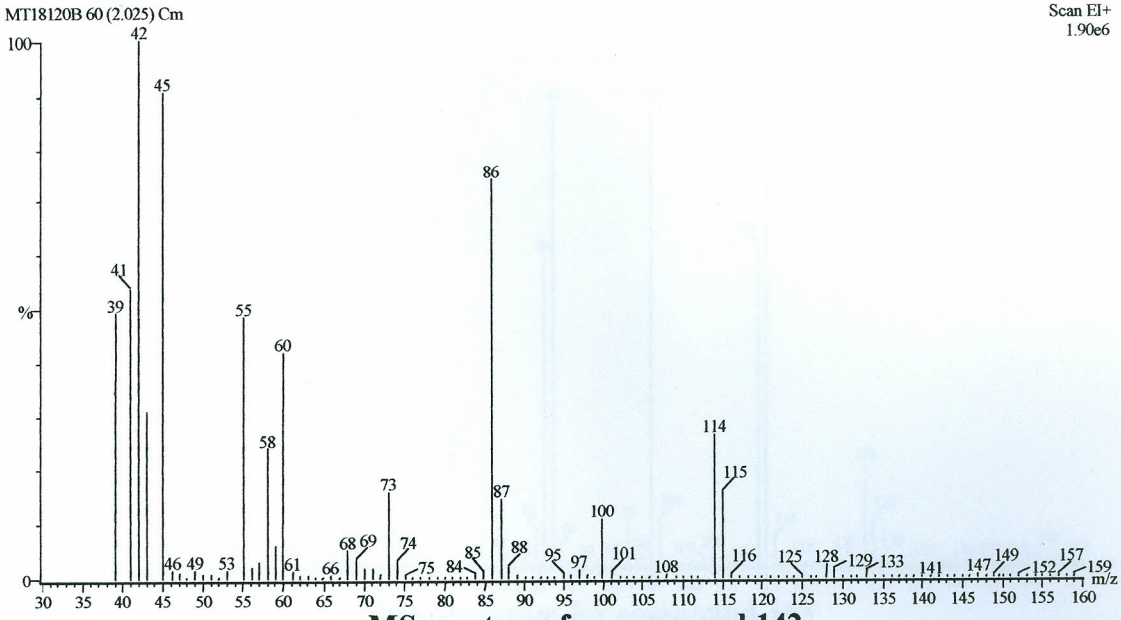


MS spectrum for compound 152b

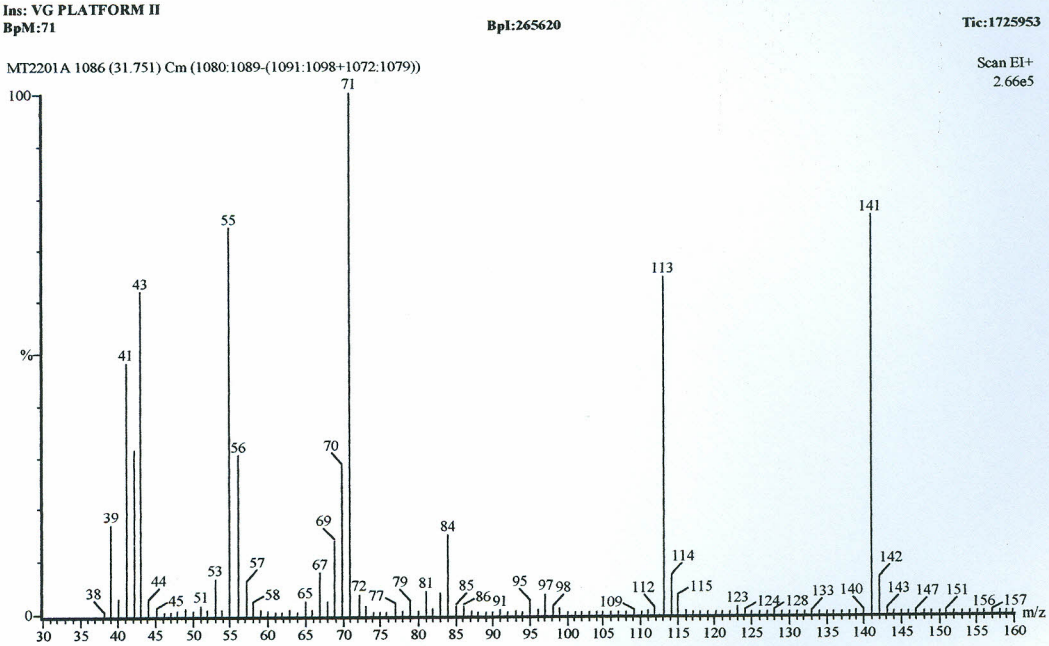


MS spectrum for compound 150

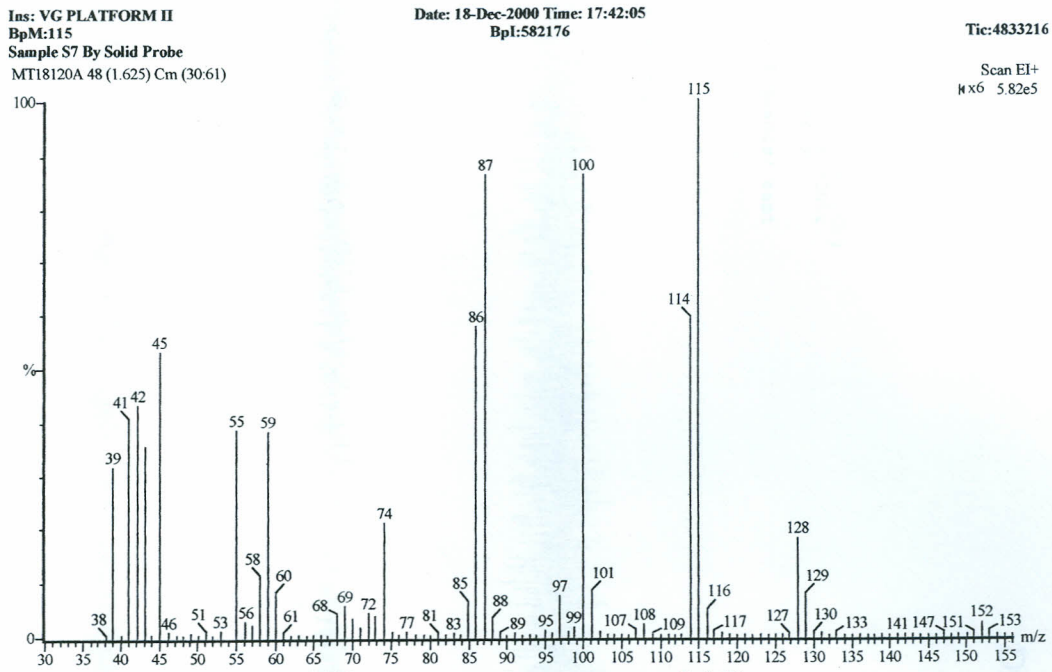
I



MS spectrum for compound 142

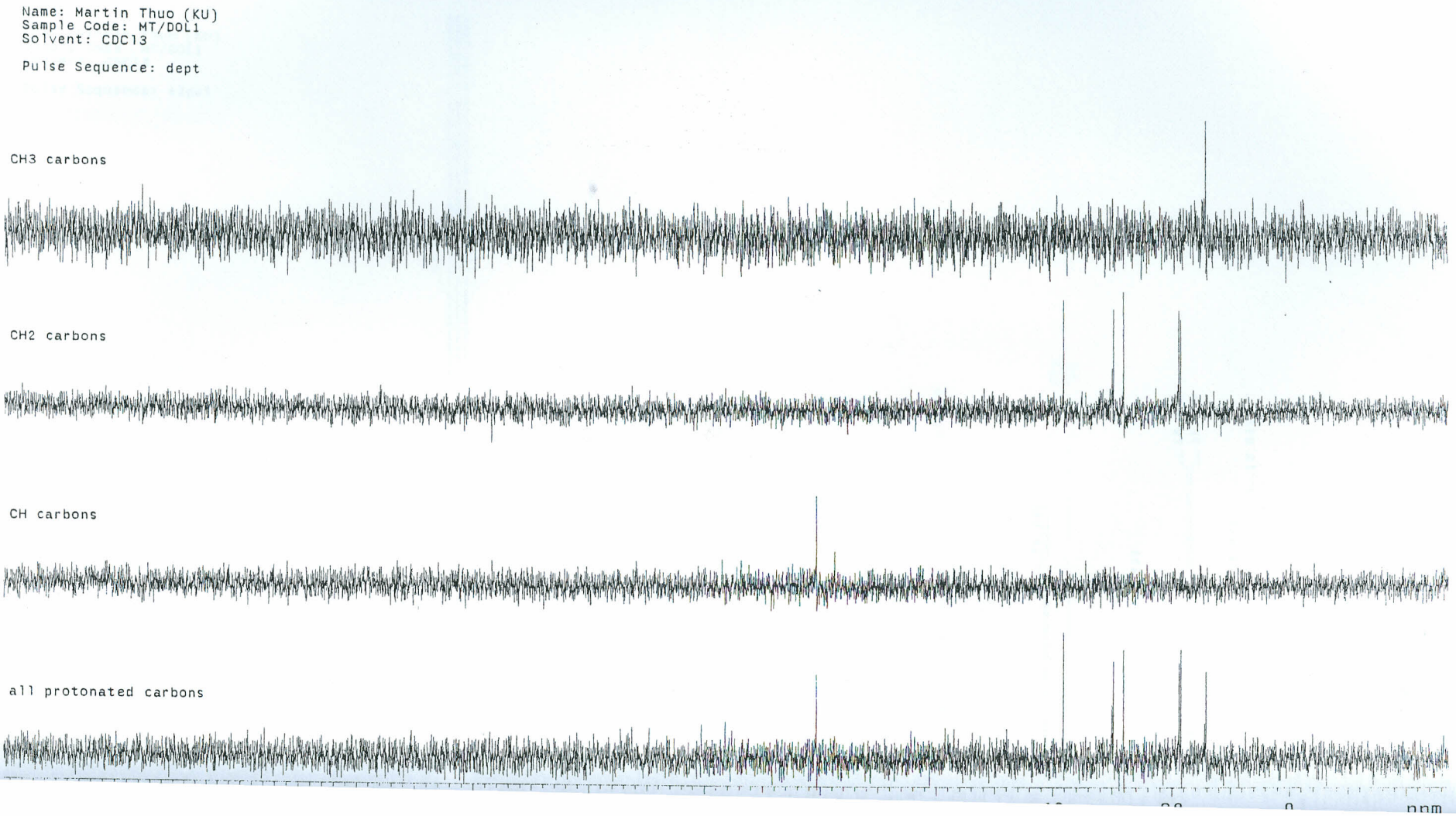


MS spectrum for compound 146

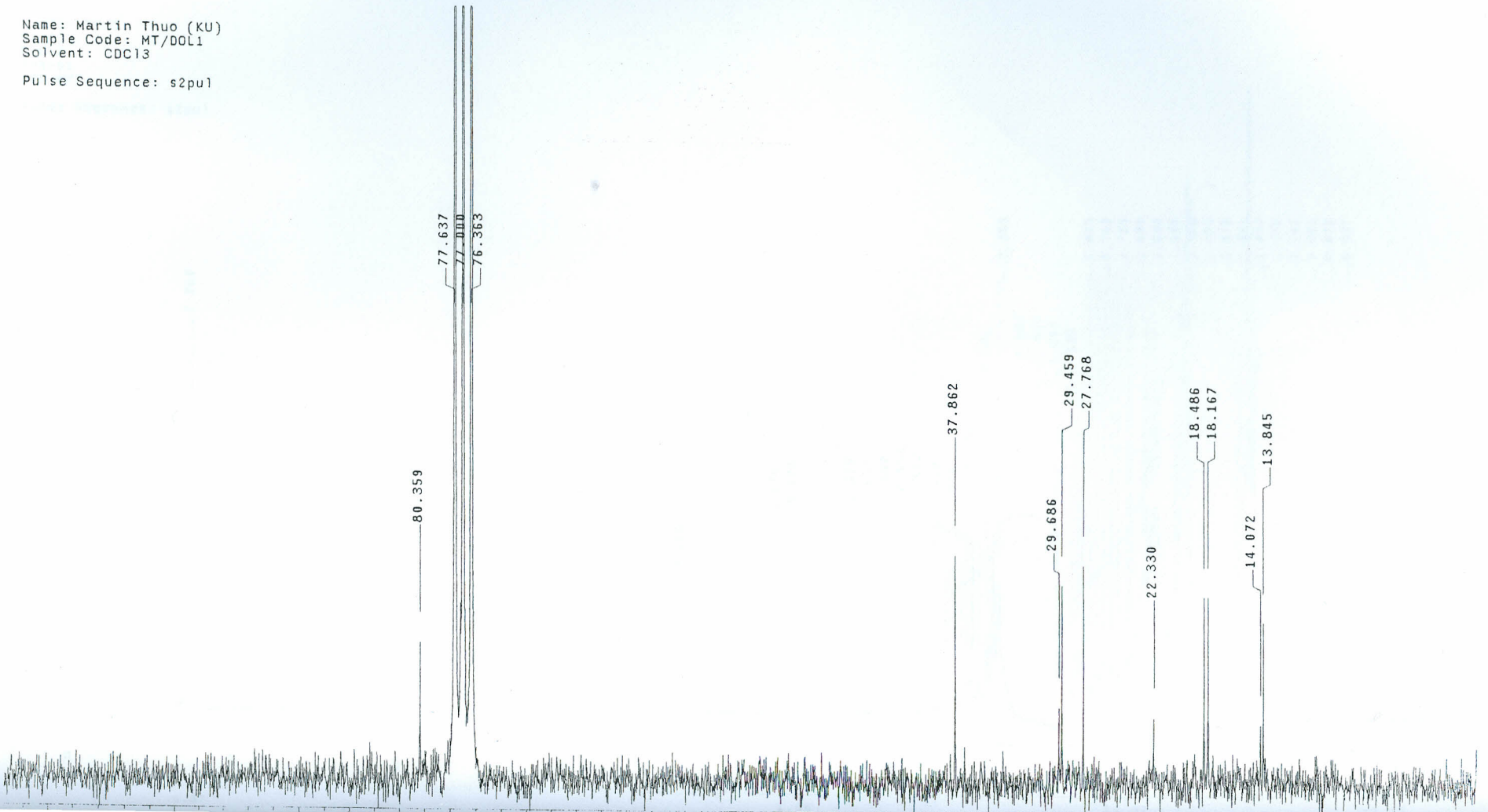


MS spectrum for compound 143

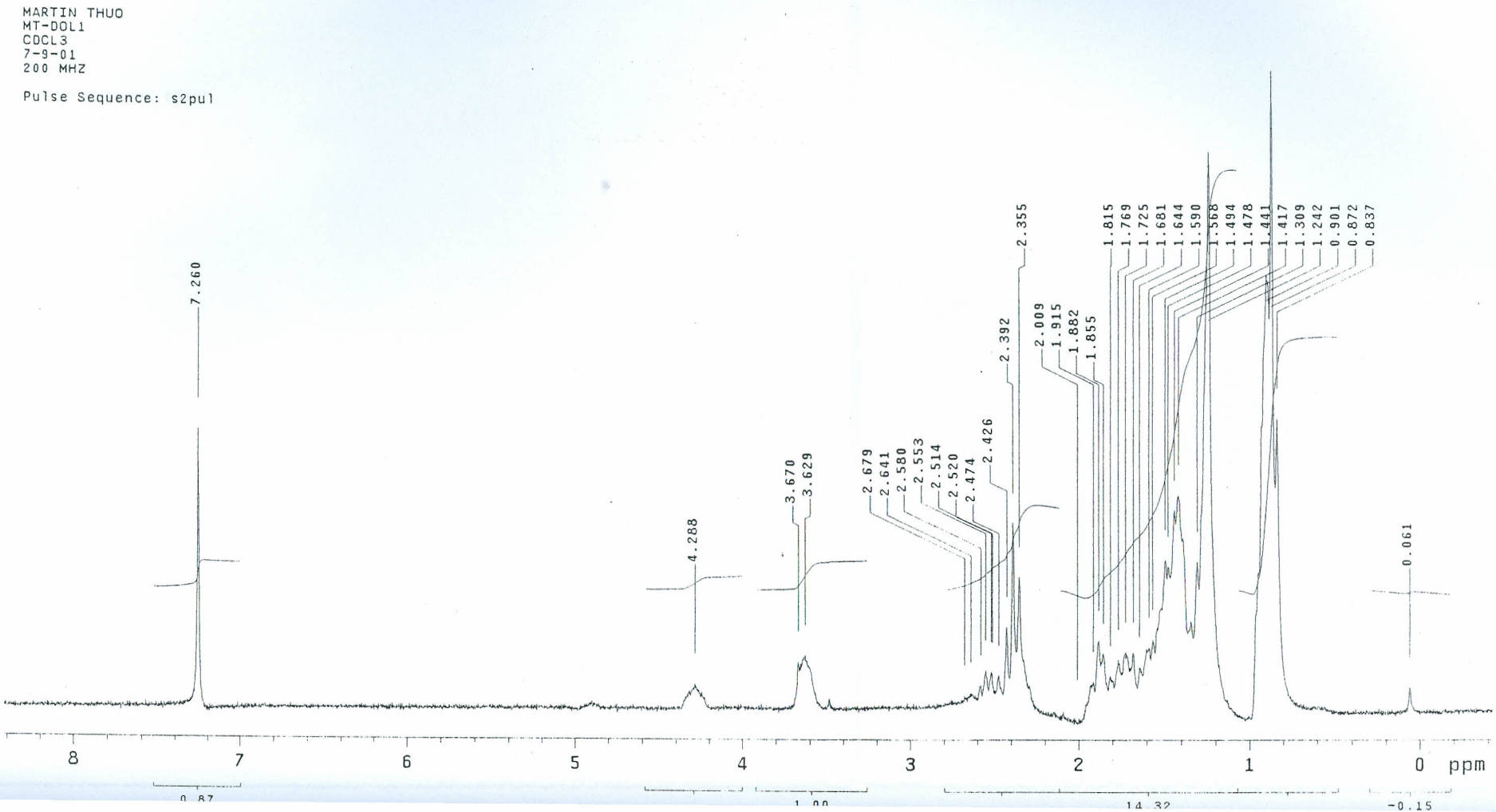
DEPT NMR spectrum of compound **10**.



^{13}C NMR spectrum of compound **10**.



^{1}H NMR spectrum of compound **10**.

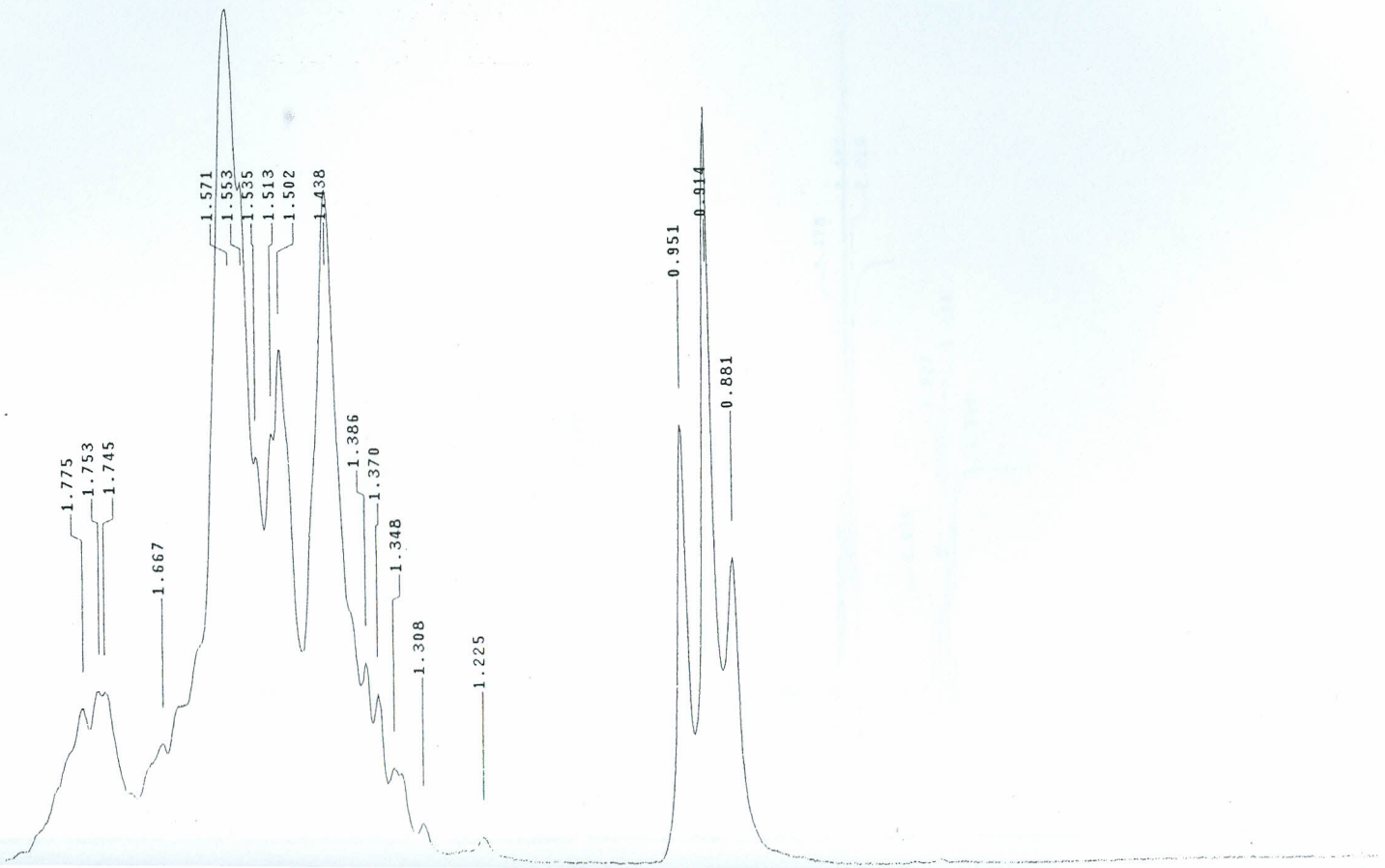


^{13}C NMR spectrum of compound **139**.

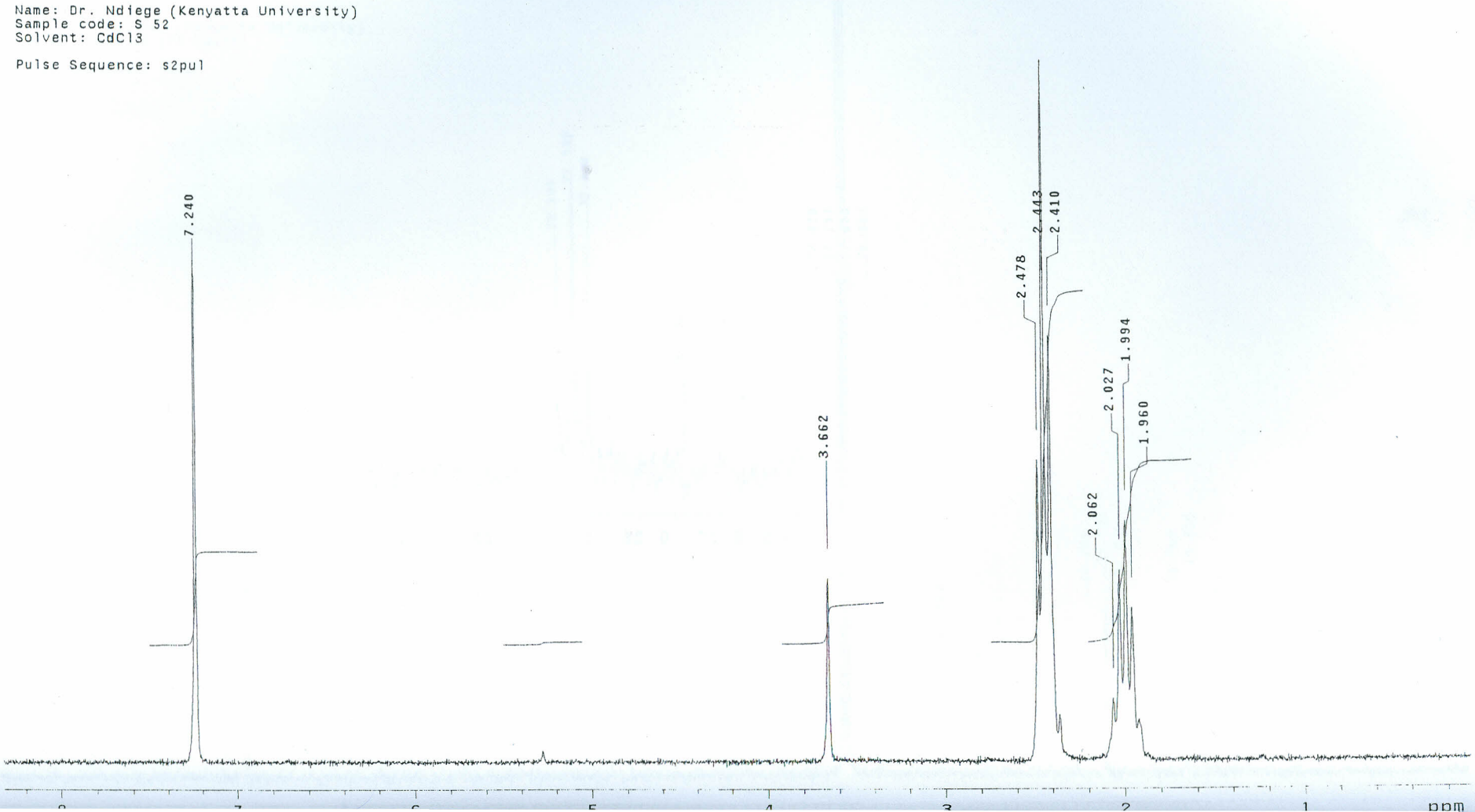
^{1}H NMR spectrum of compound **139**.

Name: Martin Thuo (Kenyatta University)
Sample code: MT81CPD2
Weight: 5mg

Pulse Sequence: s2pul



^{1}H NMR spectrum of compound **143**.



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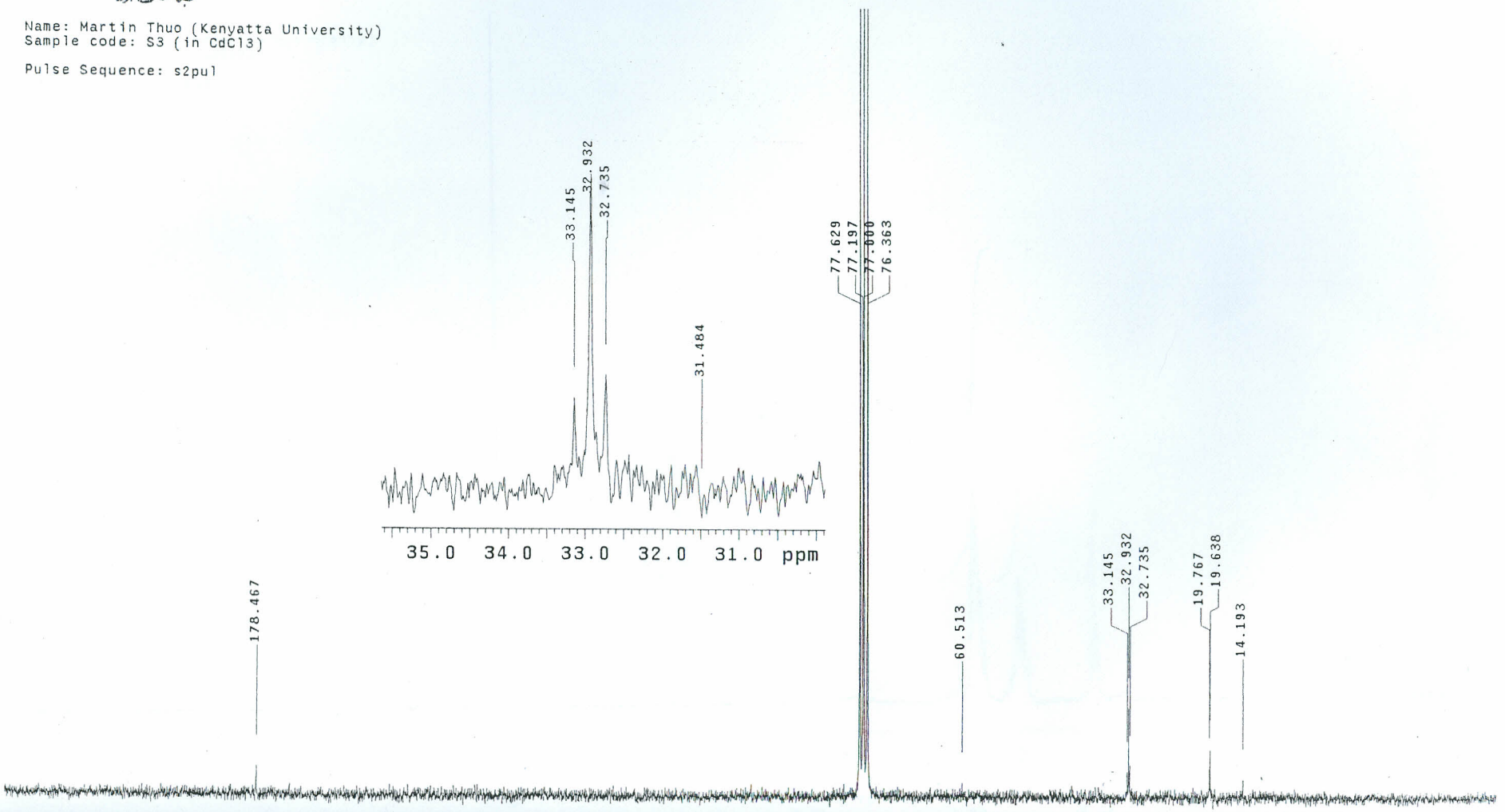

^{1}H NMR spectrum of contaminated compound **144**.