Synthesis and Analysis of Zingerone Analogues as Fruit Fly Attractants

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Date of Submission: 9 October 2015

Declaration of Originality

I, Benjamin L. Hanssen, declare that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Benjamin L. Hanssen Date

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Acknowledgements

I would like to thank my supervisors Ian Jamie, Joanne Jamie, Soo Jean Park, and Phil Taylor who have provided much support and guidance throughout this year. They have been an invaluable source of knowledge and given me great insight into what research can involve.

Soo deserves special acknowledgement for her immeasurable help, especially at the beginning as I was settling in with the project and lab. I cannot imagine how much harder this year would have been without Soo's support and practical experience and knowledge.

I would also like to thank Chris McRae for the use of and fixing the DSC amongst other things, without which the vapour pressure work would have been that much more difficult.

Thank you to my friends Gin and Nathan for their company in the lab, the discussions that made periods of setbacks and monotony bearable, and their support and encouragement to make it through the year.

Last but not least, I thank my family for all their never-ending support and encouragement this year.

Table of Abbreviations

ADI	Acceptable daily intake
APVMA	Australian Pesticide and Veterinary Medicines Authority
ARfD	Acute reference dose
ATR-IR	Attenuated total reflectance-infrared
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DSC	Differential scanning calorimetry
EAG	Electroantennography
EI	Electron ionisation
GC-FID	Gas chromatography-flame ionisation detector
GC-MS	Gas chromatography-mass spectrometry
MAT	Male annihilation technique
MT	Mass trapping
NMR	Nuclear magnetic resonance
SAR	Structure-activity relationship
TLC	Thin layer chromatography

Abstract

Fruit fly species have a devastating impact on food production in Australia and other countries. With new restrictions on the use of certain organophosphate insecticides, other control methods, such as male annihilation technique (MAT), will be more important for crop protection. MAT depends on effective male lures to attract pest insects to toxicants. Zingerone is a natural product that is attractive to males of *Bactrocera jarvisi*, a species that responds only weakly to other lures. In a step towards more effective lures, a series of zingerone analogues were synthesised and characterised. Many analogues were synthesised by an Aldol-hydrogenation synthetic route. Volatility is considered an important factor in lure attraction and the measurement of the volatility of the analogues by differential scanning calorimetry showed that fluorinated compounds have a 7-10-fold greater vapour pressure than corresponding non-fluorinated compounds. Unexpectedly, the acetyl and formyl esters of zingerone were half as volatile as zingerone. Electroantennography experiments with *B. tryoni* and *B. jarvisi* indicated that fluorinated compounds generally produce a greater response and the two species have vastly different preferences for the methylenedioxy moiety. Limited laboratory cage bioassays conducted with *B. tryoni* indicated that some of the fluorinated compounds tended to elicit a positive response.

Chapter 1. Introduction

Fruit flies are a significant pest of horticultural crops, and of particular concern are those species that have an impact upon food production. The Queensland fruit fly (*Bactrocera tryoni*) is the most serious pest fruit fly species in Australia with a very wide variety of host crops, ranging from citrus to nuts, tomatoes, and stone fruits.[1-3] This species of fruit fly is also known to infest at least 60 wild, non-commercial plants.[1] *B. tryoni* is present across northern Australia from Broome to north-west Queensland and eastern Australia from Cape York to south of Sydney. It is also present intermittently in Adelaide and Victoria.[1, 4] In Australia, Jarvis' fruit fly (*B. jarvisi*) is a moderate pest fruit fly present from northern Australia to northern New South Wales.[4-6] It is known to infest at least 83 wild and commercial hosts, of which mangoes are the most susceptible commercial crop.[5-6] Recent restrictions on the use of insecticides to control fruit flies,[2, 7-8] such as *B. tryoni* and *B. jarvisi*, means that other control methods will be more heavily relied on for the management of fruit flies. Alternative control methods include the use of chemical lures to attract male fruit flies to traps.[1-2]

Zingerone is one such chemical lure that has shown potential for attracting *B. tryoni* and especially *B. jarvisi*.[5-6] In this study, a series of zingerone analogues were synthesised and characterised. The aims of this study were to synthesise the chosen zingerone analogues, measure their vapour pressures, and evaluate their biological activity using electroantennography (EAG) and laboratory cage bioassays.

1.1 Fruit Flies – Life Cycle and Economic Importance

Fruit flies cause direct damage to fruit crops as a result of adult flies laying eggs under the surface of the fruit. Within days, the eggs hatch into larvae, which consume the internal structures of the fruit.[1, 3, 9-10] Fruit may also be damaged by subsequent microbial decay as a result of the fruit flies penetrating the surface of the fruit.[1, 3] Figure 1 shows the damage caused by fruit fly larvae and microbial decay in infested fruit. At the end of the larval stage, the larvae leave the fruit to pupate in the ground.[9] Adult flies emerge from the ground to complete the life cycle. The fruit fly life cycle can be completed in 3 to 4 weeks under favourable conditions, with many generations per year in tropical locations.[3, 9] Temperature is a major factor affecting the spread and severity of *B. tryoni* infestations, such that in temperate locations there may be only one generation per year.[1, 3] Climate change is predicted to extend the range of *B. tryoni* further into the southern states of Australia.[11] This is expected to dramatically increase fruit fly management costs and severely threaten the sustainability and existence of the fruit fly free status in South Australia.

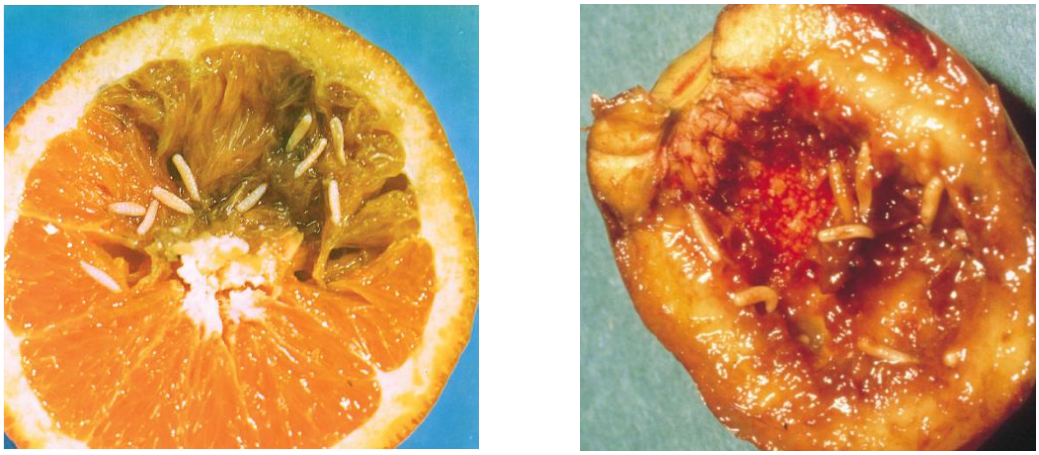


Figure 1 Images of damage caused by fruit fly larvae and microbial decay.
Left sourced from [12] and right sourced from [13].

The economic cost of fruit flies in Australia, particularly from *B. tryoni* damage, is substantial. In 2000, the annual cost of *B. tryoni* damage was estimated to be $28.5 million.[11] The direct costs to government and industry in fruit fly management was an estimated $128 million over the period from 2003 to 2008, which included direct control costs, post-harvest treatments, and monitoring of fruit fly populations.[1, 3] The annual value of Australian horticulture susceptible to fruit fly damage is estimated to be $4.8 billion, which would be severely threatened without effective fruit fly management.[3] Due to the ability of *B. tryoni* to be transported long distances and rapidly colonise new environments, it is a significant biosecurity risk both domestically and internationally. This affects the ability of Australian produce to access domestic and international markets.[3] Many markets will only permit produce that has been further treated after harvest. This results in an increased cost to growers as well as potentially inferior quality produce, which reduces overall profitability.

While *B. tryoni* is an endemic and serious pest fruit fly that is a quarantine risk for other countries, there are also other fruit flies that can cause damage to crops. An estimated 46 endemic and exotic species of fruit fly are considered to be significant economic threats to Australian horticulture.[3] Many of these are exotic species from South-East Asia and the South Pacific. Apart from the endemic Queensland fruit fly (*B. tryoni*), the invasive Oriental fruit fly (*B. dorsalis*), Melon fly (*B. cucurbitae*), Papaya fruit fly (*B. dorsalis* (former *B. papaya*)), and Mediterranean fruit fly (*Ceratitis capitata*) (already present in Perth, Western Australia) are major threats to Australian horticulture.

While the previous discussion has been concerned with the threat of fruit flies to Australia, the same points are applicable to other tropical and subtropical countries where fruit flies, such as *B. dorsalis* and *B. cucurbitae*, would be considerable threats to food security.

1.2 Fruit Fly Control Methods

1.2.1 Organophosphate Insecticides

Numerous methods have been used to control fruit fly populations and minimise damage to crops. Insecticides have traditionally been the control method of choice to prevent crop damage. The insecticides may be applied to fruit only, the entire tree, or the ground. When applied to fruit or the entire tree as cover sprays, the insecticide is intended to kill adult fruit flies before oviposition into the fruit. Under tree spraying of insecticides is instead intended to kill the larvae after leaving infested fruit or adult flies emerging after pupating.[2]

These insecticides are often contact insecticides that only require contact rather than ingestion. These contact insecticides are typically organophosphates, such as dimethoate, fenthion, and malathion, which are insect cholinesterase inhibitors.[2] According to Dominiak and Ekman[2], cover spraying with dimethoate or fenthion has been the most important method to control *B. tryoni*. However, organophosphates are hazardous to human health.[1-2] Exposure to very large amounts of organophosphate insecticides can cause severe hypotension[14] and several serious neurotoxic effects[15], which can result in death. The levels of organophosphates that consumers are typically exposed to, however, are substantially lower than the amount that causes acute life threatening illnesses. Due to health and chemical residue concerns, the Australian Pesticide and Veterinary Medicines Authority (APVMA) conducted a review into the use of dimethoate. In 2007 the APVMA decreased the acceptable daily intake (ADI) of dimethoate from 0.02 mg kg^{-1} day^{-1} to 0.001 mg kg^{-1} day^{-1}.[1-2] Due to the lower ADI and acute reference dose (ARfD) value of 0.02 mg kg^{-1} body weight, the APVMA determined that many common use patterns of dimethoate would exceed the ADI and/or ARfD.[2] As a result, the use of dimethoate has been severely restricted, cancelled, or suspended for many crops. The APVMA is also reviewing fenthion and it is likely that the use of fenthion will be similarly severely restricted.[1-2] The restriction of the use of dimethoate and fenthion, which are important insecticides for controlling fruit flies, raises serious concerns about the future sustainability of Australian horticulture and food security more broadly.

1.2.2 Alternative Control Methods: MT and MAT

The restrictions on the use of organophosphate use means that other fruit fly control methods will be more heavily relied on to protect crops. Mass trapping (MT) and male annihilation technique (MAT) are two related control methods that use a chemical lure to attract adult fruit flies to a trap, removing the fruit flies from the population. While these traps use an insecticide, the insecticide is not applied to crops and is contained inside the trap.

In MT, the lure is often food bait, such as hydrolysed protein or orange-ammonia solution.[16] It has been generally accepted that MT captures more female flies than male flies, but this view is changing; one study observed that hydrolysed protein traps captured on average a ratio of 5.71:1 male to female flies.[16] This same study observed that an orange-ammonia trap was even more skewed towards capturing male flies with on average a ratio of 40.1:1 male to female flies. Another study had a smaller difference between male and female fly captures for hydrolysed protein traps with a ratio of male to female flies of 1.2-1.5:1.[17] The number of flies captured by MT using food baits is significantly lower than other lure-based traps.[16-17] The low capture rates may be due to the presence of high molecular weight components in the lure that are not sufficiently volatile to be detected by the adult fruit flies at reasonable distances.[1]

Similar to MT, MAT also uses a chemical lure to attract adult fruit flies. A MAT trap uses the lure to attract flies to the trap where they are killed by an insecticide present in the trap.[1-2, 18-19] The lures used in MAT differ significantly from those used in MT, in that the lure is not a food bait, but is instead a specific lure that only attracts mature adult male fruit flies.[1-2, 16-19] Despite only attracting male flies, the advantage of MAT over MT is that the capture rates are much higher, which means that fewer MAT traps are needed to adequately cover an area.[16-17] MAT alone has successfully eradicated *B. dorsalis* from the Island of Rota and *B. cucurbitae* from Easter Island.[10]. A MAT trap typically uses an absorbent material to hold both the lure and the insecticide. The type of material affects the rate of release of the lure as well as the longevity of the trap. Various materials have been used including caneite blocks (compressed particle board),[2] cotton wicks,[2] bucket traps,[20] canec disks,[20] Min-U-Gel,[20] and moulded paper fibre (Amulet).[21] MAT is able to reduce fruit damage by removing adult male flies from the population, which decreases the number of mated females and thus the number of ovipositions and larvae that cause crop damage.[18]

While MAT has higher capture rates than MT, it alone can be insufficient to completely protect crops from fruit fly damage or eradicate a species from an area. A combination of MAT with other control methods, such as MT, may be more able to provide complete fruit fly control than MAT alone. Lloyd, *et al.*[18] implemented an area-wide management programme for the control of *B. tryoni* in the Central Burnett district of Queensland. This programme used a combination of MAT traps, protein-baited MT traps, and orchard hygiene. After 4 years, this programme reduced average trap catches by 95% during the peak summer/spring period. However, fruit fly infestation in backyard fruit was reduced by only 64%.

The success of MAT and similar lure-based control methods for fruit fly management relies heavily on the use of an attractive and effective lure. The development of more attractive and effective lures is highly desirable for lure-based control methods as it would allow for more

rapid population reduction, greater population reduction, and lower trap density. More effective traps are also desirable for fruit fly population monitoring as well as control. Fruit fly population monitoring is important for quarantine, determining the geographical range of various fruit fly species, and to more confidently demonstrate pest-free status.[4-5, 22] More effective lures would be able to detect smaller populations of fruit flies, which would be beneficial in more rapidly identifying the incursion of exotic and endemic fruit flies into sensitive areas.

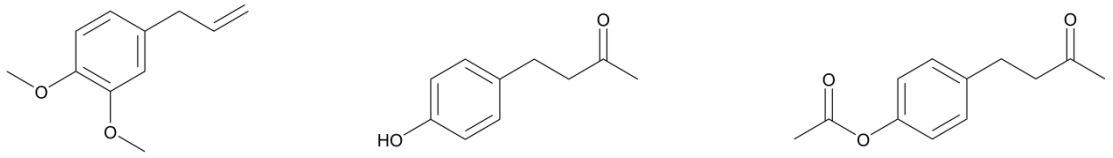
1.3 The Chemical Basis of Lure Attraction

The reason for the attraction of male fruit flies to male chemical lures, such as those used in MAT, is not completely understood. Many hypotheses have been proposed to explain this attraction. The most generally accepted hypothesis is that the lure is related to male pheromones and thus sexual selection.[10, 22-24] The lure is believed to be attractive to male fruit flies because the consumption of the lure enhances the efficacy of the male pheromone blend towards female flies. The lure may be released unaltered or may be chemically modified before release. This pheromone hypothesis is supported by observations that males will compulsively feed on the lure; the lure is transported to and accumulates in the rectal gland with other pheromones; the lure can be released with pheromones during calling; and pheromone blends with the lure have a greater female response than those without the lure.[1-2, 10, 22-26] It should be noted, however, that little is known about fruit fly pheromones and sexual selection in the *Bactrocera* genus.[22] Other reasons for the attraction of male flies to certain chemical lures includes as an allomone to deter predators,[1, 10, 25] as a stimulant to increase energy metabolism or male competitiveness,[1, 23-24] or the lure may have other physiological effects on male flies such as an earlier mating time.[25]

1.4 Methyl Eugenol and Raspberry Ketone as Chemical Lures

In Australia, there are 108 recorded species of fruit fly from the family Tephritidae. Many of these species are present in Queensland, and of these 91 species belong to the *Bactrocera* genus and 13 species belong to the related genus *Dacus*.[4] In Australia, only a few species of *Bactrocera* are pests or potential pests.[4] Despite the large number of species in each genus, many of these species will respond to one of two male chemical lures, but never significantly to both lures.[5, 10, 22-24, 26-27] These two male chemical lures are methyl eugenol (4-allyl-1,2-dimethoxybenzene) and raspberry ketone (4-(4-hydroxyphenyl)-2-butanone). Their chemical structures are shown in Figure 2. The attraction of male fruit flies to methyl eugenol was first discovered by Howlett in 1912, who observed that oil of citronella was attractive to male *Dacus diversus* and *D. zonatus*. Later investigations showed that the active lure was methyl eugenol.[10, 22] There is some concern that methyl eugenol is a carcinogen as it has been observed that

methyl eugenol causes hepatic tumours in mice and rats.[28] However, the level and risk of human exposure to methyl eugenol is expected to be minimal.[29-30] When it was discovered that raspberry ketone is a fruit fly lure is less certain, but certainly by 1959 it was used as a lure for *B. tryoni* in Australia.[31] At a similar time, thousands of compounds were being screened in Hawaii for attractiveness to *B. dorsalis*, *B. cucurbitae*, and *C. capitata*.[10, 22] From this screening, anisyl acetone (4-(4-methoxyphenyl)-2-butanone) was found to be attractive to *B. cucurbitae*. Following this observation, a similar compound, cuelure (4-(4-acetoxyphenyl)-2-butanone), was found to be a superior lure to anisyl acetone.[10, 32] As shown in Figure 2, cuelure is very similar to raspberry ketone in that it is the acetyl ester of raspberry ketone. There are also structural similarities between methyl eugenol and raspberry ketone/cuelure. All 3 lures have a central benzene ring, an aliphatic carbon chain, and an oxygen-containing substituent in the *para* position.



Methyl Eugenol
4-Allyl-1,2-dimethoxybenzene

Raspberry Ketone
4-(4-Hydroxyphenyl)-2-butanone

Cuelure
4-(4-Acetoxyphenyl)-2-butanone

Figure 2 Chemical structures of methyl eugenol, raspberry ketone, and cuelure.

While methyl eugenol, raspberry ketone, and/or cuelure are attractive to many *Bactrocera* and *Dacus* species, approximately 50% of species do not respond to any known lure, including some pest species.[1, 5, 22, 27] Therefore, new lures must be developed that are attractive to these non-responding species and ideally also more attractive to methyl eugenol and raspberry ketone/cuelure responding species.

1.5 Lure Attractiveness

The underlying reason for the attraction of male fruit flies to methyl eugenol and raspberry ketone/cuelure is probably related to their incorporation into pheromones and therefore to sexual selection. The extent of attractiveness is strongly determined by the chemical and physical properties of the lure and effective binding at the receptor binding site in the insect antenna. While fruit flies may have multiple receptors and proteins involved in the detection of lure compounds, in this discussion, all such receptors and proteins will be referred to as a single entity. The attractiveness of lures is probably a combination of intrinsic attractiveness and the volatility of the lures. Therefore, investigations into the development of new lures should consider both the intrinsic attractiveness and volatility of any proposed lures.

1.5.1 Intrinsic Attractiveness

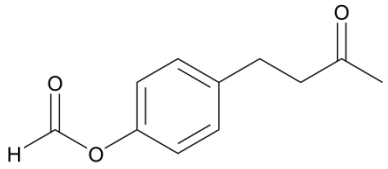
Intrinsic attractiveness refers to the preference of the receptor in the insect antenna, which detects stimuli in the atmosphere, for particular compounds with specific functional groups or particular functional group geometries, positions, or other structural features. The receptor that detects male chemical lures has evolved to detect these specific compounds.[22, 27, 33-35] Therefore, for a lure to be detected and elicit a response typical of male lures, it must be capable of interacting with the relevant receptor. The protein structure of these receptors is not known and there is only limited information from structure-activity relationship (SAR) studies regarding the structure of the binding site. Based on the similarities between methyl eugenol and raspberry ketone/cuelure, it is likely that most of the *Bactrocera* and *Dacus* lure receptors require that the lure molecule has a central benzene ring, an aliphatic carbon chain, and an oxygen-containing substituent in the *para* position. In light of this, new lures should include these important functional groups. Performing an SAR investigation by varying the functional groups in potential lures could provide valuable information on the structure of the receptor binding site.

1.5.2 Volatility

In addition to intrinsic attractiveness, volatility would seem to be a critical factor in the overall attractiveness of male chemical lures. Since the lures are detected in the vapour phase by male fruit flies, it is necessary for the lure to vaporise into the atmosphere and be transported to the location of the flies. Thus, if the volatility of a lure can be increased, more of the lure will be available for detection, which would result in improved attraction at a greater distance from the source of the lure. However, as discussed previously, the intrinsic attractiveness is also crucial for attraction and a more volatile lure will not necessarily be more attractive if increasing the volatility affects the important structural features of the lure.

The difference in attraction between raspberry ketone and cuelure is a prime example of the effect of volatility. Since cuelure has an acetyl ester group instead of the phenolic group of raspberry ketone, cuelure is significantly more volatile. It has been estimated that the release rate of cuelure from traps is 20 times greater than the release rate of raspberry ketone.[10] Given that cuelure and raspberry ketone have very similar structures, the greater attractiveness of cuelure compared to raspberry ketone is most likely due to the higher volatility of cuelure. Similarly, methyl eugenol is generally accepted to be more strongly attractive to methyl eugenol-responsive species than is cuelure to cuelure-responsive species.[22] The greater attractiveness of methyl eugenol compared to cuelure is also most likely to be due to volatility. In one study that compared the vaporisation of lures in outdoor tests, 99.3% of cuelure remained after 12 months, while only 70.9% of methyl eugenol remained.[36]

In the search for more attractive lures similar to cuelure, the formyl ester of raspberry ketone (melolure, 4-(4-formoxyphenyl)-2-butanone) was found to be twice as effective as cuelure in attracting *B. cucurbitae*.[9] The chemical structure of melolure is shown in Figure 3. Later studies supported this observation with melolure found to be approximately 1.7 times more effective in attracting *B. cucurbitae*[37] and *B. tryoni*[27] than cuelure. Similar to cuelure, melolure is an ester of raspberry ketone with greater volatility than raspberry ketone. Since melolure has a smaller substituent (formyl group) than the acetyl group of cuelure, melolure is more volatile than both cuelure and raspberry ketone. A smaller substituent has a reduced surface area and intermolecular interactions, which increases volatility. Thus, the order of attractiveness of these lures is the same as the order of volatility: melolure > cuelure > raspberry ketone, which supports volatility as a critical factor in lure attraction to cuelure-responsive species, such as *B. tryoni*. As noted previously, a more volatile lure does not ensure greater attractiveness as the intrinsic attractiveness is also a significant factor. Royer[27] observed that *B. neohumeralis* (lesser Queensland fruit fly) is less attracted to melolure than cuelure, even though the very closely related *B. tryoni* (Queensland fruit fly) is more attracted to melolure.[27]



Melolure
4-(4-Formoxyphenyl)-2-butanone

Figure 3 Chemical structure of melolure.

1.5.2.1 Determining Vapour Pressure by Differential Scanning Calorimetry

Despite the importance of volatility in determining the attractiveness of lures to male fruit flies, there are very little quantitative data concerning the volatility of lures or the relationship between volatility and attractiveness. Differential scanning calorimetry (DSC) is reported as a convenient technique for determining vapour pressure. DSC has been successfully used to determine the vapour pressures of 1-octanol,[38] thiodiglycol,[39] several lower alkyl phosphonates,[40-41] several fatty acids,[42-43], and three 2-dialkyl aminoethanethiol compounds.[44]

With DSC, the boiling point of the lures is determined under a range of known pressures. At the boiling point of a compound, the vapour pressure of the compound is equal to the applied pressure. The temperature-pressure data can be fitted to the Antoine Equation (1) to obtain a vapour pressure curve.[45]

$$\log P = A - \frac{B}{T+C} \quad (1)$$

where P is pressure (kPa), T is temperature (K), and A, B, and C are the Antoine parameters.

This relationship then allows the vapour pressure of the compound to be determined at any desired temperature.

1.5.2.2 Increasing Volatility in Lures by Structural Modifications

More attractive lures may be developed by increasing the volatility of lures. The substitution of hydrogen atoms for fluorine atoms is one known method of achieving greater volatility. The size of a fluorine substituent is often approximated as being the same as a hydrogen substituent.[46] However, the van der Waals radius of fluorine is 1.47 Å, whereas the radii of hydrogen and oxygen are 1.20 and 1.52 Å respectively.[47] Nevertheless, the size of a fluorine substituent is smaller than most other substituents. Studies by Schlosser and Michel[46] and Michel and Schlosser[48] demonstrated that the substitution of a hydrogen by a fluorine had a minimal biological effect in relation to taste and odour, unlike substitution of a hydrogen by a methyl group. The capability of fluorine to increase volatility is most likely due to its very low polarisability.[49-52] The very low polarisability of fluorine results from its tightly held valence electrons, which are not easily affected by external electric fields, such as those of dipoles or ions.[51-52] While the C-F bond can be a strong dipole due to the large difference in electronegativities of carbon and fluorine, this effect is usually outweighed by the very low polarisability of fluorine.[53] Low polarisabilities result in low surface energies and weaker intermolecular forces especially dispersion and induced dipole interactions.[50, 52] Reduced intermolecular forces may also result from repulsions between several close fluorine atoms, which can repel each other due to a high charge density from the tightly bound valence electrons.[54] This means that the organofluorine compound will tend to have a lower boiling point and higher volatility.

1.6 Chemical Lure and Receptor SAR Investigations

Many studies have been conducted in an attempt to discover more attractive alternatives to methyl eugenol and cuelure. Melolure is one example of a lure observed to be generally more attractive than the related existing lure, cuelure. These studies have often been conducted as SAR investigations using model *Bactrocera* species, such as *B. cucurbitae* and *B. dorsalis*, in order to more completely understand the relevant receptor.

1.6.1 Methyl Eugenol Receptor

Metcalf, *et al.*[33] conducted an SAR investigation into the effect of substitution on the methyl eugenol receptor of *B. dorsalis*. In this study, the response of *B. dorsalis* to analogues of methyl eugenol with different functional groups instead of the allyl group was examined. It was observed that there is an optimal size of this primary substituent as three-atom chains were found to produce the strongest responses. Hydrophobicity and electron-donating capability of

this primary substituent were also important with higher hydrophobicities and electron-donating capability generally yielding stronger responses. Replacing the *para* methoxy group with methyl and chlorine substituents produced a compound that elicited no response, which indicated that the *para* position was the primary electronic site. The presence of the second methoxy group in the *meta* position was crucial for a very strong response. A similar study by Metcalf, *et al.*[34], also observed consistent responses of *B. dorsalis* to various non-polar 3,4-dimethoxyphenyl compounds. Mitchell, *et al.*[55] found the methyl eugenol receptor of *B. dorsalis* to be highly selective. Tests using several analogues of methyl eugenol with different functional groups instead of the allyl group were unsuccessful as no analogue was as effective as methyl eugenol. This indicated that effective attractants for *B. dorsalis* must be sterically and electronically similar to methyl eugenol. Metcalf, *et al.*[35] conducted an extensive study into the attraction of substituted benzyl acetates to *B. dorsalis*. In this study, the critical role of the aromatic ring for effective attraction was demonstrated as a saturated cyclohexane analogue showed no response, unlike unsaturated analogues. This is expected as the benzene ring is conserved across attractive methyl eugenol and raspberry ketone analogues. Interestingly, the introduction of a fluorine substituent in the *ortho*, *meta*, or *para* position of benzyl acetate did not significantly alter the attractiveness to *B. dorsalis* compared to unsubstituted benzyl acetate. Later studies also showed that fluorine substituents on the benzene ring are not as attractive as methyl eugenol to *B. dorsalis* males.[56] On the other hand, studies with fluorinated allyl chains instead of fluorinated benzene rings observed that a single fluorine atom on the terminal carbon atom in 4-[(2*E*)-3-fluoroprop-2-en-1-yl]-1,2-dimethoxybenzene was as attractive as methyl eugenol to *B. dorsalis*.[57] Overall, the methyl eugenol receptor in *B. dorsalis* appears to interact with lures through the benzene ring, unsaturated 3 atom chain, and the two adjacent methoxy groups on the benzene ring (Figure 4).[35]

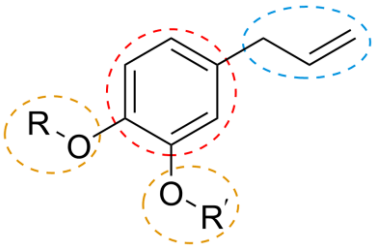


Figure 4 Structural features involved in binding to the *B. dorsalis* receptor.

1.6.2 Raspberry Ketone Receptor

SAR investigations involving the raspberry ketone receptor of *B. cucurbitae* have also been conducted. Metcalf, *et al.*[34] observed that *B. cucurbitae* responds strongly to non-polar 4-hydroxyphenyl compounds. Although *B. cucurbitae* was attracted to 4-methoxyphenyl compounds,[34] 3,4-dimethyoxyphenyl compounds produced no response.[35] Metcalf, *et al.*[34]

state that the primary receptor site of *B. cucurbitae* interacts with the carbonyl group of the butanone chain. However, response strongly depended on the position of the carbonyl group relative to the benzene ring with the carbonyl group separated from the benzene ring by two atoms being most attractive. A study by Metcalf, *et al.*[35] supports the importance of the carbonyl group in *B. cucurbitae* attraction. The same study also showed that the response of unsaturated butenone analogues was substantially reduced compared to saturated butanone analogues, suggesting that a flexible chain is required for effective receptor binding. Carboxylic acid methyl esters were found to be 3 to 1000 times more attractive to *B. cucurbitae* than free carboxylic acids, which indicates that hydrophobic interactions between the receptor and butanone chain are critical.[34] While a hydrophobic butanone chain may be required, Metcalf, *et al.*[35] observed that a hydrophilic *para* substituent is also necessary. The size of the hydrophilic *para* substituent affects response with smaller substituents generally favoured.[35] Like *B. dorsalis*, the introduction of a fluorine substituent in the *ortho*, *meta*, or *para* position of benzyl acetate did not significantly alter the attractiveness to *B. cucurbitae* compared to unsubstituted benzyl acetate.[35] Overall, the raspberry ketone receptor in *B. cucurbitae* appears to interact with lures through the benzene ring, a small hydrophilic *para* substituent, and the carbonyl group of the butanone chain (Figure 5).[35]

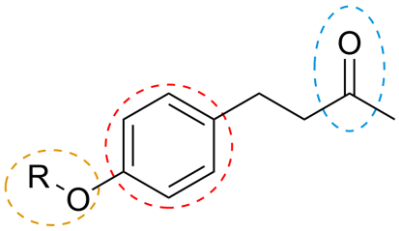
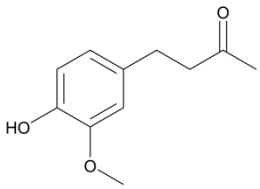


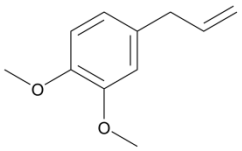
Figure 5 Structural features involved in binding to the *B. cucurbitae* receptor.

1.7 Zingerone as a Chemical Lure

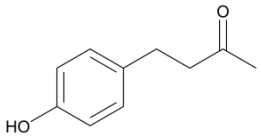
The structural similarities between methyl eugenol and raspberry ketone – central benzene ring, aliphatic carbon chain, and oxygen-containing substituent in the *para* position, suggests that these features may be universally important for effective lure attraction in *Bactrocera* and *Dacus*. Another naturally-occurring compound, zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone), shares these features with both methyl eugenol and raspberry ketone. As shown in Figure 6, zingerone is most similar to raspberry ketone in that the two compounds have a common phenolic group in the *para* position, benzene ring, and butanone chain; but zingerone is also similar to methyl eugenol with a methoxy group in the *meta* position to the carbon chain. Given the structural similarities between zingerone and the lures methyl eugenol and raspberry ketone/cuelure, it might be expected that zingerone is also attractive to *Bactrocera* and *Dacus* species as a male chemical lure.



Zingerone
4-(4-Hydroxy-3-methoxyphenyl)-2-butanone



Methyl Eugenol
4-Allyl-1,2-dimethoxybenzene



Raspberry Ketone
4-(4-Hydroxyphenyl)-2-butanone

Figure 6 Chemical structures of zingerone, methyl eugenol, and raspberry ketone.

Zingerone is the component responsible for the pungency of cooked ginger,[22] but not raw ginger as zingerone is the thermal degradation product of gingerol. Zingerone has also been found in the flowers of certain orchid species, such as *Bulbophyllum patens* and *Bulbophyllum baileyi*, where it was found to attract some species of *Bactrocera*.[58-60] Tan and Nishida[58] noted that both methyl eugenol and raspberry ketone responsive species were attracted to the flowers of *Bulbophyllum patens*, which was expected based on the structural similarities between methyl eugenol, raspberry ketone, and zingerone. This is a significant observation because as discussed previously no fruit fly species is known to significantly respond to both methyl eugenol and raspberry ketone. While this is still true, it suggests that some methyl eugenol and raspberry ketone responsive species can be attracted by a single lure. More unexpected was the observation that zingerone could attract fruit fly species not known to respond to any lures.

In Australia, *B. jarvisi* does not readily respond to methyl eugenol or raspberry ketone and perhaps has only a very weak attraction to cuelure.[4-5] While its response to existing lures may be very weak, zingerone is attractive to *B. jarvisi*.[5, 27] Over a five year period, up to 99.6% of fruit flies captured in zingerone traps in northern Queensland were male *B. jarvisi*.[5] The same study found that only 0.1% of zingerone captured fruit flies were *B. tryoni*, which indicates that zingerone may not be attractive to all lure responsive species. A study by Royer[27] also observed *B. jarvisi* to be strongly attracted to zingerone while *B. tryoni* was only weakly attracted. In the same study, zingerone was attractive to 13 known cuelure responsive species, and seven species that only responded to zingerone, including two undescribed species of *Dacus*. In contrast to both of the above studies, one study in the Sydney region of Australia over a two year period observed zingerone to be reasonably attractive to *B. tryoni*.[6] Cuelure, however, captured a significantly greater number of *B. tryoni* than zingerone. Figure 7 summarises the results of this study and illustrates that while zingerone captured significantly fewer *B. tryoni*, zingerone is still an effective lure for this species. Similar to Royer[27], Dominiak, *et al.*[6] observed that

zingerone captured a greater diversity of fruit fly species compared to both methyl eugenol and cuelure, however, the capture rates of these other species was very low.[6] The greater attraction of *B. tryoni* to zingerone in this study compared to other studies was suggested to be possibly due to the different climatic conditions between Sydney and Cairns. The greater numbers of fruit flies in northern Queensland may also alter the sensitivity of certain species to different lures.[6] Experimental design was also cited as a possible reason for the difference in attraction – Fay[5] used a non-competitive design, whereas Dominiak, *et al.*[6] used a competitive design. This might suggest that zingerone, unlike cuelure, only has a short range of attraction, such that in the competitive design it was cuelure that first attracted *B. tryoni* to the trapping area before being attracted by zingerone at a closer distance.[6]

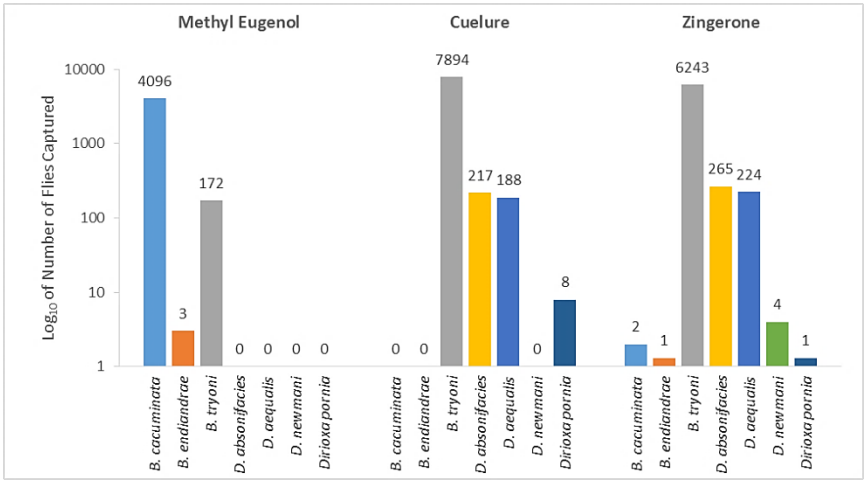


Figure 7 Number of fruit flies captured by methyl eugenol, cuelure, and zingerone in the Sydney, Australia region over a two year period by species. Data from Dominiak, *et al.*[6].

1.7.1 Reasons for Zingerone Attraction

The similarities between methyl eugenol, raspberry ketone, and zingerone would suggest that the reason for the attraction of male flies to zingerone would also be comparable. Indeed, Khoo and Tan[25] showed that male *B. cucurbitae* fed zingerone or cuelure attracted a greater number of female flies than zingerone or cuelure deprived male flies, which suggests that zingerone has a similar effect on male pheromones as cuelure. Similarly, Kumaran, *et al.*[24] observed that zingerone-fed and cuelure-fed male *B. tryoni* elicited a significantly stronger female response during calling than unfed males, but cuelure-fed males had a stronger response than zingerone-fed males. However, when the female response to male rectal glands was investigated, it was found that zingerone-fed and lure-unfed males were not significantly different, even though cuelure-fed male rectal glands elicited a strong female response. While zingerone does modify the pheromone blend similar to cuelure, these results indicate that the zingerone pheromone blend is not more attractive to females than unaltered pheromone blend. Instead of improving the male pheromone blend, the attraction of male fruit flies to zingerone may alternatively be explained by higher energy metabolism as a result of zingerone consumption. Kumaran, *et al.*[23]

observed that lure feeding of *B. tryoni* resulted in many gene expression changes across a number of different metabolic pathways, which was accompanied by increased physical activity. Higher levels of physical activity may allow lure-fed male flies to outcompete lure-unfed males by calling for a longer period of time or more intensely. Thus, the attraction of male fruit flies to zingerone may be because zingerone is a physical activity stimulant rather than a favourable pheromone blend modifier.

1.8 Implications of Zingerone for Fruit Fly Control

The capability of zingerone to attract non-responding species and methyl eugenol/raspberry ketone/cuelure responsive species is a significant discovery for fruit fly management and control. A lure such as zingerone would be invaluable for the control and population monitoring of non-responding pests like *B. jarvisi* as well as methyl eugenol and raspberry ketone/cuelure responding species that also respond to zingerone. Exotic fruit fly species that are a threat to horticulture in Australia and other countries may also be zingerone responsive, and thus, zingerone could be a vital lure for the detection of exotic fruit fly incursions. While zingerone is only weakly attractive to *B. tryoni* and some other pest species compared to cuelure, the attractiveness of zingerone can likely be improved by developing analogues with greater intrinsic attractiveness and volatility, analogous to the substantial improvement in attractiveness achieved with cuelure, the acetyl ester of raspberry ketone.

1.9 Aims and Scope of Study

A series of zingerone analogues will be synthesised and characterised as potential fruit fly attractants for *B. tryoni* and *B. jarvisi*, including fluorinated compounds. Many of the analogues will be synthesised by existing literature methods. Using DSC, the vapour pressure of the compounds will be measured and vapour pressure curves for each compound constructed. The EAG responses of *B. tryoni* and *B. jarvisi* to the zingerone analogues will be obtained. Laboratory cage bioassays will be conducted to evaluate the behavioural response of *B. tryoni* to the analogues. A comparison of the analogue structures with the available vapour pressure, EAG, and bioassay data will be performed.

Chapter 2. Experimental Methods

2.1 Synthesis of Zingerone Analogues

Table 1 shows the chemical name and structure of zingerone and analogues investigated in this study, as well as unique bold numbers that will be used to refer to these compounds in the text. Other synthesised compounds referred to in this study are identified in Table 2. Zingerone (**1**) was purchased from Sigma-Aldrich and purified by short-path distillation for vapour pressure measurements. Compounds **7** and **15** were purchased from Sigma-Aldrich and used without further purification. The justification for the choice of these compounds is given in Section 3.1.1.

Table 1 Zingerone analogues investigated in this study.

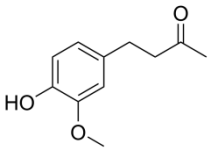
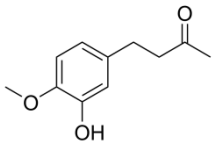
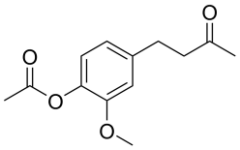
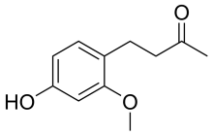
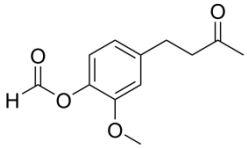
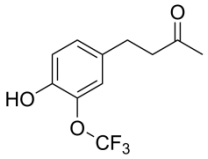
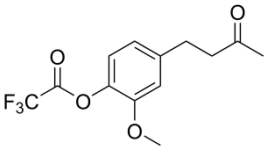
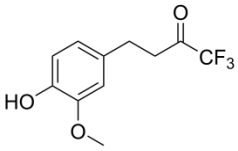
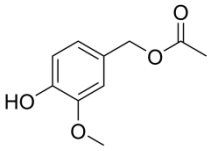
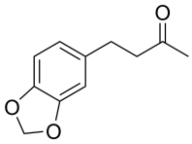
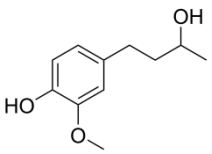
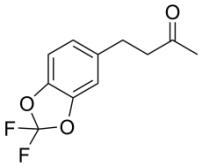
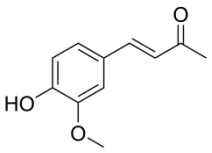
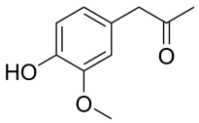
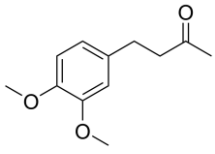
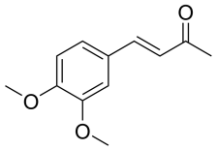
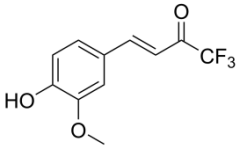
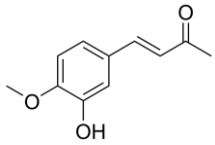
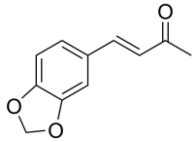
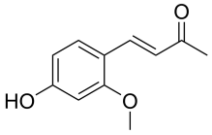
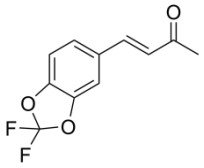
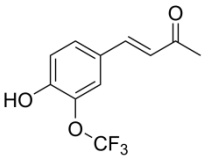
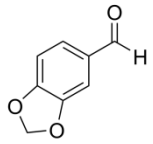
Number	Name	Chemical Structure	Number	Name	Chemical Structure
1	Zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone)		**9b**	Isozingerone 4-(3-Hydroxy-4-methoxyphenyl)-2-butanone	
2	4-(4-Acetoxy-3-methoxyphenyl)-2-butanone		**10b**	4-(4-Hydroxy-2-methoxyphenyl)-2-butanone	
3	4-(4-Formoxy-3-methoxyphenyl)-2-butanone		**11b**	4-(4-Hydroxy-3-(trifluoromethoxy)-phenyl)-2-butanone	
4	4-(4-Trifluoroacetoxy-3-methoxyphenyl)-2-butanone		**12b**	1,1,1-Trifluoro-4-(4-hydroxy-3-methoxyphenyl)-2-butanone	
5	4-Hydroxy-3-methoxybenzyl acetate		**13b**	4-(3,4-Methylenedioxy-phenyl)-2-butanone	
6	Zingerol (4-(4-Hydroxy-3-methoxyphenyl)-2-butanol)		**14b**	4-(3,4-(Difluoromethylene-dioxy)phenyl)-2-butanone	
7	Dehydrozingerone (4-(4-Hydroxy-3-methoxyphenyl)but-3-en-2-one)		**15**	4-Hydroxy-3-methoxyphenylacetone	
8b	Methylzingerone 4-(3,4-Dimethoxyphenyl)-2-butanone				

Table 2 Other numbered synthesised compounds in this study.

Number	Name	Chemical Structure	Number	Name	Chemical Structure
8a	4-(3,4-Dimethoxyphenyl)but-3-en-2-one		**12a**	1,1,1-Trifluoro-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one	
9a	4-(3-Hydroxy-4-methoxyphenyl)but-3-en-2-one		**13a**	4-(3,4-Methylenedioxyphenyl)-but-3-en-2-one	
10a	4-(4-Hydroxy-2-methoxyphenyl)but-3-en-2-one		**14a**	4-(3,4-(Difluoromethylene-dioxy)phenyl)but-3-en-2-one	
11a	4-(4-Hydroxy-3-(trifluoromethoxy)-phenyl)but-3-en-2-one		**16**	Piperonal 3,4-Methylenedioxy-benzaldehyde	

General Procedure

^{1}H, ^{13}C, and ^{19}F nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance DPX 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR, 101 MHz for ^{13}C NMR, and 376 MHz for ^{19}F NMR. $CDCl_3$ was used as the solvent for all NMR samples. ^{1}H NMR chemical shifts are reported in parts per million (δ) referenced to the proton signal of the deuterated solvent ($CDCl_3$: 7.26 ppm). ^{13}C NMR chemical shifts are reported in parts per million (δ) referenced to the carbon signal of the deuterated solvent ($CDCl_3$: 77.16 ppm). ^{19}F NMR chemical shifts are reported in parts per million (δ) referenced to the fluorine signal of trifluoroacetic acid (-76.55 ppm). The following abbreviations are used to describe the NMR data – singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), multiplet (m), and broad (br). Low resolution mass spectra were recorded on a Shimadzu GCMS-2010 using electron ionisation (EI) (70 keV). Infrared spectra were recorded using a Thermo Scientific Nicolet iS5 FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. Peak positions from ATR-IR spectra are given in wavenumbers, $\tilde{\nu}$, (cm^{-1}). Flash column chromatography was performed using a Biotage Isolera Four over normal phase Merck 60 silica gel (40-60 μm) packed in a Biotage cartridge. The progress of all reactions was monitored with thin layer chromatography (TLC) and was performed using Merck TLC silica gel 60 F_{254} on aluminium sheets (0.2 mm) and visualised with ultraviolet light at 254 nm. Solvents were removed under reduced pressure using a Büchi Rotavapor R-200, Büchi V-500 vacuum pump, and Büchi B-490 heating bath set to a temperature of 40 °C. Drying following solvent removal

was performed with an Alcatel Pascal 2005 SD high vacuum pump. All reagents were purchased from Sigma-Aldrich, Merck, or Alfa-Aesar and used without further purification.

4-(4-Acetoxy-3-methoxyphenyl)-2-butanone (2)

Acetic anhydride (0.80 mL, 8.5 mmol, 1.5 eq) was added to zingerone (1.01 g, 5.20 mmol, 1.0 eq) and cooled to 0 °C. Pyridine (0.60 mL, 7.4 mmol, 1.5 eq) was added to the colourless solution, which was then heated to 80 °C and refluxed for 2 hours. The colourless solution was again cooled to 0 °C and hydrochloric acid (20.0 mL, 0.5 mol L^{-1}) was added. The colourless aqueous solution was extracted with DCM (2 × 20.0 mL). The organic layers were combined, washed with $NaHCO_3$ solution (2 × 20.0 mL, 10% (w/v)), and dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure to give the crude product as a pale yellow oil, which was purified by flash column chromatography (eluted with 10-40% ethyl acetate in hexane) to give the product as a white solid (1.01 g, 4.27 mmol, 82.2%, mp 43-44 °C (lit. mp 40-42 °C[61]), R_f: 0.16 (4:1 (v/v) hexane:ethyl acetate), elemental analysis C: 66.37% H: 6.97% (calc. C: 66.09% H: 6.83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.15 (3H, s, $COCH_3$), 2.30 (3H, s, $COCH_3$), 2.76 (2H, t, *J* = 7.3 Hz, CH_2), 2.88 (2H, t, *J* = 7.4 Hz, CH_2), 3.81 (3H, s, OCH_3), 6.74 (1H, dd, *J* = 1.8, 8.0 Hz, Ar-H), 6.79 (1H, d, *J* = 1.7 Hz, Ar-H), 6.93 (1H, d, *J* = 8.0 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.8 ($\underline{C}H_3COO$), 29.7 (alkyl), 30.3 (alkyl), 45.3 ($CO\underline{C}H_2$), 56.0 (OCH_3), 112.8 (Ar), 120.5 (Ar), 122.8 (Ar), 138.1 (Ar), 140.2 (Ar), 151.0 (Ar), 169.4 ($CH_3\underline{C}OO$), 207.9 (CO) ppm. Although no literature spectral data were available, experimental spectral data were consistent with that expected for the title compound.

4-(4-Formoxy-3-methoxyphenyl)-2-butanone (3)

To a solution of formic acid (0.29 mL, 7.69 mmol, 1.5 eq) in DCM (20.0 mL), DMAP (62.9 mg, 0.515 mmol, 0.1 eq) was added, followed by zingerone (1.00 g, 5.15 mmol, 1.0 eq). The colourless solution was cooled to 0 °C and DCC (1.59 g, 7.71 mmol, 1.5 eq) was added very slowly over a period of 2.5 hours. The white suspension was stirred for 5 minutes at 0 °C and then at room temperature for 4.5 hours. The white precipitate was removed by filtration, and the solvent was removed under reduced pressure to give the crude product as a yellow oil. This was purified by flash column chromatography (eluted with 0-25% ethyl acetate in hexane) to give the product as a colourless oil (*impure* 1.12 g, 98.0%, containing approximately 5% zingerone by ^{1}H NMR and GC-FID, R_f: 0.21 (3:1 (v/v) hexane:ethyl acetate)). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.15 (3H, s, $COCH_3$), 2.76 (2H, t, *J* = 7.5 Hz, CH_2), 2.88 (2H, t, *J* = 7.4 Hz, CH_2), 3.82 (3H, s, OCH_3), 6.76 (1H, dd, *J* = 1.5, 8.1 Hz, Ar-H), 6.82 (1H, s, Ar-H), 6.99 (1H, d, *J* = 8.1 Hz, Ar-H), 8.24 (1H, s, CHO) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 29.6 (alkyl), 30.2 (alkyl), 45.2 ($CO\underline{C}H_2$), 56.0 (OCH_3), 113.0 (Ar), 120.6 (Ar), 122.5 (Ar), 137.2 (Ar), 140.8 (Ar), 150.7 (Ar), 159.4 ($H\underline{C}OO$), 207.7 (CO) ppm. GC-MS (EI) *m/z* (% of base peak): 222

($M^{\bullet+}$, 6.8), 194 ($M^{\bullet+}$-CO, 37.6), 151 ($M^{\bullet+}$-CO-$COCH_3$, 16.2), 137 ($M^{\bullet+}$-CO-CH_2COCH_3, 100.0), 124 (9.7), 122 (9.1), 119 (22.3), 91 (25.2), 79 (8.5), 77 (15.5), 65 (10.8), 51 (13.9). IR-ATR $\tilde{\nu}_{max}$: 2940 (C-H), 1738 (C=O, aldehyde), 1711 (C=O, ketone), 1604 (Ar C-C), 1508 (Ar C-C), 1311, 1151, 1125, 1095, 1030 cm^{-1}.

4-(4-Trifluoroacetoxy-3-methoxyphenyl)-2-butanone (4)

Trifluoroacetic anhydride (0.86 mL, 6.2 mmol, 1.2 eq) was added to an oven-dried flask containing zingerone (1.00 g, 5.15 mmol, 1.0 eq) under an inert argon atmosphere at room temperature. The orange solution was stirred at 90 °C for 3 hours. Trifluoroacetic acid was removed under reduced pressure and the crude product was purified by flash column chromatography (eluted with 0-30% ethyl acetate in hexane) to yield the product as a yellow liquid (0.816 g, 2.81 mmol, 54.6%, R_f: 0.23 (5:1 (v/v) hexane:ethyl acetate)). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.16 (3H, s, $COCH_3$), 2.78 (2H, t, J = 7.5 Hz, CH_2), 2.90 (2H, t, J = 7.4 Hz, CH_2), 3.83 (3H, s, OCH_3), 6.79 (1H, dd, J = 1.6, 8.1 Hz, Ar-H), 6.85 (1H, fine d, J = <1.5 Hz, Ar-H), 7.02 (1H, d, J = 8.1 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 29.7 (alkyl), 30.2 (alkyl), 45.1 (CO<u>C</u>H_2), 56.1 (OCH_3), 113.2 (Ar), 114.8 (q, J = 286.7 Hz, <u>C</u>F_3COO), 120.6 (Ar), 121.7 (Ar), 136.6 (Ar), 141.9 (Ar), 150.3 (Ar), 155.6 (q, J = 43.3 Hz, CF_3<u>C</u>OO), 207.6 (CO) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ -75.23 ppm. GC-MS (EI) *m/z* (% of base peak): 290 ($M^{\bullet+}$, 53.3), 247 ($M^{\bullet+}$-$COCH_3$, 100.0), 233 ($M^{\bullet+}$-CH_2COCH_3, 24.2), 229 (17.6), 150 ($M^{\bullet+}$-$COCH_3$-CF_3CO, 28.7), 119 (18.7), 91 (28.9), 79 (16.3), 77 (34.1), 69 (37.3), 65 (18.9), 51 (22.3). IR-ATR $\tilde{\nu}_{max}$: 2944 (C-H), 1800 (C=O, ester), 1716 (C=O, ketone), 1608 (Ar C-C), 1510 (Ar C-C), 1356, 1221, 1127, 1113, 1031 cm^{-1}.

4-Hydroxy-3-methoxybenzyl acetate (5)

To vanillyl alcohol (1.00 g, 6.49 mmol, 1.0 eq) was added potassium fluoride (0.500 g, 8.61 mmol, 1.3 eq), then glacial acetic acid (15.0 mL, 0.262 mol, 40 eq). The colourless solution was stirred at 80 °C for 6.5 hours. Water (60.0 mL) was then added and the crude product was extracted with ethyl acetate (3 ×60.0 mL). The combined organic layers were washed with $NaHCO_3$ solution (60.0 mL, 5% (w/v)), then brine (60.0 mL) and dried with $MgSO_4$. The solvent was then removed under reduced pressure to yield the crude product as a brown oil. The crude product was purified by flash column chromatography (eluted with 0-40% ethyl acetate in hexane) to yield the product as a very pale yellow oil (1.02 g, 5.18 mmol, 79.9%, R_f: 0.30 (3:1 (v/v) hexane:ethyl acetate)). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (3H, s, CH_3COO), 3.91 (3H, s, OCH_3), 5.02 (2H, s, $COOCH_2$), 5.66 (1H, br s, OH), 6.89 (3H, m, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 21.2 (<u>C</u>H_3COO), 56.1 (OCH_3), 66.7 (COO<u>C</u>H_2), 111.5 (Ar), 114.5 (Ar), 122.3 (Ar), 127.9 (Ar), 146.0 (Ar), 146.6 (Ar), 171.1 (<u>C</u>OO) ppm. Experimental spectral data were consistent with literature data.[62-63]

Zingerol (4-(4-hydroxy-3-methoxyphenyl)-2-butanol) (6)

To a solution of zingerone (1.00 g, 5.15 mmol, 1.0 eq) in methanol (2.00 mL) was added a suspension of sodium borohydride (0.150 g, 3.97 mmol, 3.1 eq) in methanol (1.00 mL). The yellow suspension was stirred at room temperature for 90 minutes. Water (10.0 mL) was then added to the stirring yellow suspension. The product was extracted with ethyl acetate (3 × 10.0 mL) and the combined organic layers were washed with saturated brine solution (3 × 30.0 mL) and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield the pure product as a very pale yellow oil (0.976 g, 4.97 mmol, 96.6%, R_f: 0.09 (3:1 (v/v) hexane:ethyl acetate)). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (3H, d, *J* = 6.2 Hz, CH_3), 1.75 (2H, m, CH_2), 2.65 (2H, m, CH_2), 3.83 (1H, m, C<u>H</u>OH), 3.88 (3H, s, OCH_3), 5.46 (1H, br s, OH), 6.69 (2H, m, Ar-H), 6.83 (1H, d, *J* = 7.7 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 23.8 (COH<u>C</u>H$_3$), 32.0 (Ar-CH_2), 41.3 (COH<u>C</u>H$_2$), 56.0 (OCH_3), 67.7 (COH), 111.1 (Ar), 114.4 (Ar), 121.0 (Ar), 134.1 (Ar), 143.8 (Ar), 146.6 (Ar) ppm. Experimental spectral data were consistent with literature data.[64]

Piperonal (3,4-methylenedioxybenzaldehyde) (16)

To a suspension of manganese dioxide (28.57 g, 0.329 mol, 10. eq) in DCM (250 mL) was added piperonyl alcohol (5.00 g, 32.9 mmol, 1.0 eq). The black suspension was heated to reflux for 6.5 hours. It was then filtered and the solvent removed under reduced pressure to yield a clear viscous oil. Further drying under high vacuum yielded the product as a white solid (4.48 g, 29.9 mmol, 90.8%, R_f: 0.42 (5:1 (v/v) hexane:ethyl acetate)). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.08 (2H, s, OCH_2O), 6.93 (1H, d, *J* = 7.9 Hz, Ar-H), 7.34 (1H, d, *J* = 0.9 Hz, Ar-H), 7.41 (1H, dd, *J* = 1.0, 8.0 Hz, Ar-H), 9.81 (1H, s, CHO) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 102.2 (OCH_2O), 107.1 (Ar), 108.5 (Ar), 128.8 (Ar), 132.0 (Ar), 148.9 (Ar), 153.2 (Ar), 190.4 (CHO) ppm. Experimental spectral data were consistent with literature data.[65-66] The crude product was sufficiently pure to be used to synthesise the methylenedioxy butenone (**13a**) by the Aldol reaction.

General Method for Synthesis of Substituted 4-Phenylbut-3-en-2-ones (8a-11a and 13a-14a)

To a solution of substituted benzaldehyde (1.00 g) in acetone (20.0 mL) was slowly added sodium hydroxide solution (5.00 mL, 10% (w/v)). The mixture was then stirred at 50 °C for 60 minutes. After removing the reaction mixture from heat, sufficient hydrochloric acid (1.0 mol L^{-1}) was added to achieve a pH of 1-2. The crude product was then extracted with ethyl acetate (3 × 30.0 mL). The combined organic layers were dried with $MgSO_4$ and the solvent removed under reduced pressure. Crude product (**8a-11a** and **13a-14a**) was sufficiently pure for the subsequent hydrogenation step.

4-(4-Hydroxy-2-methoxyphenyl)but-3-en-2-one (**10a**): reaction mixture was stirred at 50 °C for 24 hours.

4-(4-Hydroxy-3-(trifluoromethoxy)phenyl)but-3-en-2-one (**11a**): synthesised on a 0.500 g scale instead of 1.00 g scale. Reaction mixture was stirred at 50 °C for 36 hours.

4-(3,4-Methylenedioxyphenyl)but-3-en-2-one (**13a**): water (20.0 mL) was added instead of hydrochloric acid.

4-(3,4-(Difluoromethylenedioxy)phenyl)but-3-en-2-one (**14a**): water (20.0 mL) was added instead of hydrochloric acid.

1,1,1-Trifluoro-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (12a)

To a solution of vanillin (1.00 g, 6.57 mmol, 1.0 eq) in DCM (20.0 mL) was added pyrrolidine (1.08 mL, 12.9 mmol, 2.0 eq) in a single portion. The yellow solution was stirred at room temperature for 90 minutes. The brown solution was cooled to 0 °C, and then 1,1,1-trifluoroacetone (0.60 mL, 6.7 mmol, 1.0 eq) in cold DCM (5.0 mL, -78 °C) was slowly added to the solution over a period of 10 minutes. The dark brown solution was then allowed to return to room temperature. Four additional aliquots of 1,1,1-trifluoroacetone (0.60 mL, 6.7 mmol, 1.0 eq) in cold DCM (5.0 mL, -78 °C) were slowly added to the solution at 0 °C over a period of 10 minutes every 24 hours. The dark red solution was allowed to return to room temperature after each aliquot. After an additional 24 hours, a final aliquot of 1,1,1-trifluoroacetone (0.30 mL, 3.4 mmol, 0.5 eq) in cold DCM (5.0 mL, -78 °C) was slowly added to the solution at 0 °C over a period of 10 minutes. This dark red solution was then stirred at room temperature for 48 hours before glacial acetic acid (1.13 mL, 19.7 mmol, 3.0 eq) was added in a single portion. The dark red solution was washed with water (4 × 50.0 mL) and dried with anhydrous $MgSO_4$. The solvent was then removed under reduced pressure to yield the crude product as a dark red-brown solid, which was purified by flash column chromatography (eluted with 0-40% ethyl acetate in hexane). This yielded an impure yellow solid (approximately 50% pure), but was sufficiently pure for the subsequent step (2 g, <50%).

General Method for Synthesis of Substituted 4-Phenyl-2-butanones (8b-14b)

To a solution of substituted 4-phenylbut-3-en-2-one (**8a-14a**) in methanol (30.0 mL) was added powered Rh/Al_2O_3 (0.20 mol% of substituted benzaldehyde). The reaction vessel was evacuated and filled with hydrogen gas by a balloon. The mixture was stirred at room temperature for 60 minutes. The mixture was then filtered and the solvent removed under reduced pressure to yield the crude product. The crude product was purified by flash column

chromatography (eluted with 0-40% ethyl acetate in hexane) to yield pure substituted 4-phenyl-2-butanone.

4-(3,4-Dimethoxyphenyl)-2-butanone (**8b**): white solid, 1.02 g, 5.29 mmol, 81.4% overall yield, mp 55-56 °C (lit. mp 55-56 °C[61, 67]), R_f: 0.19 (4:1 (v/v) hexane:ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.14 (3H, s, $COCH_3$), 2.74 (2H, t, *J* = 7.1 Hz, CH_2), 2.85 (2H, t, *J* = 7.1 Hz, CH_2), 3.85 (3H, s, OCH_3), 3.87 (3H, s, OCH_3) 6.72 (2H, m, Ar-H), 6.79 (1H, d, *J* = 4.3 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 29.5 (alkyl), 30.3 (alkyl), 45.6 ($CO\underline{C}H_2$), 56.0 (OCH_3), 56.1 (OCH_3), 111.5 (Ar), 111.9 (Ar), 120.2 (Ar), 133.8 (Ar), 147.5 (Ar), 149.0 (Ar), 208.2 (CO) ppm. Experimental spectral data were consistent with literature data.[68-69]

4-(3-Hydroxy-4-methoxyphenyl)-2-butanone (**9b**): reaction mixture was stirred under hydrogen gas for 120 minutes instead of 60 minutes. Flash column chromatography was performed with 0-50% ethyl acetate in hexane. White solid, 1.06 g, 5.44 mmol, 82.8% overall yield, mp 36-37 °C (lit. mp 28-29 °C[70] and 41-42 °C[71]), R_f: 0.21 (3:1 (v/v) hexane:ethyl acetate), elemental analysis C: 68.00% H: 7.44% (calc. C: 68.02% H: 7.27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (3H, s, $COCH_3$), 2.71 (2H, t, *J* = 7.3 Hz, CH_2), 2.80 (2H, t, *J* = 7.3 Hz, CH_2), 3.86 (3H, s, OCH_3), 5.59 (1H, br s, OH), 6.65 (1H, dd, *J* = 2.0, 8.2 Hz, Ar-H), 6.76 (2H, m, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 29.3 (alkyl), 30.2 (alkyl), 45.4 ($CO\underline{C}H_2$), 56.1 (OCH_3), 110.8 (Ar), 114.6 (Ar), 119.8 (Ar), 134.4 (Ar), 145.1 (Ar), 145.7 (Ar), 208.3 (CO) ppm. Although no literature spectral data were available, experimental spectral data were consistent with that expected for the title compound.

4-(4-Hydroxy-2-methoxyphenyl)-2-butanone (**10b**): reaction mixture was stirred under hydrogen gas for 120 minutes instead of 60 minutes. Flash column chromatography was performed with 0-50% ethyl acetate in hexane. White solid, 1.06 g, 5.44 mmol, 82.7% overall yield, mp 113-115 °C (lit. mp 116-118 °C[72]), R_f: 0.19 (3:1 (v/v) hexane:ethyl acetate), elemental analysis C: 67.82% H: 7.34% (calc. C: 68.02% H: 7.27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (3H, s, $COCH_3$), 2.68 (2H, t, *J* = 7.4 Hz, CH_2), 2.80 (2H, t, *J* = 7.3 Hz, CH_2), 3.78 (3H, s, OCH_3), 5.01 (1H, br s, OH), 6.31 (1H, dd, *J* = 2.4, 8.1 Hz, Ar-H), 6.39 (1H, d, *J* = 2.4 Hz, Ar-H), 6.95 (1H, d, *J* = 8.1 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 24.6 (Ar-CH_2), 30.1 ($CO\underline{C}H_3$), 44.2 ($CO\underline{C}H_2$), 55.3 (OCH_3), 99.0 (Ar), 106.7 (Ar), 121.3 (Ar), 130.5 (Ar), 155.6 (Ar), 158.5 (Ar), 209.9 (CO) ppm. Although no literature spectral data were available, experimental spectral data were consistent with that expected for the title compound.

4-(4-Hydroxy-3-(trifluoromethoxy)phenyl)-2-butanone (**11b**): synthesised half scale; 15.0 mL of methanol instead of 30.0 mL. White solid, 0.483 g, 1.95 mmol, 80.2% overall yield, mp 59-61 °C, R_f: 0.24 (4:1 (v/v) hexane:ethyl acetate), elemental analysis C: 53.31% H: 3.94% (calc.

C: 53.23% H: 4.47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.14 (3H, s, $COCH_3$), 2.73 (2H, t, *J* = 7.2 Hz, CH_2), 2.83 (2H, t, *J* = 7.1 Hz, CH_2), 5.67 (1H, br s, OH), 6.93 (1H, d, *J* = 8.3 Hz, Ar-H), 7.00 (1H, dd, *J* = 1.7, 8.3 Hz, Ar-H), 7.03 (1H, br s, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 28.8 (alkyl), 30.2 (alkyl), 45.2 ($CO\underline{C}H_2$), 117.5 (Ar), 120.8 (q, *J* = 259.9 Hz, OCF_3), 121.4 (Ar), 128.0 (Ar), 133.9 (Ar), 136.4 (*J* = 1.3 Hz, $\underline{Ar}$-OCF_3), 146.3 (Ar), 208.2 (CO) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ -58.64 ppm. GC-MS (EI) *m/z* (% of base peak): 248 ($M^{\bullet+}$, 100.0), 233 ($M^{\bullet+}$-CH_3, 18.7), 191 ($M^{\bullet+}$-CH_2COCH_3, 85.8), 171 (78.9), 105 (53.9), 91 (63.8), 89 (24.5), 77 (67.8), 69 (18.3), 65 (40.0), 63 (19.8), 51 (53.1). IR-ATR $\tilde{\nu}_{max}$: 3304 (O-H), 2939 (C-H), 1693 (C=O, ketone), 1520 (Ar C-C), 1243, 1199, 1163, 1144, 1114, 819 cm^{-1}.

1,1,1-Trifluoro-4-(4-hydroxy-3-methoxyphenyl)-2-butanone (**12b**): the amount of Rh/Al_2O_3 was 0.20 mol% assuming 50% yield from first step. Reaction mixture was stirred under hydrogen gas for 120 minutes instead of 60 minutes. Product was purified by flash column chromatography (eluted with 0-40% ethyl acetate in hexane) twice. Yellow oil, 0.510 g, 2.05 mmol, 31.2% overall yield, R_f: 0.10 (4:1 (v/v) hexane:ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.93 (2H, t, *J* = 7.2 Hz, CH_2), 3.02 (2H, t, *J* = 7.3 Hz, CH_2), 3.88 (3H, s, OCH_3), 5.51 (1H, br s, OH), 6.69 (2H, m, Ar-H), 6.85 (1H, fine dd, *J* = <1.5 Hz, 7.8 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 28.2 (Ar-CH_2), 38.6 ($CO\underline{C}H_2$), 56.0 (OCH_3), 111.1 (Ar), 114.7 (Ar), 115.7 (q, *J* = 293.3 Hz, $CO\underline{C}F_3$), 121.0 (Ar), 131.3 (Ar), 144.5 (Ar), 146.7 (Ar), 190.9 (q, *J* = 35.4 Hz, $\underline{C}OCF_3$) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ -80.03 ppm. GC-MS (EI) *m/z* (% of base peak): 248 ($M^{\bullet+}$, 24.4), 137 ($M^{\bullet+}$-CH_2COCF_3, 100.0), 122 ($M^{\bullet+}$-CH_3COCF_3, 14.2), 107 (7.7), 94 (9.0), 91 (7.2), 79 (8.4), 77 (11.6), 65 (7.4), 55 (7.9), 53 (7.4), 51 (10.8). IR-ATR $\tilde{\nu}_{max}$: 3450 (O-H), 2943 (C-H), 1761 (C=O, ketone), 1613 (Ar C-C), 1515 (Ar C-C), 1203, 1127, 1032, 992, 815, 791 cm^{-1}.

4-(3,4-Methylenedioxyphenyl)-2-butanone (**13b**): reaction mixture was stirred under hydrogen gas for 90 minutes instead of 60 minutes. White solid, 1.10 g, 5.73 mmol, 86.1% overall yield, mp 44-46 °C (lit. mp 50 °C[73] and 55 °C[74]), R_f: 0.37 (5:1 (v/v) hexane:ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (3H, s, $COCH_3$), 2.71 (2H, t, *J* = 7.1 Hz, CH_2), 2.81 (2H, t, *J* = 7.1 Hz, CH_2), 5.91 (2H, s, OCH_2O), 6.62 (1H, dd, *J* = 1.4, 7.9 Hz, Ar-H), 6.67 (1H, d, *J* = 1.2 Hz, Ar-H), 6.72 (1H, d, *J* = 7.9 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 29.6 (alkyl), 30.3 (alkyl), 45.6 ($CO\underline{C}H_2$), 101.0 (OCH_2O), 108.4 (Ar), 108.9 (Ar), 121.2 (Ar), 134.9 (Ar), 146.0 (Ar), 147.8 (Ar), 208.1 (CO) ppm. Experimental spectral data were consistent with literature data.[68]

4-(3,4-(Difluoromethylenedioxy)phenyl)-2-butanone (**14b**): the crude product was purified by Kugelrohr distillation instead of flash column chromatography. Colourless liquid, 0.836 g, 3.66 mmol, 68.1% overall yield, R_f: 0.36 (5:1 (v/v) hexane:ethyl acetate). ^{1}H NMR (400 MHz,

$CDCl_3$): δ 2.14 (3H, s, $COCH_3$), 2.74 (2H, t, *J* = 7.3 Hz, CH_2), 2.88 (2H, t, *J* = 7.3 Hz, CH_2), 6.87 (1H, dd, *J* = 1.5, 8.1 Hz, Ar-H), 6.91 (1H, d, *J* = 1.6 Hz, Ar-H), 6.94 (1H, d, *J* = 8.2 Hz, Ar-H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 29.5 (alkyl), 30.2 (alkyl), 45.2 ($CO\underline{C}H_2$), 109.4 (Ar), 109.8 (Ar), 123.4 (Ar), 131.8 (t, *J* = 255.8 Hz, OCF_2O), 137.3 (Ar), 142.3 (Ar), 144.0 (Ar), 207.4 (CO) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ -50.82 ppm. GC-MS (EI) *m/z* (% of base peak): 228 ($M^{\bullet+}$, 99.9), 185 ($M^{\bullet+}$-$COCH_3$, 59.7), 171 ($M^{\bullet+}$-CH_2COCH_3, 100.0), 119 (22.8), 105 (30.6), 91 (59.7), 89 (25.7), 77 (49.3), 65 (40.8), 63 (32.9), 51 (60.6), 50 (17.7). IR-ATR $\tilde{\nu}_{max}$: 2935 (C-H), 1715 (C=O, ketone), 1498 (Ar C-C), 1447, 1232, 1143, 1035, 805, 703 cm^{-1}.

2.2 Vapour Pressure Measurements

Vapour pressure measurements were conducted on a TA Instruments 2010 DSC equipped with a standard DSC cell. Vacuum was achieved with a Vacuubrand MD4 diaphragm vacuum pump between 15 kPa and 0.5 kPa and an Edwards E2M-1.5 high vacuum pump between 0.5 kPa and 0.15 kPa. The pressure was regulated by a needle valve to balance inflow and outflow. The absolute pressure was measured by an Edwards Active Pirani Gauge APG-L-NW16.

Calibration of the DSC was performed in accordance with ASTM E967-08.[75] Temperature calibration was conducted with indium and lead. The pressure gauge was calibrated after temperature calibration of the DSC by measuring the boiling point of 1-octanol under different reduced pressures between atmospheric and 0.14 kPa and comparing the measured pressure to literature pressure-boiling data for 1-octanol.[76]

Samples of 8-14 mg were weighed on a micro-analytical balance with a precision of ±0.01 mg and placed in hermetic aluminium pans (TA Instruments) and sealed hermetically with hermetic lids (TA Instruments) that had pinholes of various sizes. For pressures between atmospheric and 7 kPa, pinholes of 75 µm were used. Use of pressures below 7 kPa required pinholes smaller than 75 µm. These pinhole lids were prepared by manually punching the lids with a needle and the pinhole size was determined by microscopy. For pressures between 7 kPa and 1 kPa, pinholes ranging from approximately 290 µm to 340 µm were used. Below 1 kPa, use of larger pinholes ranging from approximately 460 µm to 540 µm was necessary. Due to reduced thermal contact between the pans and the sample and reference platforms at reduced pressures, thermally conductive paste was applied to the pans and platforms for pressures <7 kPa.

The operation of the DSC for vapour pressure measurements was performed in accordance with ASTM E1782-14 with a modified pressure range (15 kPa to 0.15 kPa). After achieving the desired pressure, the sample in the DSC was rapidly heated to approximately 50 °C below its expected boiling point and allowed to equilibrate. Once equilibrated, heating at 5 °C min^{-1} was

initiated and maintained until a stable baseline was achieved after the sample boiled. The pressure was measured when the sample began to boil.

The pressure-boiling point data from the DSC measurements were fitted to the Antoine Equation (1) to obtain a vapour pressure curve for each compound.

$$\log P = A - \frac{B}{T+C} \quad (1)$$

where P is pressure (kPa), T is temperature (K), and A, B, and C are the Antoine parameters.

The Antoine parameters were calculated by iterative least squares nonlinear regression using MATLAB R2015a (The MathWorks, Inc.). The vapour pressure and volatility of the compounds at room temperature (298.15 K) were calculated using the Antoine Equation (1) and equation (2) respectively.

$$Volatility = \frac{PM}{RT} \times 10^6 \quad (2)$$

where *volatility* is given in mg m^{-3}, P is pressure (kPa), M is molar mass (g mol^{-1}), R is the ideal gas constant (J K^{-1} mol^{-1}), and T is temperature (K).

2.3 Biological Testing

2.3.1 Electroantennography (EAG)

Whole male Queensland fruit fly (*B. tryoni*) and Jarvis' fruit fly (*B. jarvisi*) heads were removed and placed between the electrodes of a Syntech EAG Combi probe antenna holder and held in place with electrode gel. Humidified air was used as the carrier for the compound vapours and controlled by a Syntech Stimulus Controller CS-55. The EAG signal was passed through a Syntech IDAC 4 and analysed using GC-EAD 2010 software version 1.2.2. A simple diagram of the EAG configuration is shown in Figure 8.

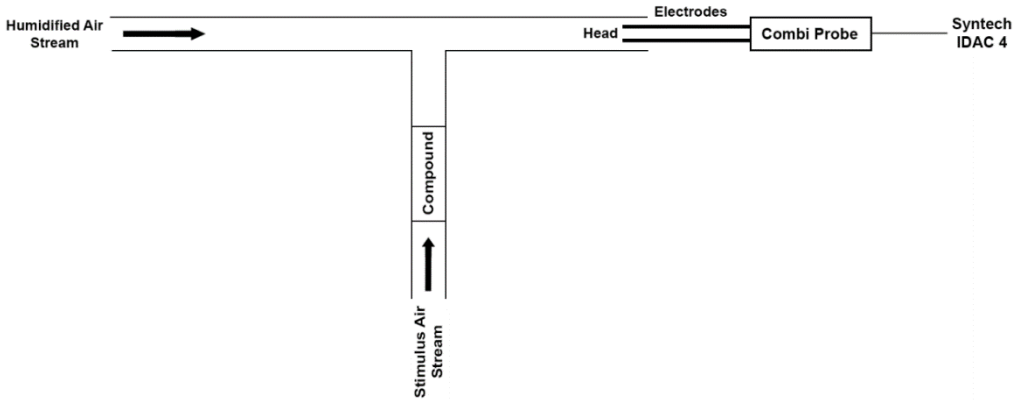


Figure 8 Simple diagram of EAG configuration.

2.3.2 Laboratory Cage Bioassays

2.3.2.1 Preliminary Cage Bioassay

Three 20-L screened cages containing approximately 300-500 12 day old sexually mature male and female *B. tryoni* were placed in a temperature controlled room (25 °C). Four to six hours into photophase two or three folded filter papers containing 5 ± 1 mg of compounds **1**-**5**, **7**-**10b**, and **12b**-**15** were positioned in the cages. Attraction of flies to compounds was monitored visually over 0.5-1.0 hours.

2.3.2.2 Large Cage Bioassay

Ten Lynfield traps were placed in a 3×3×3 m mesh cage in an environment controlled room (25 °C, 65% humidity). Each trap contained a sticky insert and a rolled filter paper under the lid which was treated with 20 ± 1 mg of compound, except for the control which was untreated. The compounds were investigated in two groups – compounds **2**-**8b** and **9b**-**15**, with each group having 7 traps for compounds and 1 trap each for a control, zingerone (**1**), and cuelure. Traps were placed in the cage in random positions in a 3-4-3 grid. At the beginning of photophase, 250 male and 250 female sexually mature *B. tryoni* of age 13-19 days were released into the cage. The number of male and female flies caught by the traps after 4 hours and 24 hours was counted. Two replicates were performed.

Chapter 3. Results and Discussion

3.1 Selection and Synthesis of Zingerone Analogues

3.1.1 Selection of Zingerone Analogues

Numerous studies have investigated raspberry ketone and methyl eugenol analogues as fruit fly attractants. Some of these studies have conducted basic structure-activity relationship (SAR) investigations into the raspberry ketone and methyl eugenol receptors. The identification of zingerone as a fruit fly attractant is much more recent than for raspberry ketone, cuelure, and methyl eugenol, and therefore no studies have examined zingerone analogues as potential attractants. Furthermore, no SAR or volatility studies have been conducted for zingerone or analogues relating to fruit fly attraction. As described in the Introduction chapter, the presence of a benzene ring, small hydrophilic *para* substituent, and carbonyl group appear to be important for attraction to raspberry ketone analogues. Similarly, the presence of a benzene ring, unsaturated 3 atom chain, and two adjacent methoxy groups on the benzene ring, appear to be important for methyl eugenol analogue attraction.

An initial range of zingerone analogues was proposed, where the analogues were subdivided into three categories based on the type of change to the basic zingerone structure – changes related to the phenol, the central ring, and carbon chain. Figure 9 illustrates some of the proposed zingerone analogues, including esters, an amide, various substituted benzenes, chain extensions, chain contractions, and fluorinated functional groups. These proposed analogues were chosen for a basic SAR investigation to allow a greater understanding of the important features for binding to the receptor and to increase volatility compared to zingerone.

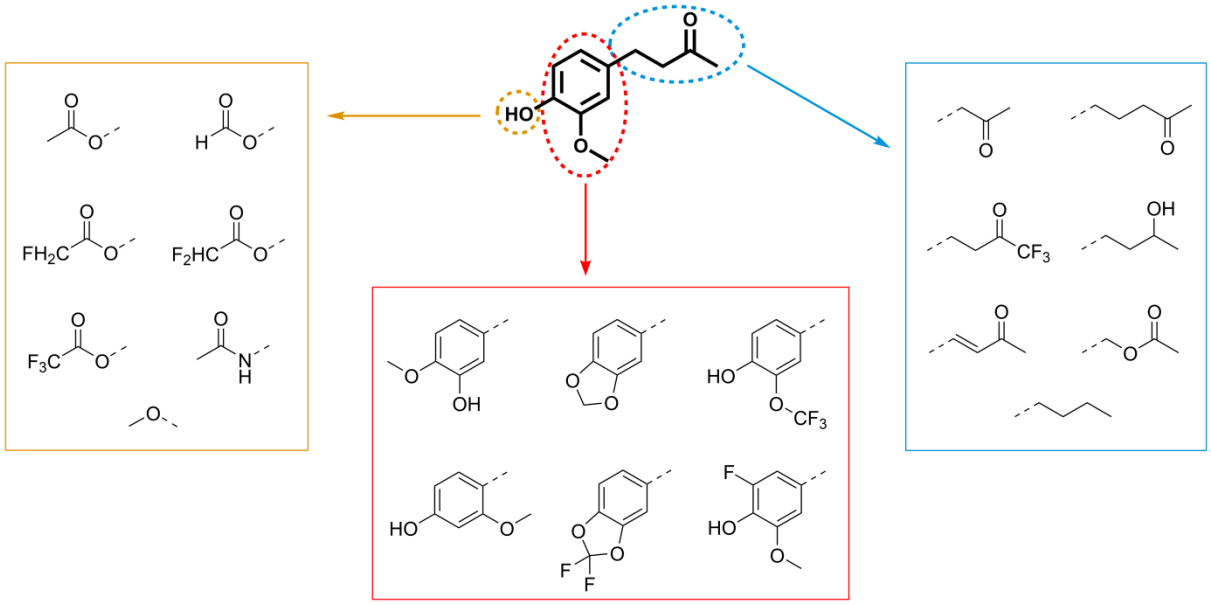


Figure 9 Structures of potential zingerone analogues. Dashed bonds refer to the remainder of the zingerone molecule.

From an SAR perspective, the acetyl and formyl esters were chosen to examine the importance of the free phenolic group (esters remove hydrogen bond donation and decrease hydrogen bond accepting properties) and begin to investigate steric factors around the phenolic oxygen. The amide was chosen as an isostere of the acetyl ester. The methyl ether was chosen as methylation of phenolic groups removes hydrogen bond donating properties and it has less steric bulk than the acetyl or formyl esters. Two of the proposed analogues have the phenolic and methoxy groups in different positions on the benzene ring, which allows the importance of the *para* and *meta* positions to be investigated. The methylenedioxy functional group was chosen as a potential zingerone analogue because the presence of the ring reduces possible conformations of the *para* and *meta* substituents; a particular conformation may be critical for effective receptor binding and attraction. Similarly, the butenone analogue rigidifies the carbon chain restricting the conformations of the chain. The chain extension and contraction analogues move the position of the carbonyl group; the carbonyl group has been shown to be important for attraction in raspberry ketone SAR studies. The butanol analogue was proposed as it replaces the carbonyl group with a secondary alcohol and would allow the importance of the carbonyl group to be confirmed. Similarly, the carbonyl group is completely removed in the butane analogue. The difference in attraction to the butanol and butane analogues would suggest whether the carbonyl group is important for dipole-dipole interactions. The benzyl acetate analogue was proposed as it is an isostere of zingerone with a methylene group adjacent to the carbonyl replaced by an oxygen atom yielding an ester.

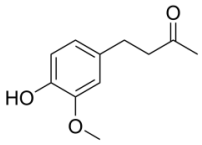
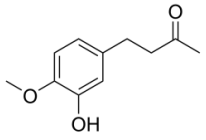
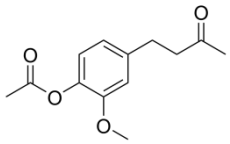
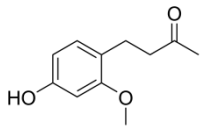
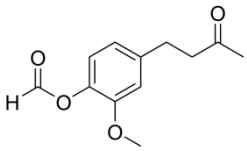
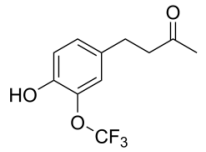
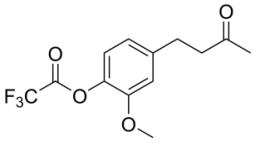
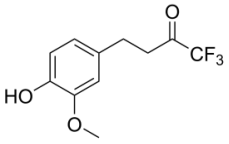
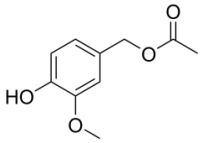
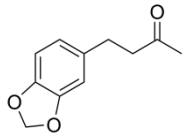
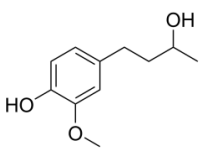
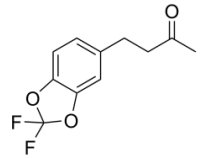
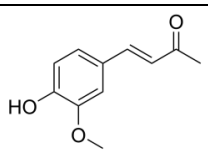
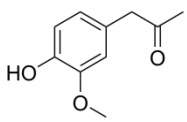
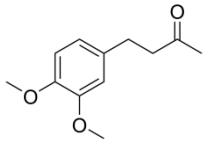
The proposed analogues, especially the fluorinated analogues, were also chosen for having potentially greater volatility compared to zingerone. The importance of volatility in constructing an effective fruit fly attractant relates to the requirement for the attractant compound to vaporise and be transported through the air in order to be detected by a fruit fly. Some analogues with fluorine functional groups were chosen because the presence of fluorine in organic compounds is known to increase volatility by reducing intermolecular interactions and is likely due to the very low polarisability of fluorine.[49-52] The strong electronegativity of fluorine will also affect the electronic properties of adjacent functional groups. The proposed fluorinated analogues include mono-, di-, and trifluoroacetyl esters, a difluoromethylenedioxy analogue, trifluoromethoxy- and fluorobenzene analogues, and a trifluorobutanone analogue. The diversity of fluorine positions allows for the impact of fluorination on attraction and volatility to be evaluated.

An alternate strategy to increase volatility is by eliminating the intermolecular hydrogen bonding present in zingerone. Intermolecular hydrogen bonding is a significant contributor to the total intermolecular interactions between neutral organic molecules. Since the strength of the intermolecular interactions between molecules determines the volatility of a compound, removal of this hydrogen bonding would increase volatility. The effectiveness of this can be seen by the acetylation of raspberry ketone to the acetyl ester cuelure. Indeed, cuelure is

estimated to be 20 times as volatile as raspberry ketone.[10] Many of the proposed analogues do not possess the phenolic group of zingerone, including all the phenolic esters, the amide analogue, the methylated phenol analogue, and the two methylenedioxy analogues.

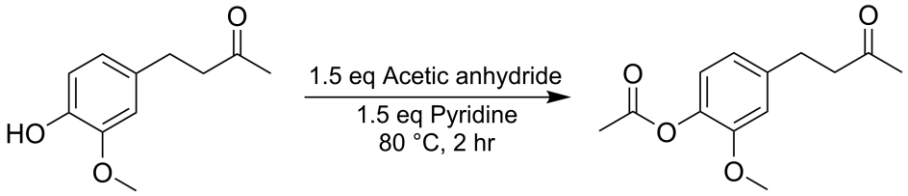
A final list of zingerone analogues to be investigated is presented in Table 1 and was chosen by considering the feasibility of synthesising the proposed analogues, accessibility of starting materials and reagents, and still allowing for a moderate diversity of functional groups/structures. From the list of proposed analogues, the pentanone analogue was also not chosen as there was no obvious synthetic route to this compound. The amide and fluorobenzene analogues were not selected due to the inaccessibility of starting materials. The mono- and difluoroacetyl esters were not selected in favour of the trifluoroacetyl ester, which would be most different from the acetyl and formyl esters.

Table 3 Zingerone analogues investigated in this study.

Number	Name	Chemical Structure	Number	Name	Chemical Structure
1	Zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone)		**9b**	Isozingerone 4-(3-Hydroxy-4-methoxyphenyl)-2-butanone	
2	4-(4-Acetoxy-3-methoxyphenyl)-2-butanone		**10b**	4-(4-Hydroxy-2-methoxyphenyl)-2-butanone	
3	4-(4-Formoxy-3-methoxyphenyl)-2-butanone		**11b**	4-(4-Hydroxy-3-(trifluoromethoxy)-phenyl)-2-butanone	
4	4-(4-Trifluoroacetoxy-3-methoxyphenyl)-2-butanone		**12b**	1,1,1-Trifluoro-4-(4-hydroxy-3-methoxyphenyl)-2-butanone	
5	4-Hydroxy-3-methoxybenzyl acetate		**13b**	4-(3,4-Methylenedioxy-phenyl)-2-butanone	
6	Zingerol (4-(4-Hydroxy-3-methoxyphenyl)-2-butanol)		**14b**	4-(3,4-(Difluoromethylene-dioxy)phenyl)-2-butanone	
7	Dehydrozingerone (4-(4-Hydroxy-3-methoxyphenyl)but-3-en-2-one)		**15**	4-Hydroxy-3-methoxyphenylacetone	
8b	Methylzingerone 4-(3,4-Dimethoxyphenyl)-2-butanone				

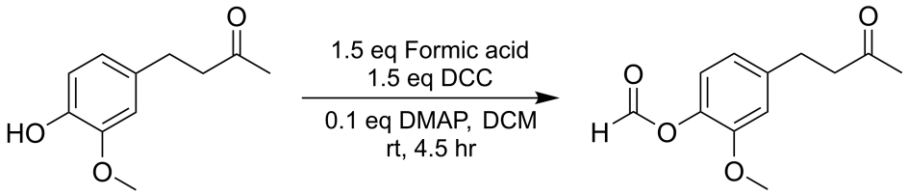
3.1.2 Proposed Synthetic Methods

The synthetic procedures selected for all the analogues were based on literature procedures and generally relied on readily available reagents. The chosen route for the synthesis of the acetyl ester of zingerone (**2**) was the acetylation of zingerone by acetic anhydride in the presence of pyridine (Scheme 1). This method was adapted from Verley and Bölsing[77]. It has not been used for the synthesis of the acetyl ester (**2**), but **2** is a known compound.



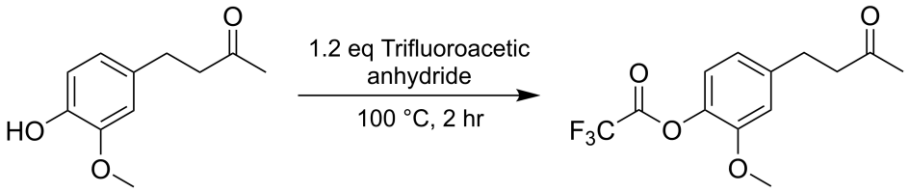
Scheme 1 Synthetic method for the synthesis of the acetyl ester (**2**).

Synthesis of the formyl ester (**3**) using an analogous method to above was not chosen due to the lack of commercial availability of formic anhydride and its risk in handling – readily decomposing to carbon monoxide. Instead, esterification of zingerone with formic acid was considered to be a more appropriate route. Steglich esterification was chosen due to the availability of the necessary reagents and previous experience in synthesising the formyl ester of raspberry ketone (melolure) by the same method. This reaction uses DCC to couple formic acid and zingerone. The reaction scheme for **3** is shown in Scheme 2 and is adapted from the procedure in Neises and Steglich[78]. The formyl ester (**3**) is a novel compound and therefore has not been previously synthesised.



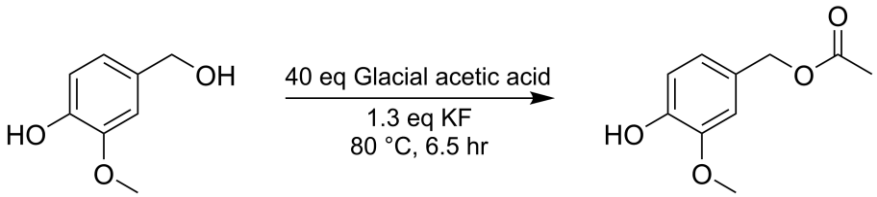
Scheme 2 Synthetic method for the synthesis of the formyl ester (**3**).

Synthesis of the trifluoroacetyl ester (**4**), which is a novel compound, was proposed to occur in neat trifluoroacetic anhydride, as illustrated in Scheme 3. Pyridine was not regarded as necessary due to the greater reactivity of trifluoroacetic anhydride over acetic anhydride. The absence of pyridine would also make subsequent workup easier to perform. The chosen method is adapted from Clark and Simons[79].



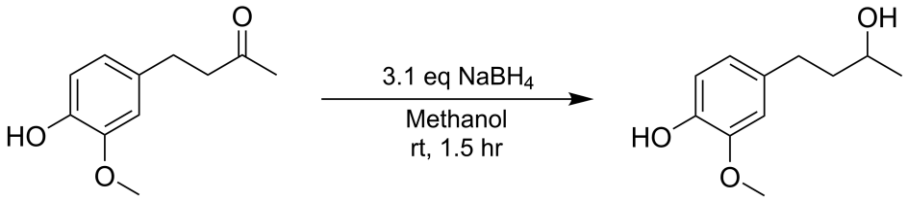
Scheme 3 Synthetic method for the synthesis of the trifluoroacetyl ester analogue (**4**).

Synthesis of the benzyl acetate (**5**), which is a known compound, was proposed to occur by selectively acetylating the commercially available compound, 4-hydroxy-3-methoxybenzyl alcohol, with acetic acid in the presence of potassium fluoride (Scheme 4), using the method of Bosco, *et al.*[80]. Although Bosco, *et al.*[80] did not synthesise **5**, they have used this method to successful acetylate various primary and secondary alcohols in the presence of phenols.



Scheme 4 Synthetic method for the synthesis of the benzyl acetate analogue (**5**).

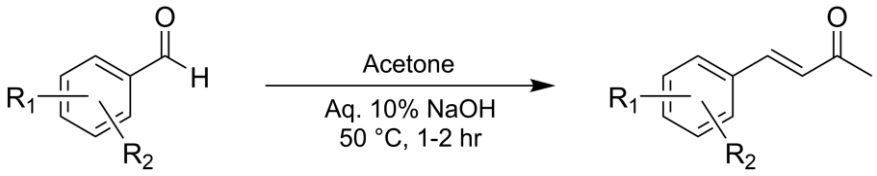
Sodium borohydride is commonly used for reduction of ketones to alcohols, and is a readily available, safer alternative to other stronger reducing agents, such as lithium aluminium hydride. Thus, zingerol (**6**) was proposed to be synthesised by the procedure used by Kitayama, *et al.*[81], as shown in Scheme 5. Zingerol (**6**) is a known compound and has been previously synthesised by this method.



Scheme 5 Synthetic method for the synthesis of zingerol (**6**).

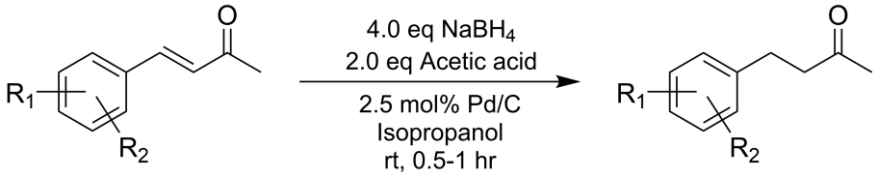
Synthesis of the butenones of methylzingerone (**8a**), isozingerone (**9a**), the 2-methoxy analogue (**10a**), the trifluoromethoxy analogue (**11a**), the methylenedioxy analogue (**13a**), and the difluoromethylenedioxy analogue (**14a**) was expected to be efficiently conducted by an Aldol condensation of commercially available benzaldehydes with acetone, followed by hydrogenation. The trifluorobutenone analogue (**12a**) was proposed to be synthesised by replacing acetone with 1,1,1-trifluoroacetone. Due to commercial unavailability, it was necessary to synthesise the methylenedioxy analogue (**13a**) starting material (piperonal, **16**). There are many examples of this Aldol-hydrogenation route in the literature, such as the

synthesis of zingerone (**1**),[64, 82-85] methylzingerone (**8b**),[68-69] and the methylenedioxy analogue (**13b**).[68] Scheme 6 presents the general method for the proposed Aldol synthesis of **8a**-**14a** and is adapted from the literature examples above. Analogues **8a**-**10a** and **12a**-**13a** are known compounds, whereas the trifluoromethoxy analogue (**11a**) and the difluoromethylenedioxy analogue (**14a**) are novel compounds.



Scheme 6 Synthetic method for the synthesis of **8a** (R_1 = 4-OCH_3, R_2 = 3-OCH_3), **9a** (R_1 = 4-OCH_3, R_2 = 3-OH), **10a** (R_1 = 4-OH, R_2 = 2-OCH_3), **11a** (R_1 = 4-OH, R_2 = 3-OCF_3), **12a** (trifluoroacetone instead of acetone, R_1 = 4-OH, R_2 = 3-OCH_3, 1,1,1-trifluorobut-3-en-2-one), **13a** (R_1 = R_2 = 3,4-OCH_2O), and **14a** (R_1 = R_2 = 3,4-OCF_2O).

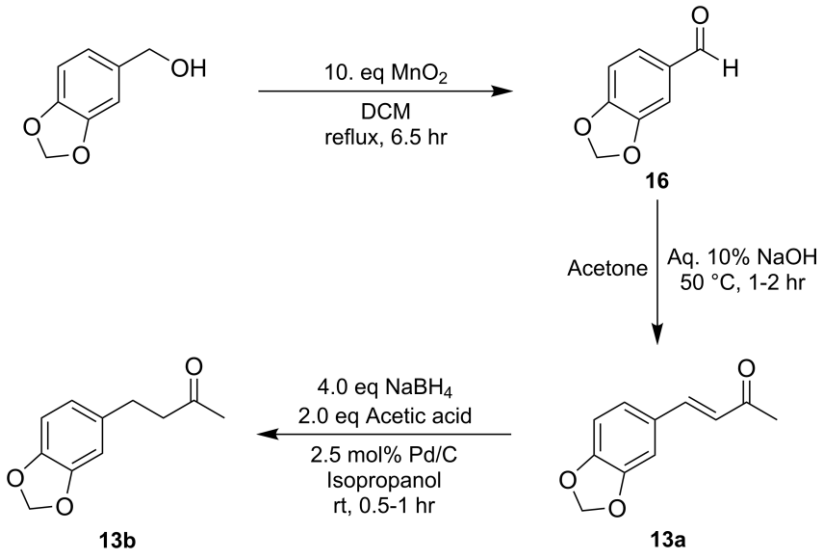
Synthesis of the butanones methylzingerone (**8b**), isozingerone (**9b**), the 2-methoxy analogue (**10b**), the trifluoromethoxy analogue (**11b**), the methylenedioxy analogue (**13b**), and the difluoromethylenedioxy analogue (**14b**) was expected to be readily obtained by hydrogenation of the unsaturated intermediates (**8a**-**14a**). Various methods have been reported for such reductions of the alkene selectively over the carbonyl group, including with sodium borohydride and palladium,[86] triethylsilane and palladium,[87] and catalytic hydrogenation.[64, 68-69, 82-85, 88] The sodium borohydride and palladium catalysed reduction was chosen because it used only solid reagents, which are easier to handle, and did not use extremely flammable hydrogen gas. Scheme 7 presents the synthesis of **8b**-**14b** by the sodium borohydride and palladium catalysed reduction of Russo, *et al.*[86]. Analogues **8b**-**10b** and **13b** are known compounds, whereas the trifluoromethoxy analogue (**11a**), trifluorobutanone analogue (**12b**), and the difluoromethylenedioxy analogue (**14a**) are novel compounds.



Scheme 7 Synthetic method for the synthesis of **8b** (R_1 = 4-OCH_3, R_2 = 3-OCH_3), **9b** (R_1 = 4-OCH_3, R_2 = 3-OH), **10b** (R_1 = 4-OH, R_2 = 2-OCH_3), **11b** (R_1 = 4-OH, R_2 = 3-OCF_3), **12b** (R_1 = 4-OH, R_2 = 3-OCH_3, 1,1,1-trifluoro-2-butone), **13b** (R_1 = R_2 = 3,4-OCH_2O), and **14b** (R_1 = R_2 = 3,4-OCF_2O).

Synthesis of the methylenedioxy analogue (**13b**) was proposed to occur by oxidation of a commercially available alcohol (piperonyl alcohol), followed by Aldol condensation with acetone and hydrogenation. Manganese dioxide was regarded as the oxidant of choice for the oxidation due to its availability and weak oxidation properties; oxidising the primary alcohol only to an aldehyde and not further to a carboxylic acid. Manganese dioxide is also a

heterogeneous oxidant, which simplifies workup as the excess and spent oxidant can be removed by filtration. The synthesis of the methylenedioxy analogue (**13b)** from piperonyl alcohol is illustrated in Scheme 8 where the oxidation reaction is adapted from Poli and Giambastiani[65], the Aldol reaction is adapted from various literature examples, and the hydrogenation reaction is from Russo, *et al.*[86]. Piperonal (**16**) is a known compound and has been previously synthesised by this method.



Scheme 8 Synthetic method for the synthesis of the methylenedioxy analogue (**13b**) from piperonyl alcohol.

3.1.3 Discussion of Synthetic Methods

The reactions performed in this study were not optimised due to time constraints and it was not a major aim of this study, and can likely be improved.

The synthesis of the acetyl ester (**2**) by the acetylation of zingerone with acetic anhydride and pyridine proceeded well and purification by flash column chromatography was sufficient, giving a yield of 82.2%. The formation of **2** was identified by the disappearance of the ^{1}H NMR phenolic group signal and appearance of the methyl group signal from the acetyl ester.

Synthesising the formyl ester (**3**) was more troublesome in that the reaction did not go to completion and the yield was variable despite the proportion of formic acid and DCC being increased. Extended reaction times also did not improve the reaction progress. Although the reaction did not achieve completion, the conversion was typically 95% by GC-FID and ^{1}H NMR within 3-6 hours. The formation of the formyl ester (**3**) was characterised by the disappearance of the ^{1}H NMR phenolic group signal and appearance of the formyl ester signal. The greatest difficulty with this reaction lay with the separation of the formyl ester (**3**) from zingerone, the starting material. The incomplete reaction would be acceptable if the product could be adequately separated. However, due to the very similar polarity of zingerone and **3**, separation by column chromatography was inadequate. On a TLC plate (hexane-ethyl acetate

mobile phase), zingerone and the formyl ester (**3**) have identical R_f values. While very slow flash column chromatography was found to give some separation of the two components, this was minimal, with zingerone contaminating all but the very last fractions. This could be the result of zingerone trailing due to exchange between the phenolic group of zingerone and the silica. If this was the case, addition of acid to the mobile phase would prevent the trailing. However, acid was not added due to the risk of hydrolysing the ester. The most successful attempt to synthesise the formyl ester (**3**) had a very good yield of 98.0% but contained approximately 5% zingerone.

Derivatisation of the unreacted zingerone in the impure formyl ester (**3**) was attempted to change the polarity of the zingerone contaminant, but derivatisation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, methyl iodide, and trifluoroacetic anhydride to produce trimethylsilyl-zingerone, methylzingerone (**8b**), and the trifluoroacetyl analogue (**4**) respectively, were all unsuccessful and appeared to promote the degradation of the ester **3** to zingerone. Kugelrohr distillation was also attempted but the proportion of zingerone was consistently 5-10%. This inability to separate the two components by distillation may indicate that the volatility of zingerone and **3** is similar or they form an azeotrope, or that the formyl ester (**3**) is not thermally stable at elevated temperatures and is degrading to zingerone.

Given the difficulty of separating zingerone and **3** it would seem a better strategy would be to use a synthetic method that went to completion. Optimisation of the Steglich esterification may be able to achieve a greater conversion of zingerone to the formyl ester (**3**).

The synthesis of the trifluoroacetyl ester (**4**) was complicated by purification rather than the actual reaction. The reaction of zingerone with neat trifluoroacetic anhydride at 90 °C for 3 hours appeared to reach completion based on TLC. In ^{1}H NMR, the formation of **4** was identified by the disappearance of the phenolic group signal. Given that a trifluromethyl group is strongly electron-withdrawing, the trifluoroacetyl ester (**4**) would be expected to be extremely susceptible to hydrolysis even without a catalyst. In light of this anticipated instability, measures were implemented to prevent the ester coming into contact with moisture during and after the reaction. Initially, distillation was the chosen purification method; however, like the formyl ester (**3**), the proportion of zingerone remained at 5-10% even after several rounds of distillation. The trifluoroacetyl ester (**4**) should be much more volatile than zingerone as it is fluorinated, thus, it seems probable that the ester was thermally unstable and degrading to zingerone. Supporting this is the observation that occasionally during distillation if the temperature was increased some of the yellow oil would begin to become brown and solidify. Silica column chromatography was then considered as a purification method, but flash column chromatography caused some hydrolysis such that all of the ester fractions contained zingerone.

Silica is naturally weakly acidic and may be catalysing the hydrolysis of the ester with any adsorbed water. The silica can be buffered to a variety of pH values, and while neutral silica would reduce the hydrolysis catalysis, the high susceptibility of the ester to hydrolysis means that it is likely to hydrolyse at any pH whilst water is present. The water adsorbed to the silica can be removed by drying the silica. Drying the silica did result in less hydrolysis, but the silica, which was activated by drying, catalysed many side reactions, forming at least 20 different products as determined by GC-MS. Despite this, some fractions were pure, providing a yield of 54.6% of the trifluoroacetyl ester (**4**).

Solid-phase supports were investigated as an alternative to distillation or silica column chromatography. The reason for considering solid-phase supports was to exploit the different properties of zingerone and the trifluoroacetyl ester (**4**). Unlike **4**, zingerone can act as a hydrogen bond donor or proton donor due to the weakly acidic phenolic group, either of which may be able to bind zingerone to a solid-phase support. The difficulties with silica column chromatography meant that silica was excluded as a potential solid-phase support. Alumina was also excluded due to its tendency to adsorb some water. Clays were then considered as they can have acidic or basic properties and have various metal cations and silicates, which may be able to bind zingerone through hydrogen bonding. The clay, montmorillonite K10, was also investigated as a solid-phase support for the purification of the trifluoroacetyl ester (**4**). While montmorillonite K10 is acidic[89] and therefore would not deprotonate zingerone and bind zingerone through electrostatic attraction, the presence of many metal cations and silicates could still allow for binding through hydrogen bonding. Indeed, the montmorillonite K10 completely removed zingerone from a DCM solution of impure trifluoroacetyl ester (**4**). The recovery of pure **4** was a modest 66.2% from 50.0 mg of an impure mixture containing 5-10% zingerone. The use of montmorillonite for removing zingerone was limited to the trifluoroacetyl ester (**4**) as it was completely unsuccessful for purifying the impure formyl ester (**3**). The reasons for this where not fully investigated. The mechanism of the binding of zingerone by montmorillonite is not known, but may be indeed be due to the proposed hydrogen bonding, which is absent from the trifluoroacetyl ester (**4**). This, however, cannot explain the unsuccessful purification of the formyl ester (**3**) under identical conditions.

The synthesis of the benzyl acetate analogue (**5**) by the selective acetylation reaction presented in Scheme 4 proceeded well, with a yield of 79.9%. The ^{1}H NMR spectrum of the crude product demonstrated the high selectivity of this method for the acetylation of a benzyl alcohol as only signals arising from the benzyl acetate (**5**) were visible. The downfield shift of the benzyl methylene proton signal indicated the formation of **5**.

The sodium borohydride reduction of zingerone to zingerol (**6**) was very successful with a relatively short reaction time and very high yield of 96.6%. This yield is that of the crude product, which by ^{1}H NMR was very pure and further purification was unnecessary. The formation of zingerol (**6**) was identified by the disappearance of the butanone methyl group singlet and appearance of an upfield doublet. This reaction illustrates the selectivity of sodium borohydride for 1,2-reductions without any side reactions.

The general Aldol reaction presented in Scheme 6 was used for the synthesis of the butenones of methylzingerone (**8a**), isozingerone (**9a**), 2-methoxy analogue (**10a**), trifluoromethoxy analogue (**11a**), trifluorobutanone analogue (**12a**, using 1,1,1-trifluoroacetone), methylenedioxy analogue (**13a**), and difluoromethylenedioxy analogue (**14a**), which were then hydrogenated to the desired saturated compounds. The formation of the butenones was characterised by the disappearance of the ^{1}H NMR aldehyde signal and appearance of the alkene doublets. Overall, the Aldol reactions for all of these compounds proceeded well and demonstrated the generality of this synthetic method. Excluding some of the substituted benzaldehydes, this method is an inexpensive reaction. While the reactions generally proceeded well, in all reactions a coloured side product was generated, which increased the crude yield to 110-130% without purification. With the use of acetone as both a reactant and the solvent, it is suspected that the side product is the self-condensation of acetone rather than further condensation of a benzaldehyde-acetone adduct with either another benzaldehyde or acetone molecule.

The Aldol reactions were typically rapid, often being complete within 2 hours with the exception of the 2-methoxy analogue (**10a**) and trifluoromethoxy analogue (**11a**). Compound **10a** required a reaction time of approximately 24 hours. The increased reaction time can be rationalised by considering the difference between the starting material for the 2-methoxy analogue (**10a**) (4-hydroxy-2-methoxybenzaldehyde) and the other substituted benzaldehydes (3,4-disubstituted benzaldehydes). With 4-hydroxy-2-methoxybenzaldehyde, the methoxy group is *ortho* to the aldehyde group, which due to its spatial proximity will sterically obstruct the acetone enolate attacking the aldehyde carbonyl group. The *ortho* relationship also results in a reduction of the electrophilicity of the carbonyl carbon because the methoxy group can donate electron density through resonance, which decreases the rate of reaction. None of the other substituted benzaldehydes had a substituent *ortho* to the aldehyde and therefore could not sterically hinder the approaching acetone enolate nor donate electron density through resonance. More interestingly, the synthesis of the trifluoromethoxy analogue (**11a**) required an even greater reaction time of 36 hours. Unlike the benzaldehyde for the synthesis of the 2-methoxy analogue (**10a**), the benzaldehyde used for the synthesis of the trifluoromethoxy analogue (**11a**) (4-hydroxy-3-(trifluoromethoxy)benzaldehyde) has no benzene ring substituent

that can sterically hinder the attacking acetone enolate. Considering the electronic effects of the substituents on the electrophilicity of the carbonyl carbon, the trifluoromethoxy group is electron-withdrawing and would inductively withdraw electron density from the carbonyl carbon, increasing its electrophilicity, albeit minimally. Since the phenolic group is common to other benzaldehydes, it is not a point of difference between the synthesis of the trifluoromethoxy analogue (**11a**) and other analogues. Considering the influence of both steric and electronic factors on the rate of reaction, the reaction would be expected to be slightly more rapid than other Aldol reactions. Clearly, the reason for the much slower rate of reaction is more complex than solely the steric or electronic effects of substituents.

Initial attempts to synthesise the butenone of the trifluorobutanone analogue (**12a**) according to the Aldol reaction in Scheme 6 by replacing acetone with a solution of 1,1,1-trifluoroacetone in methanol and at room temperature rather than 50 °C were unsuccessful. Figure 10 shows the mechanism for the expected base-catalysed Aldol reaction of vanillin with 1,1,1-trifluoroacetone. The rate determining step is the formation of the 1,1,1-trifluoroacetone enolate by the removal of an acidic α-hydrogen by the hydroxide ion. Due to the strong electron-withdrawing properties of the trifluoromethyl group, the three α-hydrogens are significantly more acidic than the hydrogens of acetone. Therefore, the 1,1,1-trifluoroacetone enolate would be expected to form more rapidly than the acetone enolate, meaning that the rate of reaction for the synthesis of the trifluorobuteneone analogue (**12a**) would be proportionally greater.

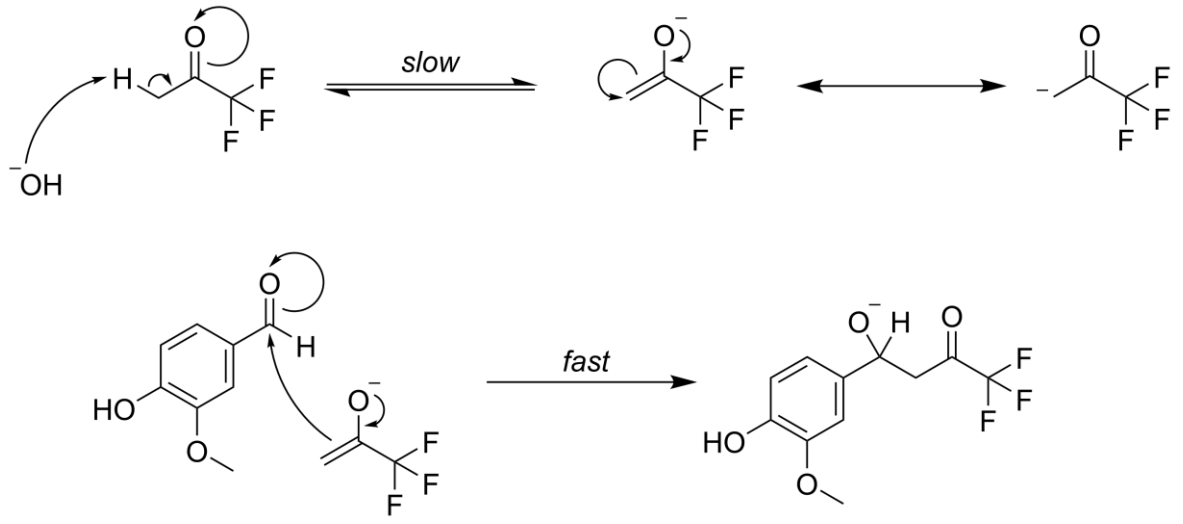


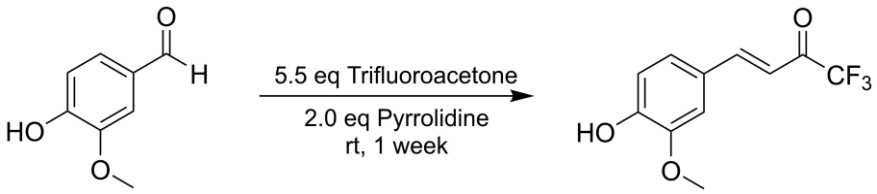
Figure 10 Reaction mechanism of the expected base-catalysed Aldol reaction of vanillin with 1,1,1-trifluoroacetone.

The reason for the greater-than-expected rate of reaction is probably also the same reason for the apparent failure of the reaction. The electron-withdrawing trifluoromethyl group dramatically increases the electrophilicity of the carbonyl such that attack by weak nucleophiles readily occurs. In an aqueous solution at 25 °C, approximately 97.2% of the

1,1,1-trifluoroacetone molecules will be hydrated as the *gem*-diol compared to only 0.14% for acetone.[90] In the hydrated form, the α-hydrogens will not be as acidic as the carbonyl form because the negative charge is not delocalised. For the base-catalysed Aldol reaction, which contains both water and the strong hydroxide nucleophile, virtually all of the 1,1,1-trifluoroacetone molecules would be expected to be hydrated. While this does not prevent the Aldol reaction because the formation of the *gem*-diol is reversible and in equilibrium with the carbonyl form, it would significantly slow the rate of reaction. The rate of reaction could be increased by adding additional 1,1,1-trifluoroacetone, reducing the concentration of water and sodium hydroxide, or by increasing the temperature. Instead of reducing the concentration of water and sodium hydroxide, both were eliminated by performing an acid-catalysed Aldol reaction with glacial acetic acid in methanol. Glacial acetic acid was chosen because it is anhydrous and is commonly used for similar Aldol reactions involving benzaldehydes and 1,1,1-trifluoroacetone.[91-94] The commonly used solvents are benzene and toluene,[91-94] but are too non-polar to dissolve vanillin. Thus, methanol was chosen as a more polar solvent to dissolve all the reactants. This should have prevented the hydration of the 1,1,1-trifluoroacetone since there was no water and the acetate ion is a weaker nucleophile than the hydroxide ion. While this would have prevented the hydration of 1,1,1-trifluoroacetone forming a *gem*-diol, the fact that under the acidic conditions 1,1,1-trifluoroacetone will readily form a hemiacetal/acetal with methanol was initially neglected.[90] Replacing the methanol solvent with DCM to prevent the formation of any hemiacetal/acetal species also appeared unsuccessful. These observations may indicate that the Aldol reaction mechanism with 1,1,1-trifluoroacetone may be more complicated than expected or other reactions, such as the dimerisation of 1,1,1-trifluoroacetone, may be occurring.

The butenone of the trifluorobutanone analogue (**12a**) was eventually synthesised by an Aldol procedure adapted from Ashworth, *et al.*[95] and Patel, *et al.*[96], still with vanillin and 1,1,1-trifluoroacetone, but with pyrrolidine as a catalyst in DCM (Scheme 9). This reaction appeared attractive as a yield of 77% was obtained with 3-cyanobenzaldehyde and 1,1,1-trifluoroacetone.[95] The reaction was believed to proceed by the formation of an enamine of 1,1,1-trifluoroacetone or the benzaldehyde, which promotes the attack of 1,1,1-trifluoroacetone on the benzaldehyde carbonyl.[95-96] Unfortunately, this reaction was not particularly successful as it required a much greater reaction time of 1 week, a larger amount of 1,1,1-trifluoroacetone (5.5 eq), and gave a low yield of less than 50%. The most significant issue with this reaction was the purification of the product, which was extremely difficult as many side products formed, such that the crude yield was 254%. Given the reactivity of the carbonyl of 1,1,1-trifluoroacetone and the large amount required, it is likely that the side products arose from competing reactions involving 1,1,1-trifluoroacetone. The difficult

purification was due to the similar polarity of some of the side products. Once the butenone of the trifluorobutanone analogue (**12a**) had been purified sufficiently, it was hydrogenated to **12b** according to Scheme 7 with an overall yield of 31.2%. The synthesis of **12a** by this route may be improved by adding molecular sieves to absorb any water, such as the water produced from the Aldol condensation reaction. Alternatively, a different reaction method such as that of Liu, *et al.*[97] could be used. In this reaction, carbon nanotube supported cerium dioxide nanoparticles were used as a catalyst for the synthesis of **12a** in a yield of 85%. This alternative synthetic method was not attempted due to time constraints and the commercial unavailability of this catalyst.



Scheme 9 Synthetic method for the synthesis of the trifluorobutenone (**12a**).

Following the Aldol synthesis of the unsaturated compounds **8a**-**14a**, hydrogenation of the alkene was necessary to yield the desired zingerone analogues: methylzingerone (**8b**), isozingerone (**9b**), the 2-methoxy analogue (**10b**), the trifluoromethoxy analogue (**11b**), the trifluorobutanone analogue (**12b**, using 1,1,1-trifluoroacetone), the methylenedioxy analogue (**13b**), and the difluoromethylenedioxy analogue (**14b**). The formation of the butanones was identified by the disappearance of the ^{1}H NMR alkene doublets and appearance of the upfield methylene triplets. Several different hydrogenation methods were attempted, including two *in situ* hydrogenations with palladium, and two catalytic hydrogenations with palladium and rhodium. The first *in situ* hydrogenation used sodium borohydride and acetic acid to generate hydrogen gas *in situ*, which then selectively reduced the alkene with a palladium catalyst.[86] This reaction was shown to be selective for the hydrogenation of 4-phenylbut-3-en-2-ones and related esters, amide, and nitrile; as well as various cyclic and acyclic aliphatic α,β-unsaturated ketones.[86] The selectivity was dependent on the polarity of the solvent with more non-polar solvents being more selective for 1,4-reduction. Due to the polarity of the butenone of methylzingerone (**8a**), the use of isopropanol instead of toluene was necessary. Several attempts at this reaction consistently yielded a mixture of methylzingerone (**8b**) and the over-reduced alcohol (4-(3,4-dimethoxyphenyl)-2-butanol) in approximately a 1:1 ratio (Figure 11). It was suspected that the method was not as selective as intended due to either the solvent polarity or the preference of sodium borohydride for 1,2-reduction. Therefore, a second *in situ* hydrogenation method was investigated for the hydrogenation of the butenone of methylzingerone (**8a**). This method utilised triethylsilane as an *in situ* hydrogen gas source and

palladium as a catalyst to reduce the alkene, and was shown not to over-reduce α,β-unsaturated esters.[87] Similar to the first method, this triethylsilane method also produced a mixture of methylzingerone (**8b**) and the over-reduced alcohol in approximately a 1:1 ratio (Figure 11).

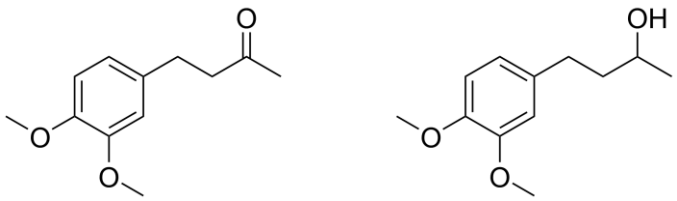
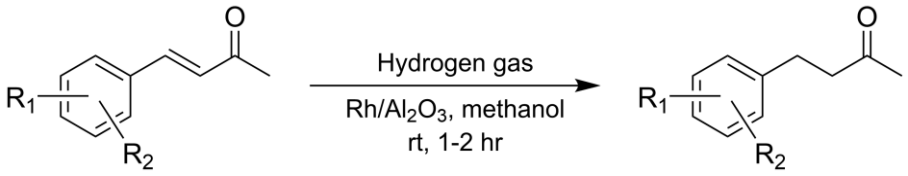


Figure 11 Structure of methylzingerone (8b) (left) and the over-reduced alcohol (4-(3,4-dimethoxyphenyl)-2-butanol) (right).

Given the poor selectivity of the *in situ* hydrogenation methods for 1,4-reduction, catalytic hydrogenation with hydrogen gas and palladium was attempted. Unfortunately, catalytic hydrogenation also produced a mixture of methylzingerone (**8b**) and the over-reduced alcohol. Despite palladium being the most commonly used catalyst for similar hydrogenations in the literature,[64, 68-69, 83-84] the particular reaction conditions used in this study resulted in unacceptably poor overall yields for methylzingerone (**8b**) as well as several other analogues. Although there are some reports of palladium over-reducing such α,β-unsaturated compounds,[64, 98] most literature studies do not report the presence of the over-reduced compound. Instead of the commonly used palladium, Roman, *et al.*[88] and Smith[85] both used rhodium on alumina for the hydrogenation of dehydrozingerone (**7**) to zingerone (**1**) and reported either a quantitative yield of zingerone or no over-reduction. When rhodium on alumina was used for the hydrogenation of the butenone of methylzingerone (**8a**), there was no detectable over-reduction, unlike the case with palladium. Similarly, for the hydrogenation of the butenones of isozingerone (**9a**), the 2-methoxy analogue (**10a**), the trifluoromethoxy analogue (**11a**), the methylenedioxy analogue (**13a**), and the difluoromethylenedioxy analogue (**14a**) using rhodium (Scheme 10), there was no detectable over-reduction. Only in the case of the butenone of the trifluorobutanone analogue (**12a**) was there possibly some over-reduction, as determined by GC-MS and NMR. The presence of the trifluoromethyl group in **12a** likely contributed to the over-reduction with rhodium, but the amount of over-reduced compound was less than palladium typically produced. While there was no detectable presence of over-reduction with the hydrogenation of the butenone of the difluoromethylenedioxy analogue (**14a**), other unidentified products were observed by TLC after hydrogenation using rhodium.



Scheme 10 Alternative synthetic method for the synthesis of **8b** (R_1 = 4-OCH_3, R_2 = 3-OCH_3), **9b** (R_1 = 4-OCH_3, R_2 = 3-OH), **10b** (R_1 = 4-OH, R_2 = 2-OCH_3), **11b** (R_1 = 4-OH, R_2 = 3-OCF_3), **12b** (R_1 = 4-OH, R_2 = 3-OCH_3, 1,1,1-trifluoro-2-butone), **13b** (R_1 = R_2 = 3,4-OCH_2O), and **14b** (R_1 = R_2 = 3,4-OCF_2O).

Apart from the hydrolysis issues identified with the trifluoroacetyl ester (**4**), only one other compound was observed to have issues with stability. In the first synthesis of the trifluorobutanone analogue (**12b**), some unusual signals were noticed in ^{1}H NMR after purification by column chromatography. However, the compound that produced those signals was removed by Kugelrohr distillation. This first batch of the trifluorobutanone analogue (**12b**) appeared to be very stable and only contained tentatively identified over-reduced compound of **12b**. However, the second synthesis of the trifluorobutanone analogue (**12b**) was purified by a slow Kugelrohr distillation, which appeared to cause the formation of this compound that had appeared previously. Over time, two fractions obtained from this distillation were slowly transformed into this compound and solidified as a white solid, as if the compound was catalysing its formation. Further spectroscopic investigations of this white solid indicated that the carbonyl group had been transformed (absence of carbonyl signals in IR and ^{13}C NMR), there were two additional equivalent protons in ^{1}H NMR at δ 2.99 ppm, which disappeared with the addition of D_2O, and the presence of a signal at 93.9 ppm in the ^{13}C NMR exhibiting $^2J_{FC}$ coupling. These data are consistent with the unidentified compound being the hydrated form of the trifluorobutanone analogue (**12b**), which is likely to readily occur considering the ease of 1,1,1-trifluoroacetone hydration.[90] However, it is unusual that the first batch of the trifluorobutanone analogue (**12b**) was seemingly more stable in that it did not experience the same complete transformation, and that the transformation became more apparent after a distillation, which would be expected to dehydrate rather than hydrate. Furthermore, when D_2O was added to a $CDCl_3$ solution of the suspected hydrate, in addition to the disappearance of the two proton singlet suspected to be the *gem*-diol protons, the amount of non-hydrated trifluorobutanone analogue (**12b**) had increased relative to the suspected hydrate. If the compound was indeed hydrated **12b**, such behaviour would not be expected. Thus, this compound is only tentatively identified as hydrated trifluorobutanone analogue (**12b**).

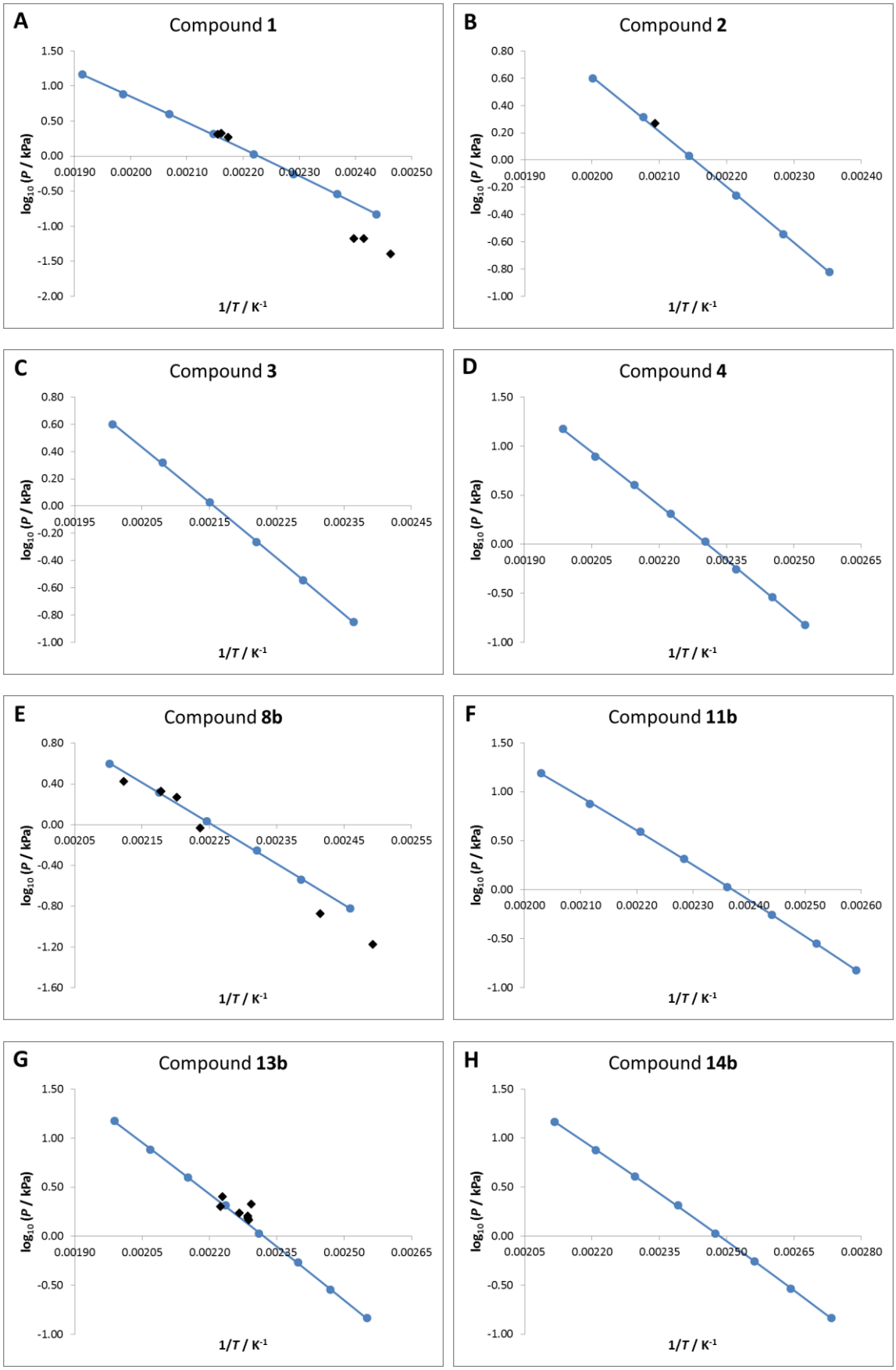
3.2 Vapour Pressure Measurements

Male fruit flies detect chemical lures in the atmosphere. This requires the lure compound to volatilise. Therefore, increasing the volatility of a lure should also improve the attraction of the lure to male fruit flies. Measuring the volatility/vapour pressure of a compound is useful for

evaluating the potential of that compound as a lure as well as providing some indication of the length of time the compound will be effective in a trap before requiring replacement.

The measurement of vapour pressure can be achieved with the use of differential scanning calorimetry (DSC). With this technique, the boiling point of a compound is determined under different reduced pressures. The use of a lid with a small pinhole is required to minimise pre-boiling vaporisation and ensure a sharp endotherm.[99] Once the pressure of the DSC system is adjusted to the desired pressure, the sample is heated at a constant rate. The sample will boil when its partial pressure at a particular temperature equals the applied pressure producing a boiling endotherm. The boiling point is measured from the onset point on the endotherm, which is the temperature where the heat flow begins to decrease from the baseline value (Figure A-1). This process is repeated at different reduced pressures to obtain a set of boiling points. This set of temperature-pressure data points can be used to construct a vapour pressure curve for that compound. The construction of the vapour pressure curve requires the fitting of an equation that appropriately models the data. The Antoine Equation (1) is one such equation that describes the relationship between vapour pressure and temperature.[45] The fitted Antoine Equation describes the partial pressures of a pure compound over a range of temperatures. While the Antoine Equation is only strictly valid over the temperature range used to fit the equation, it can be extrapolated to determine the partial pressure/vapour pressure at any temperature. The uncertainty in the extrapolated vapour pressure will increase with greater distance from the temperature validity range. In addition to comparing compounds on the basis of vapour pressure (kPa or Pa), compounds can be compared on a mass per volume basis with the volatility equation (2), which is derived from the ideal gas equation.

The vapour pressures of eight zingerone analogues (zingerone (**1**), the acetyl ester (**2**), the formyl ester (**3**), the trifluoroacetyl ester (**4**), methylzingerone (**8b**), the trifluoromethoxy analogue (**11b**), the methylenedioxy analogue (**13b**), and the difluoromethylenedioxy analogue (**14b**)) were determined using this DSC technique. The obtained temperature-pressure data are tabulated in Table A-1, where T is the measured onset point, P_{exp} is the experimental pressure, and P_{calc} is the pressure calculated by the Antoine Equation using the relevant parameters from Table 4. The vapour pressure curves for the compounds are shown in Figures 12A-H, where blue circles represent experimental data points, blue lines are the fitted Antoine Equation curves, and black diamonds are available literature data. Table 4 presents the Antoine parameters and validity range for each of the measured compounds. Table 5 shows the experimental or extrapolated normal boiling points, vapour pressure, volatility, and vapour pressure relative to zingerone (**1**) for each of the compounds.



Figures 12A-H Vapour pressure curves for zingerone (**1**), the acetyl ester (**2**), the formyl ester (**3**), the trifluoroacetyl ester (**4**), methylzingerone (**8b**), the trifluoromethoxy analogue (**11b**), the methylenedioxy analogue (**13b**), and the difluoromethylenedioxy analogue (**14b**). Blue circles represent experimental data points, blue lines are the fitted Antoine Equation curve, and black diamonds are available literature data. References for literature data: A [100-104], B [61], E [61, 104-108], and G [100, 104, 106, 109-112].

Table 4 Antoine parameters and validity range for compounds **1**-**4**, **8b**, **11b**, **13b**, and **14b**.

Compound	Antoine Parameters			
	A	B	C	Validity Range
1	6.842163	2457.631516	-89.818662	410.4 K to 522.6 K
2	7.755923	3202.359988	-51.818821	425.1 K to 499.9 K
3	7.731833	3158.757615	-55.111380	423.0 K to 498.6 K
4	7.316142	2702.339969	-64.020593	396.1 K to 503.7 K
8b	8.347736	3444.697922	-31.037649	406.7 K to 475.9 K
11b	6.540607	2096.619364	-101.250770	386.0 K to 492.8 K
13b	7.193963	2672.791488	-59.369448	392.1 K to 503.1 K
14b	6.518348	2093.708494	-81.330456	366.0 K to 472.5 K

Table 5 Normal boiling points, vapour pressures, volatility, and relative vapour pressures for compounds **1**-**4**, **8b**, **11b**, **13b**, and **14b**.

Compound	Normal Boiling Point / K	Vapour Pressure at 298.15 K / Pa	Volatility at 298.15 K / mg m^{-3}	Relative Vapour Pressure
1	598.0 [a]	0.011	0.87	1.0
2	608.7 [a]	0.0057	0.54	0.51
3	606.8 [a]	0.0054	0.49	0.49
4	572.9 [a]	0.059	7.0	5.4
8b	590.4	0.028	2.4	2.5
11b	559.0	0.078	7.8	7.0
13b	581.8	0.10	7.8	9.0
14b	540.8	0.73	67	66

[a] Extrapolated from Antoine Equation.

The vapour pressure of all compounds was not able to be measured due to time constraints. Since the main purpose of investigating zingerone analogues was to develop more volatile analogues to zingerone, vapour pressure measurement was prioritised for compounds that were expected to be more volatile than zingerone, including fluorinated compounds and compounds without the phenolic group.

The least squares nonlinear regression fitted the Antoine Equation well to the experimental data as the average absolute percentage difference was only 1.51% with a maximum of 4.73% (Table A-1). Since the expected vapour pressure of these analogues is <0.001 kPa, the range of pressures were chosen to be as close to this expected vapour pressure of <0.001 kPa as feasible. Thus, a range 15 kPa to 0.15 kPa was chosen. While 0.15 kPa is beyond the stated range of ASTM E1782-14 (0.2 kPa to 2 MPa), calibrations with 1-octanol were still in agreement with the literature values of Wilhoit and Zwolinski[76] at even lower pressures.

The normal boiling point (atmospheric pressure, 101.3 kPa) was also measured experimentally as this is a standard property of a compound, but was not used for the curve fitting. While determining the normal boiling point for methylzingerone (**8b**), the trifluoromethoxy analogue (**11b**), the methylenedioxy analogue (**13b**), and the difluoromethylenedioxy analogue (**14b**)

was straightforward, for zingerone (**1**), the acetyl ester (**2**), and the trifluoroacetyl ester (**4**) the boiling endotherms were exceedingly broad and shallow suggesting thermal decomposition of these compounds.[38-39, 41] In the case of the formyl ester (**3**), the boiling points at atmospheric pressure and the first two pressures could not be measured due to abnormal boiling endotherm shapes. While this could be due to the impurity of the sample (contained approximately 5% zingerone by ^{1}H NMR and GC-FID), it could also be the result of thermal decomposition or some combination of impurity and decomposition. Considering the less than desirable purity of the formyl ester (**3**), the obtained data should be interpreted with some caution. Similarly, the boiling endotherms of the acetyl ester (**2**) and methylzingerone (**8b**) at the first two pressures had abnormal shapes that affected the accuracy of the measured onset point. Although a value for the onset point could be determined, the data was inconsistent with the other data points and was omitted from the curve fit.

The plotted vapour pressure curves are shown in Figures 12A-H for each of the analysed compounds. For zingerone (**1**), the acetyl ester (**2**), methylzingerone (**8b**), and the methylenedioxy analogue (**13b**), literature boiling points at reduced pressures were available. These data are also plotted on the appropriate curves as black diamonds. Six literature values were available for zingerone (**1**), three of which closely agreed with the data obtained in this study. The one literature data point available for the acetyl ester (**2**) agreed very well with the experimental data. For methylzingerone (**8b**), the six literature data points approximately agree with the experimental data. With the methylenedioxy analogue (**13b**) all the 7 literature data points are concentrated around the middle of the experimental data. Although the literature data have low precision, they agree reasonably well with the experimental data obtained in this study. Another literature data point was excluded because it deviated considerably from both the experimental data and the other literature data.[113] The disagreement between literature data and the data obtained in this study is probably due to the method used to obtain the literature data. The literature data was measured from vacuum distillations, which could be imprecise due to differences in pressure between the distilling sample and the pressure gauge or temperature/pressure fluctuations.

In Table 5, the normal boiling points, vapour pressure, volatility, and vapour pressure relative to zingerone (**1**) are presented. The relative vapour pressure values in Table 5 highlight the significant impact of fluorination on the vapour pressure of a compound. In this study, replacing several hydrogen atoms with fluorine atoms on only one carbon resulted in a 7-10-fold increase in vapour pressure compared to the corresponding non-fluorinated compounds. Despite the strong dipoles of fluorinated functional groups resulting from the electronegativity

of fluorine, the low polarisability of fluorine[49-52] clearly has a significantly more influential effect on intermolecular interactions and thus volatility.

The large relative vapour pressure of the difluoromethylenedioxy analogue (**14b**) is likely due both to the presence of fluorine and the methylenedioxy moiety as the methylenedioxy analogue (**13b**) also has a high relative vapour pressure despite not being fluorinated. While the reason for the greater volatility of the methylenedioxy moiety is probably due to the removal of the hydrogen bonding present in zingerone (**1**), which contributes to intermolecular interactions, this cannot completely explain the observed increase in volatility as the non-hydrogen bonding compound, methylzingerone (**8b**), only has a relative vapour pressure of 2.5. Comparing the non-fluorinated methylenedioxy analogue (**13b**) and methylzingerone (**8b**), **13b** is a slightly smaller molecule than **8b** (192.21 g mol^{-1} and 208.25 g mol^{-1} respectively), which means that there would be fewer van der Waals interactions between **13b** molecules. In addition to van der Waals interactions, dipole-dipole interactions would also be expected to be significant contributors to the total intermolecular bonding. The conformational restrictions of the methylenedioxy analogue (**13b**) due to the fused rings may prevent the molecules from achieving appropriate conformations for dipole-dipole interactions. In the case of methylzingerone (**8b**), there is more flexibility in the two methoxy groups that may allow the molecules to achieve conformations that result in stronger dipole-dipole interactions, which would decrease volatility.

The lower volatility of the acetyl ester (**2**) and the formyl ester (**3**) compared to zingerone (**1**) was unexpected as the esters do not have the intermolecular hydrogen bonding present in **1** and therefore should be more volatile. While the same reason concerning the greater van der Waals interactions resulting from a larger mass can be argued, the volatility of the raspberry ketone esters compared to raspberry ketone (Table 6, Park, *et al.*, unpublished data) demonstrate that this is likely to only be a minor factor. The raspberry ketone ester series follows the expected trend in that removing the hydrogen bonding contribution to the intermolecular bonding increases the volatility and the replacement of hydrogen with fluorine further improves volatility. Considering the relative vapour pressure of the trifluoroacetyl ester of raspberry ketone compared to raspberry ketone, the corresponding trifluoroacetyl ester of zingerone (**4**) is not as volatile as might be expected. Clearly, the volatility of the zingerone esters is impacted by an effect greater than hydrogen bonding. Furthermore, zingerone is more volatile than raspberry ketone despite possessing an additional functional group.

One possible explanation for these observations is the presence of intramolecular hydrogen bonding in zingerone. Intramolecular hydrogen bonding between the phenolic and methoxy groups in zingerone would account for the greater volatility of zingerone compared to raspberry

ketone as it reduces intermolecular hydrogen bonding and counteracts the increased intermolecular bonding due to the methoxy group (Figure 13). Without the phenolic group that was counteracting the methoxy group, the presence of the ester group means that volatility is reduced because the ester and methoxy groups increase the intermolecular bonding (Figure 13). With the raspberry ketone esters, the intermolecular hydrogen bonding must be a dominant factor contributing to the total intermolecular bonding, therefore removing the hydrogen bonding increases the volatility. While intramolecular hydrogen bonding is a plausible hypothesis, no experimental evidence supports this hypothesis. If there was intramolecular hydrogen bonding in zingerone, the infrared OH stretch in the solid or liquid phase should be broader compared to the relatively narrower stretch of raspberry ketone. Both solid and liquid phase infrared spectra of zingerone and raspberry ketone had OH stretches with similar shapes. In support of this negative result is the crystal structure of the closely related compound, vanillin, which also has the 4-hydroxy-3-methoxybenzene moiety and does not show any intramolecular hydrogen bonding.[114] Although the crystal structure of zingerone has not been determined, it would also be expected to not show any intramolecular hydrogen bonding.

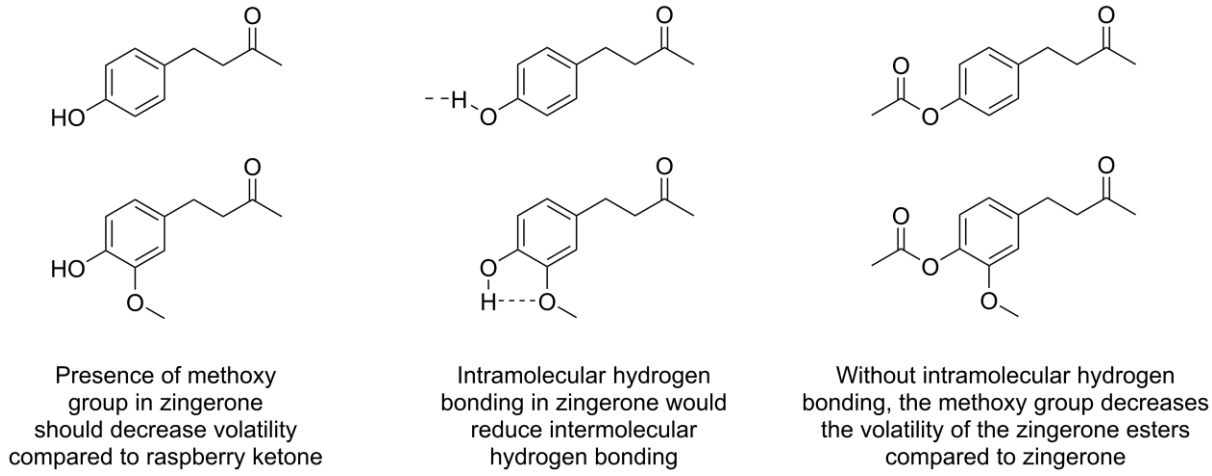


Figure 13 Summary of the intramolecular hydrogen bonding hypothesis for the lower volatility of the zingerone esters compared to zingerone.

An alternative explanation for the observed trends is that the presence of the methoxy group affects the packing of the molecules in the solid or liquid state. If the slight bulk of the methoxy group increases the distance between interacting molecules, the intermolecular hydrogen bonding will be reduced, which would account for the greater volatility of zingerone compared to raspberry ketone. Assuming the intermolecular hydrogen bonding is significantly reduced, the presence of an ester group increases the intermolecular bonding overall because the additional van der Waals and dipole-dipole interactions are greater contributors than hydrogen bonding was in zingerone. Thus, the altered molecular packing hypothesis is consistent with the observed volatility trends. Molecular modelling and crystal structures of zingerone (**1**) and

the acetyl (**2**), formyl (**3**), and trifluoroacetyl (**4**) esters would be valuable in further investigating the volatility trends and packing hypothesis.

Table 6 Vapour pressure data of raspberry ketone and esters. Data from Park, *et al*. (unpublished data).

Compound	Vapour Pressure at 298.15 K / Pa	Vapour Pressure Relative to Raspberry Ketone	Vapour Pressure Relative to Zingerone
Raspberry ketone	0.00180	1.00	0.16
Cuelure	0.00754	4.19	0.68
Melolure	0.00959	5.33	0.86
Raspberry ketone trifluoroacetyl ester	0.995	553	90

Determining the volatility of some compounds at room temperature required extrapolating the vapour pressure curve beyond the triple point. This assumes that the intermolecular bonding in the liquid phase is identical or equivalent to the bonding in the solid phase. It is unlikely that this assumption would be true. Although this further increases the uncertainty associated with the extrapolation, since most of the compounds have melting points close to 50 °C, the uncertainties resulting from extrapolation beyond the triple point to 25 °C should be acceptably small.

Considering the uncertainties associated with extrapolation, it would be beneficial to support this DSC data with data obtained from another technique, such as gas saturation.[39, 41, 44, 115] The benefit of the gas saturation technique is that it can be performed at or close to room temperature. This technique also requires a detection method that is sufficiently sensitive to detect low volatility compounds, such as those in this study.

3.3 Biological Testing

The measurement of the vapour pressure of a compound is not sufficient to understand its potential use as a fruit fly lure. While volatility appears to be an important factor in lure attraction, the attraction of a compound to a fruit fly is also affected by the presence and selectivity of an appropriate receptor on the fly and the behavioural response to the detection of this compound. The evaluation of compounds as potential lures therefore requires biological testing. In this study, electroantennography (EAG) and laboratory cage bioassays were used to evaluate the zingerone analogues as potential lures.

3.3.1 Electroantennography (EAG)

The EAG technique involves placing the antenna of an insect between two electrodes. The antenna may still be connected to the whole immobilised insect, or only the head, or entirely removed from the insect. When a chemical stimulus is exposed to the antenna, if there are receptors that detect the chemical stimulus, the depolarisation of multiple neurons is measured as a fluctuation in the voltage between the two electrodes. If the insect antenna cannot detect

the chemical stimulus because there are no appropriate receptors, there will be no voltage fluctuation. The benefit of EAG is that many compounds can be rapidly screened in a much shorter period of time than cage or field bioassays. Another benefit of EAG is that fewer flies are required than cage or field bioassays. The disadvantage of EAG, however, is that it provides no indication of the behavioural response of a fruit fly to a compound; the ability of a fly to detect a particular compound does not mean that the fly will actively seek the source of the compound, which is necessary for an effective lure.

EAG was used in this study to evaluate the ability of male Queensland fruit fly (*B. tryoni*) and Jarvis' fruit fly (*B. jarvisi*) to detect the compounds **1-15**. Due to time constraints, only a small number of replicates were performed. Representative EAG traces are presented for a male *B. tryoni* in Figure 14 and a male *B. jarvisi* in Figure 15.

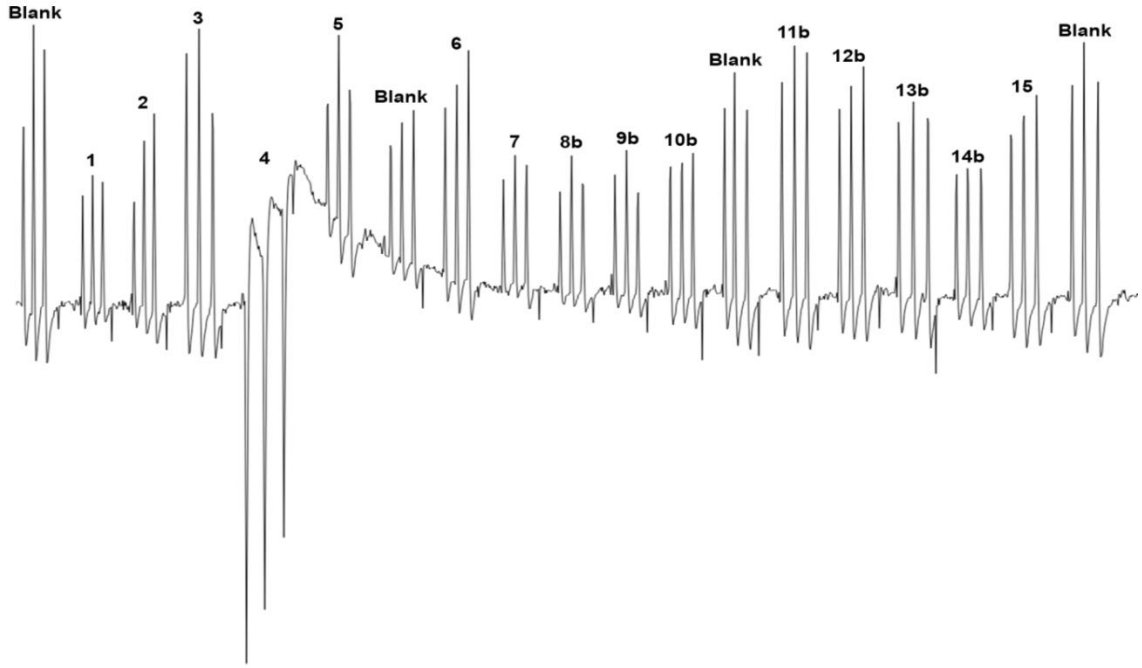


Figure 14 Representative EAG trace of a male *B. tryoni* with compounds **1-15**.

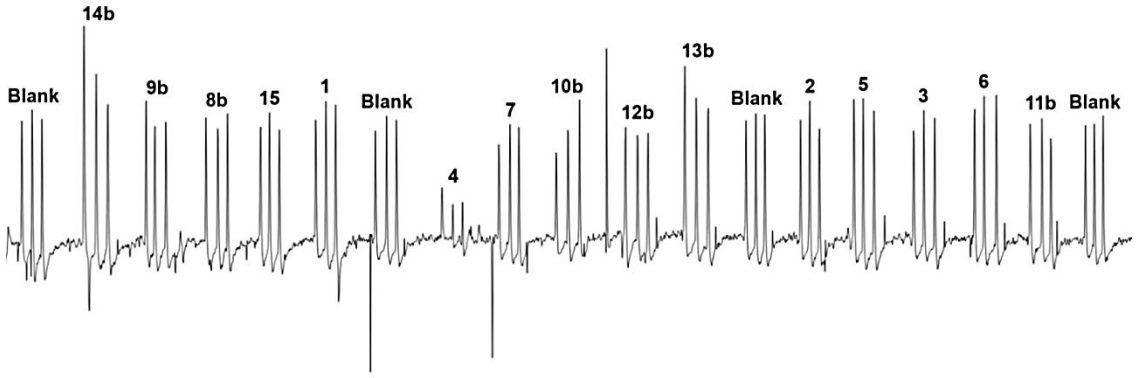


Figure 15 Representative EAG trace of a male *B. jarvisi* with compounds **1-15**.

As expected, a comparison of the EAG traces in Figure 14 and Figure 15 shows that the EAG signal varies between compounds and between species. The response of *B. tryoni* and *B. jarvisi* to zingerone (**1**) was similar and generally equal to the blank. The fluorinated compounds (trifluoroacetyl ester (**4**), trifluoromethoxy analogue (**11b**), trifluorobutanone analogue (**12b**), and difluoromethylenedioxy analogue (**14b**)) tended to produce signals that were equal to or greater than a blank, and were often the strongest signals. This is likely due to the increased volatility of these compounds, which meant that a greater amount of the compound was reaching the antenna and thus producing a stronger signal. The increased signal may also indicate that these compounds have a superior receptor fit.

Even though the trifluoroacetyl ester (**4**) had a strong EAG signal, this was limited to early runs as the signal rapidly decreased (Figure 15) and became negative (Figure 14). Figure 14 also shows that exposure of the antenna to **4** had an enduring effect on the antenna, which was visible as a slow return to the previous baseline value. Due to the use of humidified air, this is likely due to the presence of trifluoroacetic acid that was generated from the hydrolysis of the ester (**4**). The volatile and acidic properties of trifluoroacetic acid would explain the very strong EAG response in Figure 14.

For *B. tryoni*, the trifluoromethoxy analogue (**11b**) typically gave a slightly greater signal than the blank, whereas for *B. jarvisi*, the response was approximately equal to that of the blank. This is despite **11b** being 7.0 times more volatile than zingerone. Similar to the trifluoromethoxy analogue (**11b**), the response of the trifluorobutanone analogue (**12b**) was comparable to the blank for *B. jarvisi* and slightly lower with *B. tryoni*.

A main point of difference between the two species was with the methylenedioxy analogue (**13b**) and the difluoromethylenedioxy analogue (**14b**). Despite the relatively high vapour pressures, with *B. tryoni* the EAG response for **13b** was slightly lower than the blank and the response of **14b** was consistently even lower and often one of the lowest signals in the run. On the other hand, with *B. jarvisi* the methylenedioxy analogue (**13b**) consistently had a greater response than the blank and the response to the difluoromethylenedioxy analogue (**14b**) was even superior to **13b**. The decrease in signal intensity across the three stimulus pulses for **13b** and **14b** is likely due to saturation of the receptor with these compounds. These results indicate that for *B. tryoni* the methylenedioxy moiety has a poor receptor fit, whereas for *B. jarvisi* the receptor binding site is favourable for this moiety. Similar to *B. tryoni*, the Melon fly (*B. cucurbitae*) is also a cuelure responder and was found not to be attracted to a methylenedioxy analogue of benzyl acetate.[35] This might suggest that the methylenedioxy moiety is disfavoured by cuelure responding species.

The acetyl ester (**2**) and formyl ester (**3**) had variable signals for both species and were typically equal to or lower than the blank signal, which may be due to their relatively low volatility. Unlike *B. tryoni*, the EAG response of the benzyl acetate analogue (**5**) with *B. jarvisi* was often greater than a blank. Unlike *B. tryoni*, *B. jarvisi* has a reasonably strong attraction to zingerone,[5, 27] therefore the higher response of *B. jarvisi* to the benzyl acetate analogue (**5**) may be because this compound is an isostere of zingerone. The responses of both species to zingerol (**6**), dehydrozingerone (**7**), methylzingerone (**8b**), isozingerone (**9b**), the 2-methoxy analogue (**10b**), and the propanone analogue (**15**) were similar in that the responses were generally equal to or lower than a blank.

Additional EAG experiments with these compounds should be conducted with both fruit fly species with an improved signal-to-noise and signal-to-blank ratios, greater number of replicates, female flies as well as male flies, and flies of different ages and batches.

3.3.2 Laboratory Cage Bioassays

Bioassays are necessary to investigate the behavioural response of the fruit flies to the compounds and determine whether they would be an effective attractant. While field bioassays would be preferred to cage bioassays, due to time constraints only laboratory cage bioassays could be performed with *B. tryoni*. Field bioassays are preferred because the compounds are tested under real environmental conditions where attractants are likely to be deployed and used for fruit fly control and monitoring. As a result of time and availability of pupae, only bioassays with *B. tryoni* could be performed. While *B. jarvisi* would likely have a higher response to these zingerone analogues than *B. tryoni*, the lower response of *B. tryoni* to zingerone suggests that there is greater potential for improvement with respect to *B. tryoni* than with *B. jarvisi*.

3.3.2.1 Preliminary Cage Bioassay

A preliminary cage bioassay with *B. tryoni* was conducted on compounds **1**-**5**, **7**-**10b**, and **12b**-**15** which were available at the time to quickly determine whether a more robust cage bioassay would be appropriate. As this was a preliminary bioassay, the conditions were not controlled and the number of flies attracted to the compounds was not counted. In this bioassay, there was a relatively strong and rapid response to zingerone (**1**) and a rapid moderate response to the benzyl acetate analogue (**5**), which is an isostere of zingerone (**1**). All the other compounds tested (**2**-**4**, **7**-**10b**, and **12b**-**15**) elicited at most a very weak response from *B. tryoni* despite relatively high volatility of some of these compounds. This demonstrates that volatility is only one factor affecting the effectiveness of an attractant; receptor fit and behavioural response are also critical factors. A comparison of zingerone (**1**) and cuelure indicated that cuelure had a greater response than **1**, but the fruit flies did not appear to leave **1** in favour of cuelure.

The results of this preliminary bioassay justified a more robust bioassay as *B. tryoni* was responding to zingerone (**1**) and the benzyl acetate (**5**).

3.3.2.2 Large Cage Bioassay

This cage bioassay was intended to be a more robust bioassay than the preliminary, with all compounds tested in two replicates. In brief, ten sticky Lynfield traps were placed in a 3×3×3 m mesh cage with 250 male and 250 female *B. tryoni*. The 15 compounds to be investigated were separated into two groups – compounds **2**-**8b** and **9b**-**15**, with each group having 7 traps for compounds and traps for a control, zingerone (**1**), and cuelure. The bioassay results for two replicates of compounds **1**-**8b** at 4 hours and 24 hours are shown in Table 7. The bioassay results for two replicates of compounds **1** and **9b**-**15** at 4 hours and 24 hours are shown in Table 8.

Table 7 Cage bioassay results for compounds **1**-**8b**.

Compound	Replicate 1				Replicate 2			
	4 Hours		24 Hours		4 Hours		24 Hours	
	Males	Females	Males	Females	Males	Females	Males	Females
1	3	1	10	4	1	3	4	4
2	4	0	8	2	3	0	3	2
3	1	0	8	1	1	0	2	2
4	18	0	27	2	5	0	13	1
5	3	1	6	2	0	0	3	1
6	1	3	5	6	2	0	2	0
7	3	1	5	3	1	0	2	2
8b	2	0	8	5	0	0	2	1
Cuelure	12	0	15	2	1	0	2	1
Control	16	0	45	4	0	2	2	3

Table 8 Cage bioassay results for compounds **1** and **9b**-**15**.

Compound	Replicate 1				Replicate 2			
	4 Hours		24 Hours		4 Hours		24 Hours	
	Males	Females	Males	Females	Males	Females	Males	Females
1	1	0	4	3	1	0	1	2
9b	2	2	6	4	3	0	9	4
10b	3	0	6	1	6	0	10	3
11b	9	1	17	2	2	1	11	3
12b	2	0	3	2	0	1	2	7
13b	1	0	4	1	3	1	9	3
14b	11	0	17	2	19	1	35	5
15	3	3	17	23	3	0	12	8
Cuelure	5	0	9	2	6	0	16	2
Control	7	0	21	4	3	0	5	1

An examination of the number of flies caught by the cuelure and blank control traps indicates that this bioassay is anomalous or requires more replication. The cuelure trap caught predominately male *B. tryoni* as expected, but the capture rate of 0.4-6.4% was much lower

than anticipated for cuelure. The blank control also caught predominately males and in reasonably high numbers, especially for replicate one where the control caught more flies than cuelure. The male preference for the control trap may indicate previous contamination of the trap with cuelure but the low capture rate of cuelure and the variable control capture rate between the two replicates suggest that contamination is unlikely. Alternatively, a greater number of replicates may be necessary to account for intrinsic variation.

Considering the issues with the cuelure and control traps, little can be concluded from this bioassay and certainly conclusions cannot be reached. However, some trends were apparent in the results in Table 7 and Table 8. In Table 7, the trifluoroacetyl ester (**4**) had the highest number of captured males in both replicates despite the likely rapid hydrolysis of **4** to zingerone (**1**) and trifluoroacetic acid. This hydrolysis should have reduced the attraction of the trifluoroacetyl ester (**4**) trap to the level of the zingerone (**1**) trap or below. However, between 4 hours and 24 hours, the trifluoroacetyl ester (**4**) still captured additional male *B. tryoni*. Table 8 shows that between 4 hours and 24 hours in replicate one, the propanone analogue (**15**) captured an additional 14 males and 20 females, more than any other compound. Unfortunately, this was not repeated in replicate two and is therefore an anomaly. Despite the difluoromethylenedioxy analogue (**14b**) producing a weak EAG signal, it had the highest capture rate of any compound in Table 8 for both replicates. While this might suggest that the behavioural response of *B. tryoni* to **14b** is strong even though its detection by the antennae of male fruit flies was weak, the variable number of flies caught, and the issues with the cuelure and control traps mean that this cannot be concluded. It should also be noted that for the formyl ester (**3**) there may be some small additional response associated with the low-level of zingerone (**1**) impurity, but if this additional response is present it would only be very minor.

Compared to the preliminary bioassay, the results of this cage bioassay are substantially different. In the preliminary bioassay, **1** produced a strong response and there was a moderate response to **5**. In the later bioassay, however, **1** and **5** had very weak responses whereas **4** and **14b** produced stronger responses.

Unfortunately little could be interpreted from this bioassay and due to time constraints the bioassay could not repeated or redesigned. In order to determine whether any of these zingerone analogues would be useful as a fruit fly attractant, further bioassays are necessary. This could be laboratory cage bioassays with *B. tryoni* and *B. jarvisi* or field bioassays. Field bioassays would also allow for the attraction of other fruit fly species to these compounds to be evaluated.

Chapter 4. Conclusions and Future Directions

Many fruit fly species are important horticultural pests in Australia and other countries. While insecticides have been commonly used, their continued use is uncertain as many organophosphate insecticides are being restricted. Alternative fruit fly control methods are therefore necessary to protect crops from fruit fly damage. Male annihilation technique (MAT) is one technique that is promising as an alternative control method. The effectiveness of MAT is dependent on the use of efficient chemical lures. The attraction of male fruit flies to chemical lures is a combination of the lure volatility, receptor fit, and behavioural response of the flies.

In this study, a series of analogues of zingerone were investigated as potential fruit fly attractants for two Australian pest fruit fly species – the Queensland fruit fly (*B. tryoni*) and Jarvis' fruit fly (*B. jarvisi*). A total of 15 compounds including zingerone were selected. Given the importance of volatility for an effective lure, four of the chosen analogues were fluorinated, which due to the low polarisability of fluorine results in increased volatility. Analogues that would provide some insight into the binding site of the relevant receptor were also chosen, such as compounds with a rigidified or shorter carbon chain, absence of hydrogen bonding functionality, and different benzene ring substitution patterns.

Considering time and commercial availability, it was decided that that zingerone (**1**), dehydrozingerone (**7**), and the propanone analogue (**15**) would be purchased and the other compounds would be synthesised. The acetyl ester (**2**) and trifluoroacetyl ester (**4**) were synthesised by reacting zingerone with acetic anhydride and trifluoroacetic anhydride respectively. The formyl ester (**3**) was instead synthesised by a Steglich esterification with formic acid and DCC. A major issue with the esters **3** and **4** was their purification. For both esters, distillation was unsuccessful and column chromatography was difficult due to the polarity of the formyl ester (**3**) and the hydrolytic instability of the trifluoroacetyl ester (**4**). The benzyl acetate was synthesised by selectively acetylating 4-hydroxy-3-methoxybenzyl alcohol with acetic acid and potassium fluoride. Reduction of zingerone with sodium borohydride yielded the alcohol, zingerol (**6**). Compounds **8b**-**14b** were synthesised by first reacting the appropriately substituted benzaldehyde with acetone in an Aldol reaction and then catalytically hydrogenating the resulting butenones (**8a**-**14b**). The Aldol reaction for the synthesis of the trifluorobutenone (**12a**) required the use of 1,1,1-trifluoroacetone instead of acetone and also a different synthetic method from the other compounds. Although the pyrrolidine-catalysed Aldol reaction used was able to synthesise some of trifluorobutenone (**12a**), the reaction was very inefficient as it required an excess of 1,1,1-trifluoroacetone, the yield was low, and purification was difficult. The Aldol reactions for the synthesis of **8a**-**11a**, **13a**, and **14a** proceeded without issues. All the unsaturated Aldol products were hydrogenated to **8b**-**14b**

with hydrogen gas and Rh/Al_2O_3 catalyst. Palladium was found to significantly over reduce the butenones to butanols. This over reduction was completely eliminated with the use of rhodium, with the exception of the trifluorobutanone analogue (**12**) where some over reduction may have occurred.

The importance of volatility to the effectiveness of an attractant means that an understanding of the volatility of a compound is useful for evaluating its potential as a fruit fly attractant. In this study, the volatility/vapour pressure was measured using a differential scanning calorimetry (DSC) technique. A vapour pressure curve of the compound is constructed from this data and can be used to determine the vapour pressure at any temperature by extrapolation. Using these curves, the vapour pressures of compounds **1**-**4**, **8b**, **11b**, **13b**, and **14b** at room temperature (298.15 K) were determined. Fluorination increased the vapour pressure 7-10-fold compared to the corresponding non-fluorinated compounds. The acetyl (**2**), formyl (**3**), and trifluoroacetyl (**4**) esters were not as volatile as expected. The esters were expected to be more volatile because esterification removes the hydrogen bonding of the phenolic group that was present in zingerone. The reason for the lower than expected volatility was shown not to be due to intramolecular hydrogen bonding in zingerone, but may instead be a consequence of the methoxy group affecting the molecular packing.

Bioassays were necessary to determine whether any of the zingerone analogues would be useful as an attractant. While EAG alone cannot show if an analogue would be a suitable attractant, EAG can indicate whether a compound can be detected by the antennae of fruit flies. The EAG responses of male *B. tryoni* and *B. jarvisi* were recorded for compounds **1**-**15**. Differences between compounds and between species were observed. The fluorinated compounds tended to produce signals that were equal to or greater than a blank, and were often the strongest signals. This is likely due to the increased volatility of these compounds. The main difference between the two species was with the responses to the methylenedioxy analogue (**13b**) and the difluoromethylenedioxy analogue (**14b**). The response of *B. tryoni* to these compounds was low compared to a blank, whereas the response of *B. jarvisi* was consistently greater than a blank. This suggested that the *B. jarvisi* receptor is able to bind the methylenedioxy moiety more favourably than the *B. tryoni* receptor.

Cage bioassays were conducted to investigate the behavioural response of *B. tryoni* to the compounds investigated in this study. A preliminary cage bioassay indicated that *B. tryoni* had a relatively strong response to zingerone (**1**) and a moderate response to the benzyl acetate analogue (**5**), but the response to the other compounds tested was very weak. This was followed by a more robust cage bioassay with *B. tryoni*, which unfortunately had issues with cuelure and control traps and therefore little could be concluded. In contrast to the EAG results, the

trifluoroacetyl ester (**4**) and the difluoromethylenedioxy analogue (**14b**) produced the strongest responses from male *B. tryoni*.

Future directions include the synthesis and investigation of the other proposed zingerone analogues in Figure 9. Other purification methods for the formyl ester (**3**) and the trifluoroacetyl ester (**4**) should be investigated in order to test **3** in a pure form and more easily obtain **4** in the quantities required for further investigation. An alternative method for the synthesis of the trifluorobutanone analogue (**12b**) should be developed and the formation of the unidentified white solid prevented. The vapour pressure of the analogues not measured in this study should be determined to complete the zingerone analogue series and more completely understand the relationship between volatility and lure attraction. Confidence in the vapour pressure data would also be strengthened by measuring vapour pressure with other techniques, such as gas saturation. EAG responses of *B. tryoni* and *B. jarvisi* to these compounds should be repeated with a greater number of replicates, female flies as well as male flies, and flies of different ages and batches. More extensive bioassays should be conducted with both *B. tryoni* and *B. jarvisi*, including additional laboratory cage bioassays and field bioassays. This would allow for the evaluation of these zingerone analogues as potential fruit fly attractants for use with MAT or biosecurity and population monitoring.

Chapter 5. References

1. Clarke, A. R.; Powell, K. S.; Weldon, C. W.; Taylor, P. W., "The ecology of *Bactrocera tryoni* (Diptera: Tephritidae): what do we know to assist pest management?" *Ann. Appl. Biol.* **2011,** *158* (1), 26-54.
2. Dominiak, B. C.; Ekman, J. H., "The rise and demise of control options for fruit fly in Australia." *Crop Protect.* **2013,** *51*, 57-67.
3. PHA *National Fruit Fly Strategy Implementation Action Plan*; Plant Health Australia: Canberra, Australia, 2010.
4. Royer, J. E.; Hancock, D. L., "New distribution and lure records of Dacinae (Diptera: Tephritidae) from Queensland, Australia, and description of a new species of *Dacus* Fabricius." *Aust. J. Entomol.* **2012,** *51* (4), 239-247.
5. Fay, H. A., "A highly effective and selective male lure for *Bactrocera jarvisi* (Tryon)(Diptera: Tephritidae)." *Aust. J. Entomol.* **2012,** *51* (3), 189-197.
6. Dominiak, B. C.; Fay, H. A.; Fanson, B. G., "Field evaluation of the efficacy of zingerone and other chemical lures on seven species of fruit fly in Sydney, New South Wales, Australia." *Plant Protection Quarterly* **2015,** *30* (2), 67.
7. *Exposure and Risk Assessment Including Occupational Health and Safety Assessment of Dimethoate*; Australian Pesticides and Veterinary Medicines Authority: 2012.
8. *Fenthion Final Review Report and Regulatory Decision*; Australian Pesticides and Veterinary Medicines Authority: 2014.
9. Metcalf, R. L.; Metcalf, E. R., *Plant Kairomones in Insect Ecology and Control*. Springer: 1992.
10. Metcalf, R. L., "Chemical ecology of Dacinae fruit flies (Diptera: Tephritidae)." *Ann. Entomol. Soc. Am.* **1990,** *83* (6), 1017-1030.
11. Sutherst, R. W.; Collyer, B. S.; Yonow, T., "The vulnerability of Australian horticulture to the Queensland fruit fly, *Bactrocera* (*Dacus*) *tryoni*, under climate change." *Crop and Pasture Science* **2000,** *51* (4), 467-480.
12. Heaven, D., "Fruit Flies." Primary Industries and Resources SA, Ed. 2006.
13. Infested fruit showing the fruit fly larvae. http://www.dpi.nsw.gov.au/agriculture/pests-weeds/insects/images/images-and-extras/ff-affected-fruit.jpg (accessed 5/10/2015).
14. Davies, J.; Roberts, D.; Eyer, P.; Buckley, N.; Eddleston, M., "Hypotension in severe dimethoate self-poisoning." *Clin. Toxicol.* **2008,** *46* (9), 880-884.
15. Senanayake, N.; Karalliedde, L., "Neurotoxic effects of organohosphorus insecticides." *New Engl. J. Med.* **1987,** *316* (13), 761-763.
16. Dominiak, B. C.; Nicol, H. I., "Field performance of Lynfield and McPhail traps for monitoring male and female sterile *Bactrocera tryoni* (Froggatt) and wild *Dacus newmani* (Perkins)." *Pest Manage. Sci.* **2010,** *66* (7), 741-744.
17. Weldon, C.; Meats, A., "Short-range dispersal of recently emerged males and females of *Bactrocera tryoni* (Froggatt)(Diptera: Tephritidae) monitored by sticky sphere traps baited with protein and Lynfield traps baited with cue-lure." *Aust. J. Entomol.* **2007,** *46* (2), 160-166.
18. Lloyd, A. C.; Hamacek, E. L.; Kopittke, R. A.; Peek, T.; Wyatt, P. M.; Neale, C. J.; Eelkema, M.; Gu, H., "Area-wide management of fruit flies (Diptera: Tephritidae) in the Central Burnett district of Queensland, Australia." *Crop Protect.* **2010,** *29* (5), 462-469.
19. El-Sayed, A.; Suckling, D.; Byers, J.; Jang, E.; Wearing, C., "Potential of "lure and kill" in long-term pest management and eradication of invasive species." *J. Econ. Entomol.* **2009,** *102* (3), 815-835.
20. Vargas, R. I.; Stark, J. D.; Kido, M. H.; Ketter, H. M.; Whitehand, L. C., "Methyl eugenol and cue-lure traps for suppression of male oriental fruit flies and melon flies (Diptera: Tephritidae) in Hawaii: effects of lure mixtures and weathering." *J. Econ. Entomol.* **2000,** *93* (1), 81-87.
21. Vargas, R. I.; Stark, J. D.; Mackey, B.; Bull, R., "Weathering Trials of Amulet Cue-Lure and Amulet Methyl Eugenol "Attract-and-Kill" Stations with Male Melon Flies

and Oriental Fruit Flies (Diptera: Tephritidae) in Hawaii." *J. Econ. Entomol.* **2005,** *98* (5), 1551-1559.
22. Tan, K. H.; Nishida, R.; Jang, E. B.; Shelly, T. E., Pheromones, male lures, and trapping of tephritid fruit flies. In *Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies*, Springer: 2014; pp 15-74.
23. Kumaran, N.; Prentis, P. J.; Mangalam, K. P.; Schutze, M. K.; Clarke, A. R., "Sexual selection in true fruit flies (Diptera: Tephritidae): transcriptome and experimental evidences for phytochemicals increasing male competitive ability." *Mol. Ecol.* **2014,** *23* (18), 4645-4657.
24. Kumaran, N.; Hayes, R. A.; Clarke, A. R., "Cuelure but not zingerone make the sex pheromone of male *Bactrocera tryoni* (Tephritidae: Diptera) more attractive to females." *J. Insect Physiol.* **2014,** *68*, 36-43.
25. Khoo, C. C. H.; Tan, K. H., "Attraction of both sexes of melon fly, *Bactrocera cucurbitae* to conspecific males–a comparison after pharmacophagy of cue-lure and a new attractant–zingerone." *Entomol. Exp. Appl.* **2000,** *97* (3), 317-320.
26. Kumaran, N.; Clarke, A., "Indirect effects of phytochemicals on offspring performance of Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae)." *J. Appl. Entomol.* **2014,** *138* (5), 361-367.
27. Royer, J. E., "Responses of fruit flies (Tephritidae: Dacinae) to novel male attractants in north Queensland, Australia, and improved lures for some pest species." *Austral Entomology* **2015**.
28. Miller, E. C.; Swanson, A. B.; Phillips, D. H.; Fletcher, L.; Liem, A.; Miller, J. A., "Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole." *Cancer Res.* **1983,** *43* (3), 1124-1134.
29. Smith, R.; Adams, T.; Doull, J.; Feron, V.; Goodman, J.; Marnett, L.; Portoghese, P.; Waddell, W.; Wagner, B.; Rogers, A., "Safety assessment of allylalkoxybenzene derivatives used as flavouring substances—methyl eugenol and estragole." *Food Chem. Toxicol.* **2002,** *40* (7), 851-870.
30. Robison, S. H.; Barr, D. B., "Use of biomonitoring data to evaluate methyl eugenol exposure." *Environ. Health Perspect.* **2006,** *114* (11), 1797-1801.
31. Drew, R., "The responses of fruit fly species (Diptera: Tephritidae) in the South Pacific area to male attractants." *J. Aust. Entomol. Soc.* **1974,** *13* (4), 267-270.
32. Beroza, M.; Alexander, B.; Steiner, L.; Mitchell, W. t.; Miyashita, D. H., "New synthetic lures for the male melon fly." *Science* **1960,** *131* (3406), 1044-1045.
33. Metcalf, R. L.; Metcalf, E. R.; Mitchell, W., "Molecular parameters and olfaction in the oriental fruit fly *Dacus dorsalis*." *Proceedings of the National Academy of Sciences* **1981,** *78* (7), 4007-4010.
34. Metcalf, R. L.; Mitchell, W. C.; Metcalf, E. R., "Olfactory receptors in the melon fly *Dacus cucurbitae* and the oriental fruit fly *Dacus dorsalis*." *Proceedings of the National Academy of Sciences* **1983,** *80* (11), 3143-3147.
35. Metcalf, R. L.; Metcalf, E. R.; Mitchell, W., "Benzyl acetates as attractants for the male oriental fruit fly, *Dacus dorsalis*, and the male melon fly, *Dacus cucurbitae*." *Proceedings of the National Academy of Sciences* **1986,** *83* (6), 1549-1553.
36. Keiser, I.; Kobayashi, R. M.; Miyashita, D. H.; Harris, E. J.; Chambers, D. L., "Comparative volatilization of three lures for male tephritids in Hawaii: methyl eugenol (oriental fruit fly), cue-lure (melon fly), and trimedlure (Mediterranean fruit fly)." *Environ. Lett.* **1974,** *7* (1), 47-52.
37. Casana-Giner, V.; Oliver, J.; Jang, E.; Carvalho, L.; Khrimian, A.; DeMilo, A.; McQuate, G., "Raspberry ketone formate as an attractant for the melon fly (Diptera: Tephritidae)." *J. Entomol. Sci.* **2003,** *38* (1), 120-126.
38. Brozena, A., "Vapor pressure of 1-octanol below 5kPa using DSC." *Thermochim. Acta* **2013,** *561*, 72-76.

39. Brozena, A.; Tevault, D. E.; Irwin, K., "Vapor pressure of thiodiglycol." *J. Chem. Eng. Data* **2014,** *59* (2), 307-311.
40. Butrow, A. B.; Seyler, R. J., "Vapor pressure by DSC: extending ASTM E 1782 below 5 kPa." *Thermochim. Acta* **2003,** *402* (1), 145-152.
41. Butrow, A. B.; Buchanan, J. H.; Tevault, D. E., "Vapor pressure of organophosphorus nerve agent simulant compounds." *J. Chem. Eng. Data* **2009,** *54* (6), 1876-1883.
42. Falleiro, R. M. M.; Silva, L. Y. A.; Meirelles, A. J.; Kraehenbuehl, M. A., "Vapor pressure data for fatty acids obtained using an adaptation of the DSC technique." *Thermochim. Acta* **2012,** *547*, 6-12.
43. Silva, L. Y. A.; Falleiro, R. M. M.; Meirelles, A. J.; Kraehenbuehl, M. A., "Determination of the vapor pressure of ethyl esters by Differential Scanning Calorimetry." *The Journal of Chemical Thermodynamics* **2011,** *43* (6), 943-947.
44. Williams, B. R.; Hulet, M. S.; Brozena, A.; Miles Jr, R. W.; Tevault, D. E., "Vapor Pressure of 2-Dialkyl Aminoethanethiols." *J. Chem. Eng. Data* **2013,** *58* (6), 1679-1684.
45. ASTM E1782-14, "Standard Test Method for Determining Vapor Pressure by Thermal Analysis." ASTM International: West Conshohocken, PA, 2014.
46. Schlosser, M.; Michel, D., "About the “physiological size” of fluorine substituents: comparison of sensorially active compounds with fluorine and methyl substituted analogues." *Tetrahedron* **1996,** *52* (1), 99-108.
47. Bondi, A., "van der Waals volumes and radii." *The Journal of Physical Chemistry* **1964,** *68* (3), 441-451.
48. Michel, D.; Schlosser, M., "Odor–structure relationships using fluorine as a probe." *Tetrahedron* **2000,** *56* (25), 4253-4260.
49. Smart, B. E., "Fluorine substituent effects (on bioactivity)." *J. Fluorine Chem.* **2001,** *109* (1), 3-11.
50. Biffinger, J. C.; Kim, H. W.; DiMagno, S. G., "The polar hydrophobicity of fluorinated compounds." *ChemBioChem* **2004,** *5* (5), 622-627.
51. Dunitz, J. D., "Organic fluorine: odd man out." *ChemBioChem* **2004,** *5* (5), 614-621.
52. Lemal, D. M., "Perspective on fluorocarbon chemistry." *The Journal of Organic Chemistry* **2004,** *69* (1), 1-11.
53. Leroux, F.; Jeschke, P.; Schlosser, M., "α-Fluorinated ethers, thioethers, and amines: Anomerically biased species." *Chem. Rev.* **2005,** *105* (3), 827-856.
54. Samuels, J. A.; Folting, K.; Huffman, J. C.; Caulton, K. G., "Structure/Volatility Correlation of Sodium and Zirconium Fluoroalkoxides." *Chem. Mater.* **1995,** *7* (5), 929-935.
55. Mitchell, W. C.; Metcalf, R. L.; Metcalf, E. R.; Mitchell, S., "Candidate substitutes for methyl eugenol as attractants for the area-wide monitoring and control of the oriental fruitfly, *Dacus dorsalis* Hendel (Diptera: Tephritidae)." *Environ. Entomol.* **1985,** *14* (2), 176-181.
56. Khrimian, A.; Siderhurst, M. S.; Mcquate, G. T.; Liquido, N. J.; Nagata, J.; Carvalho, L.; Guzman, F.; Jang, E. B., "Ring-fluorinated analog of methyl eugenol: attractiveness to and metabolism in the oriental fruit fly, *Bactrocera dorsalis* (Hendel)." *J. Chem. Ecol.* **2009,** *35* (2), 209-218.
57. Khrimian, A. P.; DeMilo, A. B.; Waters, R. M.; Liquido, N. J.; Nicholson, J. M., "Monofluoro analogs of eugenol methyl ether as novel attractants for the oriental fruit fly." *The Journal of Organic Chemistry* **1994,** *59* (26), 8034-8039.
58. Tan, K. H.; Nishida, R., "Mutual reproductive benefits between a wild orchid, *Bulbophyllum patens*, and *Bactrocera* fruit flies via a floral synomone." *J. Chem. Ecol.* **2000,** *26* (2), 533-546.
59. Tan, K. H.; Nishida, R., "Zingerone in the floral synomone of *Bulbophyllum baileyi* (Orchidaceae) attracts *Bactrocera* fruit flies during pollination." *Biochem. Syst. Ecol.* **2007,** *35* (6), 334-341.

60. Ong, P. T.; Hee, A. K. W.; Wee, S. L.; Tan, K. H., "The attraction of flowers of *Bulbophyllum* (Section *Sestochilus*) to *Bactrocera* Fruit Flies (Diptera: Tephritidae)." *Malesian Orchid Journal* **2011,** *8*, 93-102.
61. Nomura, H., "LXIV.—The pungent principles of ginger. Part I. A new ketone, zingerone (4-hydroxy-3-methoxyphenylethyl methyl ketone) occurring in ginger." *Journal of the Chemical Society, Transactions* **1917,** *111*, 769-776.
62. Allevi, P.; Ciuffreda, P.; Longo, A.; Anastasia, M., "Lipase-catalysed chemoselective monoacetylation of hydroxyalkylphenols and chemoselective removal of a single acetyl group from their diacetates." *Tetrahedron: Asymmetry* **1998,** *9* (16), 2915-2924.
63. Barbero, G. F.; Molinillo, J. M.; Varela, R. M.; Palma, M.; Macías, F. A.; Barroso, C. G., "Application of Hansch's Model to Capsaicinoids and Capsinoids: A Study Using the Quantitative Structure− Activity Relationship. A Novel Method for the Synthesis of Capsinoids." *J. Agric. Food. Chem.* **2010,** *58* (6), 3342-3349.
64. Agarwal, M.; Walia, S.; Dhingra, S.; Khambay, B. P. S., "Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/derived from *Zingiber officinale* Roscoe (ginger) rhizomes." *Pest Manage. Sci.* **2001,** *57* (3), 289-300.
65. Poli, G.; Giambastiani, G., "An epiisopicropodophyllin aza analogue via palladium-catalyzed pseudo-domino cyclization." *The Journal of Organic Chemistry* **2002,** *67* (26), 9456-9459.
66. Iinuma, M.; Moriyama, K.; Togo, H., "Various oxidative reactions with novel ion-supported (diacetoxyiodo) benzenes." *Tetrahedron* **2013,** *69* (14), 2961-2970.
67. Lapworth, A.; Wykes, F. H., "LXVI.—The pungent principles of ginger. Part II. Synthetic preparations of zingerone, methylzingerone and some related acids." *Journal of the Chemical Society, Transactions* **1917,** *111*, 790-798.
68. Dhuru, S.; Bhedi, D.; Gophane, D.; Hirbhagat, K.; Nadar, V.; More, D.; Parikh, S.; Dalal, R.; Fonseca, L. C.; Kharas, F., "Novel diarylheptanoids as inhibitors of TNF-α production." *Bioorg. Med. Chem. Lett.* **2011,** *21* (12), 3784-3787.
69. Pandey, G.; Karthikeyan, M.; Murugan, A., "New Intramolecular α-Arylation Strategy of Ketones by the Reaction of Silyl Enol Ethers to Photosensitized Electron Transfer Generated Arene Radical Cations: Construction of Benzannulated and Benzospiroannulated Compounds." *The Journal of Organic Chemistry* **1998,** *63* (9), 2867-2872.
70. Denniff, P.; Macleod, I.; Whiting, D. A., "Syntheses of the (±)-[n]-gingerols (pungent principles of ginger) and related compounds through regioselective aldol condensations: relative pungency assays." *J. Chem. Soc., Perkin Trans. 1* **1981**, 82-87.
71. Mannich, C.; Merz, K. W., "Phenolic bases derived from 1-phenyl-3-aminobutane." *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* **1927,** *265*, 15-26.
72. Berlin, S., *Zh. Obshch. Khim.* **1952,** *22*, 1998-2002.
73. McQuillin, F.; Ord, W., "Mechanisms of catalytic hydrogenation. Part I. An examination of the role of alkali and of acid." *J. Chem. Soc.* **1959**, 2902-2908.
74. Chen, Y.-L.; Barthel, W., "Some New Pyrethrin-type Esters." *J. Am. Chem. Soc.* **1953,** *75* (17), 4287-4289.
75. E967-08(2014), A., "Standard Test Method for Temperature Calibration of Differential Scanning Calorimeters and Differential Thermal Analyzers." ASTM International: West Conshohocken, PA, 2014.
76. Wilhoit, R. C.; Zwolinski, B. J., "Physical and thermodynamic properties of aliphatic alcohols." *J. Phys. Chem. Ref. Data* **1973,** *2* (1).
77. Verley, A.; Bölsing, F., "Bestimmung des Eugenols im Nelkenöl." *Berichte der Deutschen Chemischen Gesellschaft* **1901,** *34* (3), 3359-3362.
78. Neises, B.; Steglich, W., "Simple method for the esterification of carboxylic acids." *Angewandte Chemie International Edition in English* **1978,** *17* (7), 522-524.
79. Clark, R. F.; Simons, J., "Aromatic Esters of Fluorocarbon Acids." *J. Am. Chem. Soc.* **1953,** *75* (24), 6305-6306.

80. Bosco, J. J.; Raju, B. R.; Saikia, A. K., "Potassium fluoride assisted selective acetylation of alcohols with acetic acid." *Synth. Commun.* **2004,** *34* (15), 2849-2855.
81. Kitayama, T.; Isomori, S.; Nakamura, K., "Asymmetric synthesis of enantiomerically pure zingerols by lipase-catalyzed transesterification and efficient synthesis of their analogues." *Tetrahedron: Asymmetry* **2013,** *24* (11), 621-627.
82. Kim, D. S.; Kim, J. Y., "Side-chain length is important for shogaols in protecting neuronal cells from β-amyloid insult." *Bioorg. Med. Chem. Lett.* **2004,** *14* (5), 1287-1289.
83. Pabst, A.; Barron, D.; Adda, J.; Schreier, P., "Phenylbutan-2-one β-D-glucosides from raspberry fruit." *Phytochemistry* **1990,** *29* (12), 3853-3858.
84. Plourde, G. L., "Studies towards the diastereoselective spiroannulation of phenolic derivatives." *Tetrahedron Lett.* **2002,** *43* (19), 3597-3599.
85. Smith, L. R., "Rheosmin ("Raspberry Ketone") and Zingerone, and Their Preparation by Crossed Aldol-Catalytic Hydrogenation Sequences." *The Chemical Educator* **1996,** *1* (3), 1-18.
86. Russo, A. T.; Amezcua, K. L.; Huynh, V. A.; Rousslang, Z. M.; Cordes, D. B., "A simple borohydride-based method for selective 1, 4-conjugate reduction of α, β-unsaturated carbonyl compounds." *Tetrahedron Lett.* **2011,** *52* (50), 6823-6826.
87. Mandal, P. K.; McMurray, J. S., "Pd-C-induced catalytic transfer hydrogenation with triethylsilane." *The Journal of Organic Chemistry* **2007,** *72* (17), 6599-6601.
88. Roman, B. I.; Monbaliu, J. C.; De Coen, L. M.; Verhasselt, S.; Schuddinck, B.; Van Hoeylandt, E.; Stevens, C. V., "Feruloylbenzotriazole and Weinreb Amide as Bioinspired Building Blocks: A Reactivity Study towards O-, N-, S-, and C-Nucleophiles." *Eur. J. Org. Chem.* **2014,** *2014* (12), 2594-2611.
89. Laszlo, P., "Chemical reactions on clays." *Science* **1987,** *235* (4795), 1473-1477.
90. Guthrie, J. P., "Carbonyl addition reactions: Factors affecting the hydrate-hemiacetal and hemiacetal-acetal equilibrium constants." *Can. J. Chem.* **1975,** *53* (6), 898-906.
91. Davies, A. T.; Pickett, P. M.; Slawin, A. M.; Smith, A. D., "Asymmetric synthesis of tri-and tetrasubstituted trifluoromethyl dihydropyranones from α-aroyloxyaldehydes via NHC redox catalysis." *ACS Catalysis* **2014,** *4* (8), 2696-2700.
92. Zheng, C.; Li, Y.; Yang, Y.; Wang, H.; Cui, H.; Zhang, J.; Zhao, G., "Highly Efficient Asymmetric Epoxidation of Electron-Deficient α, β-Enones and Related Applications to Organic Synthesis." *Adv. Synth. Catal.* **2009,** *351* (10), 1685-1691.
93. Mead, D.; Loh, R.; Asato, A.; Liu, R., "Fluorinated retinoids via crossed aldol condensation of 1, 1, 1-trifluoroacetone." *Tetrahedron Lett.* **1985,** *26* (24), 2873-2876.
94. Hu, J.; Chen, S.; Sun, Y.; Yang, J.; Rao, Y., "Synthesis of tri-and tetrasubstituted pyrazoles via Ru (II) catalysis: intramolecular aerobic oxidative C–N coupling." *Org. Lett.* **2012,** *14* (19), 5030-5033.
95. Ashworth, I.; Hopes, P.; Levin, D.; Patel, I.; Salloo, R., "An asymmetric synthesis of a 4-substituted-1, 4-dihydropyridine." *Tetrahedron Lett.* **2002,** *43* (28), 4931-4933.
96. Patel, I.; Ashworth, I.; Levin, D. Process for asymmetric synthesis of substituted 1,4 - dihydropyridines. 2006.
97. Liu, X.-H.; Song, B.-A.; Bhadury, P. S.; Zhu, H.-L.; Cui, P.; Hou, K.-K.; Xu, H.-L., "Novel 5-(3-(substituted)-4, 5-dihydroisoxazol-5-yl)-2-methoxyphenyl derivatives: Synthesis and anticancer activity." *Aust. J. Chem.* **2008,** *61* (11), 864-869.
98. Mase, N.; Kitagawa, N.; Takabe, K., "Protection-, Salt-, and Metal-Free Syntheses of [n]-Shogaols by Use of Dimethylammonium Dimethyl Carbamate (DIMCARB) without Protecting Groups." *Synlett* **2010,** (1), 93-96.
99. Barrall, I.; Edward, M., "Precise determination of melting and boiling points by differential thermal analysis and differential scanning calorimetry." *Thermochim. Acta* **1973,** *5* (4), 377-389.
100. Berlin, A.; Sherlin, S. M., "Derivatives of zingerone. I." *Zh. Obshch. Khim.* **1948,** *18*, 1386-94.

101. Nomura, *The Science Reports of the Tohoku University. First Series (Physics, Chemistry, Astronomy) 14*, 143.
102. Lide, D. R., *CRC Handbook for Chemistry and Physics*. 90th ed.; CRC Press: Boca Raton, FL, 2010.
103. Bunce, R. A.; Reeves, H. D., "Amberlyst-15 Catalyzed Addition of Phenols to α, β-Unsaturated Ketones." *Synth. Commun.* **1989,** *19* (5-6), 1109-1117.
104. Faillebin, *Annales de Chimie* **1925,** *10* (4), 423-435.
105. Adjangba, S. M.; Billet, D., "Studies on lignans. III. Confirmation of the structure of galcatine by total synthesis." *Bull. Soc. Chim. Fr.* **1962**, 1970-77.
106. Kaufmann, A.; Radosevic, R., "Synthesis of isoquinoline derivatives. I." *J. Chem. Soc.* **1916,** *110* (1), 502-4.
107. Le Goffic, F.; Galliot, C.; Gouyette, A., "Preparation of polycyclic compounds by cyclization of aryl acetylenic alcohols." *Bull. Soc. Chim. Fr.* **1975,** *5* (6), 1343-6.
108. Koo, J., "Syntheses in the Indene Series." *J. Am. Chem. Soc.* **1953,** *75* (8), 2000-2001.
109. Nomura, H.; Nozawa, F., "Synthesis of some phenolic ketones and their tastes." *The Science Reports of the Tohoku Imperial University* **1918,** *7*, 79-92.
110. Behal, A.; Tiffeneau, M., "Phenolic Ethers with a Pseudo Allylic Chain, -(Me)C:CH, (IV). Vanillic, Veratric and Piperonylic Series." *Bull. Soc. Chim. Fr.* **1908,** *3*, 732-6.
111. Brochet; Cabaret, *Bull. Soc. Chim. Fr.* **1915,** *4* (17), 58.
112. Vavon; Faillebin, "Hydrogenation of piperonalacetone and dipiperonalacetone." *Comptes Rendus* **1919,** *169*, 65-7.
113. Semmelhack, M.; Bargar, T., "Photostimulated nucleophilic aromatic substitution for halides with carbon nucleophiles. Preparative and mechanistic aspects." *J. Am. Chem. Soc.* **1980,** *102* (26), 7765-7774.
114. Velavan, R.; Sureshkumar, P.; Sivakumar, K.; Natarajan, S., "Vanillin-I." *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.* **1995,** *51* (6), 1131-1133.
115. ASTM E1194-07, "Standard Test Method for Vapour Pressure (Withdrawn 2013)." ASTM International: West Conshohocken, PA, 2007.

Appendix

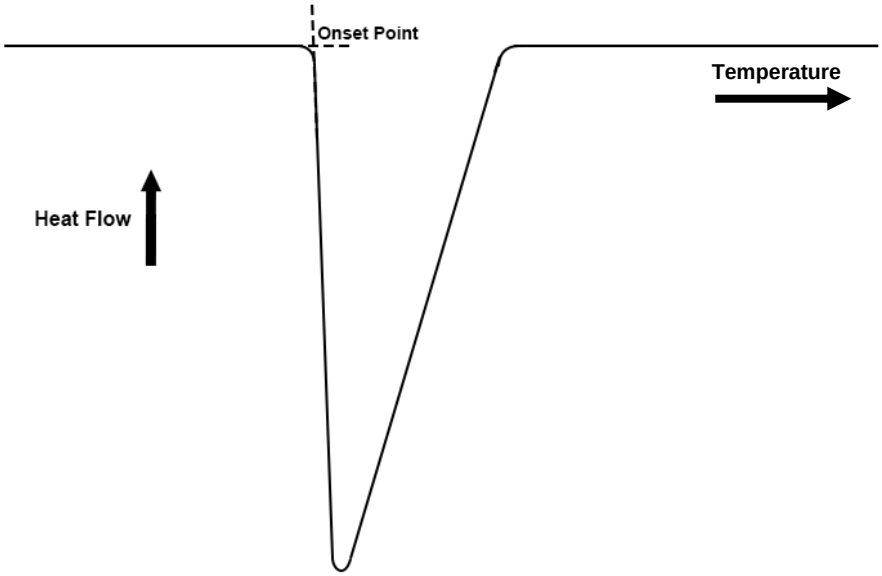


Figure A-1 Endotherm showing the onset point as the intersection of two tangents.

Table A-1 Temperature-pressure data for zingerone (**1**), the acetyl ester (**2**), the formyl ester (**3**), the trifluoroacetyl ester (**4**), methylzingerone (**8b**), the trifluoromethoxy analogue (**11b**), the methylenedioxy analogue (**13b**), and the difluoromethylenedioxy analogue (**14b**).

Compound	***T* / K**	**P_{exp} / kPa**	**P_{calc} / kPa**	**Percentage Difference** [d]
	600.6 [a,b]	atm		
	522.6	14.7	14.6	1.30
	503.5	7.73	7.97	-3.03
	483.6	4.03	3.99	0.82
1	465.7	2.07	2.01	2.90
	450.8	1.08	1.08	-0.67
	436.8	0.559	0.574	-2.56
	422.5	0.290	0.285	1.60
	410.4	0.149	0.150	-0.42
	598.9 [a,b]	atm		
	544.4 [a,c]	15.2		
	522.5 [a,c]	7.54		
	499.9	4.00	4.06	-1.40
2	481.6	2.07	2.02	2.64
	466.4	1.08	1.08	-0.09
	451.7	0.551	0.560	-1.62
	437.9	0.287	0.289	-0.61
	425.1	0.151	0.150	0.68

	- [a,c]	atm		
	- [a,c]	15.0		
	- [a,c]	7.77		
	498.6	4.00	4.06	-1.48
3 [e]	480.8	2.09	2.05	1.93
	465.0	1.07	1.06	1.06
	450.5	0.546	0.554	-1.48
	436.9	0.286	0.287	-0.19
	423.0	0.141	0.140	0.88
	576.5 [a,b]	atm		
	503.7	15.2	14.8	2.43
	486.2	7.82	8.21	-4.73
	466.4	4.03	3.98	1.20
4	449.5	2.06	2.02	1.92
	434.3	1.07	1.04	2.35
	421.9	0.557	0.581	-4.16
	407.8	0.289	0.286	1.15
	396.1	0.151	0.150	0.33
	590.4 [a]	atm		
	519.1 [a,c]	14.7		
	498.7 [a,c]	7.64		
	475.9	3.96	4.01	-1.30
8b	459.7	2.07	2.05	0.86
	445.3	1.08	1.08	0.17
	431.1	0.559	0.545	2.60
	419.2	0.289	0.297	-2.57
	406.7	0.151	0.151	0.10
	559.0 [a]	atm		
	492.8	15.6	15.3	1.61
	472.6	7.55	7.85	-3.84
	453.3	3.94	3.85	2.43
11b	437.9	2.07	2.05	1.07
	423.6	1.07	1.09	-1.51
	409.7	0.555	0.554	0.12
	396.9	0.282	0.280	0.56
	386.0	0.150	0.150	-0.28
	581.8 [a]	atm		
	503.1	15.2	14.8	2.24
	483.7	7.64	7.85	-2.76
	464.9	4.00	4.00	0.01
13b	447.4	2.08	2.02	2.84
	433.1	1.07	1.10	-2.45
	417.3	0.543	0.532	2.10
	405.0	0.288	0.290	-0.55
	392.1	0.147	0.145	1.39

	540.8 [a]	atm		
	472.5	14.7	14.6	0.74
	453.0	7.55	7.68	-1.80
	435.7	4.07	4.07	-0.07
14b	418.2	2.06	2.01	2.59
	404.2	1.07	1.08	-0.70
	390.4	0.553	0.554	-0.12
	378.6	0.293	0.298	-1.64
	366.0	0.147	0.146	1.01

[a] Data not used for Antoine Equation fit.

[b] Broad boiling endotherm, suggesting sample decomposition.

[c] Abnormal boiling endotherm shape affecting onset point measurement.

[d] Percentage difference = $100 \times (P_{exp} - P_{calc}) / P_{calc}$.

[e] Impure sample (contains approximately 5% zingerone).