Changes in Whiskey Odorants by the Lincoln County Process

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Abstract

The processing step that makes Tennessee whiskey unique from bourbon is known as the Lincoln county process (LCP). It is performed by filtering fresh whiskey distillate through a bed of maple charcoal. A model LCP system was developed, and the aroma of whiskey before and after LCP treatment was characterized. Sensory comparison of the two distillates was performed by olfactory profile analysis. Odorants in the two distillates were identified by comparative aroma extract dilution analysis (cAEDA). The odorants were then quantitated by stable isotope dilution assays (SIDA), and odor activity values (OAVs) were calculated based on the odor threshold of each odorant in an ethanol and water matrix as well as the concentration of each odorant. The LCP treatment was found to cause a decrease in the malty, rancid, fatty, and popcorn character of the distillate sample. A total of forty-nine odorants were identified in the distillate samples, nine of which are being reported for the first time in whiskey distillate. Twenty-nine odorants were quantified in the distillate samples, and all decreased between 13 and >99% as a result of LCP treatment. Four odorants, namely, (2*E,4E*)-nona-2,4-dienal (fatty), 3-methylbutanoic acid (sweaty, rancid), and corn-derived 2′-aminoacetophenone (foxy), and 2-acetyl-1-pyrroline (popcorn) dropped below their detection thresholds (OAV < 1) as a result of LCP treatment. The LCP treatment decreased lipid-derived aldehydes, organic acids, and corn-derived odorants by between 55 and >99%. The work presented here lays the groundwork for more comprehensive investigations into the process that makes Tennessee Whiskey unique from bourbon and provides an analytical toolkit to aid distillers in optimizing the flavor profile of their products.

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Introduction

Whiskey is an alcoholic beverage produced by fermenting a mixture of grains and water which is then distilled and aged in an oak barrel. Based on variations in each of these steps the final product can be one of several varieties of whiskey. Different proportions of grains can result in a rye or a bourbon whiskey and being produced in a different location can result in a different type of whiskey in the case of Scotch and Japanese whisky (note that whisky from these countries is traditionally spelled without the 'e'). Bourbon whiskey and Tennessee whiskey share many similarities, they must both be made from at least 51% corn, distilled at no more than 90% ethanol (180° proof), and stored in unused charred oak barrel at no more than 62.5% ethanol (125° proof). Tennessee whiskey is unique from bourbon whiskey due to an additional processing step known as the Lincoln county process (LCP). The LCP, also known as charcoal mellowing, is an additional processing step where fresh distillate is filtered through charcoal from the maple (*Acer sp.*) tree. While some research has been done into whiskey aroma, the only published research into how the Lincoln county process affects whiskey distillate was conducted in 1908.

The ***first objective*** of this study was to identify the odorants present in untreated and LCP treated whiskey distillate samples. This was done by using solvent assisted flavor evaporation (SAFE) to isolate volatiles and comparative aroma extract dilution analysis (cAEDA) to identify odorants and determine which of these odorants were most important to the distillate's aroma. The advantage of using SAFE over traditional distillation techniques, such as steam distillation, is the use of high vacuum and low

temperature, reducing the formation of artifacts and degradation of odorants. AEDA is a technique where the SAFE isolate is diluted 1:1 and analyzed via gas chromatography-olfactometry (GC-O) until no further odors are detected. For each odorant, it is noted the amount of dilution required before the odorant is no longer detected by GC-O thus giving insight into the importance of each odorants.

The *second objective* of this study was to quantitate changes in key odorants as a result of LCP treatment. Quantitation was performed by stable isotope dilution assay (SIDA), which is a technique that utilizes ^{13}C or ^{2}H substituted analogues of each odorant as an internal standard. Analysis of the samples was then performed via gas chromatography-mass spectrometry (GC-MS), and the isotopic standards were differentiated from the analytes based on comparison of ions. Concentrations of the analyte were calculated based on peak area in the chromatogram for ions corresponding to standard and analyte, amount of standard added to sample, and the amount of sample used for analysis. The quantitation was then confirmed by preparing aroma simulation models based on the analytical data and comparing the aroma models to original distillates through human sensory testing.

The results of this study and analytical methods developed can be used to guide Tennessee whiskey manufacturers in adjusting their process to produce whiskey with their desired flavor profile. Additionally, this study sets the groundwork for studies exploring variations in the LCP such as dwell time, charcoal to distillate ratio, and treatment temperature.

1. Literature Review

1.1 Whiskey Production

All whiskey must be made using grain and water as the starting material. Depending on the type whiskey being produced, different proportions of grains must be used. Scotch whisky is typically made from malted barley, bourbon and Tennessee whiskey must be made from at least 51% corn, and rye whiskey must be made from at least 51% rye grain[1]. Another grain that is often used in the production of whiskey is wheat, but this is rarely the primary constituent. The proportions of each grain in the recipe is commonly referred to as the "*mash bill*", and each distillery has their own mash bill that yields their unique whiskey[2].

The grain is a source of sugar that yeast can ferment into alcohol, however the sugars in grain are naturally present as starch. Yeast cannot readily ferment starch, so the grains must be processed[3]. This processing is referred to as "*mashing*" and begins by grinding the grain, thus increasing the surface area. The grains are then mixed with water and enzymes to break down starch into mono- and disaccharides. The result of this mash is a sugary mixture of grains and water that is referred to as a "*wort*"[4].

The mashing of the grains yields adequate substrate for utilization by brewer's yeast (*Saccharomyces cerevisiae)*. One reason that this species of yeast is the most commonly used is because *S. cerevisiae* is Crabtree positive, meaning that the organism will prefer to ferment sugar even in the presence of oxygen[5]. The primary product of this

fermentation is ethanol, but a myriad of odorants are produced including as ethyl esters, branched alcohols (also known as fusel oils), organic acids, aldehydes, and sulfur containing odornats[5]. The level of these odorants produced can vary based on parameters such as strain of yeast, fermentation temperature, wort pH, and availability of yeast nutrients. Fermentation takes place over approximately seven days and yields what is referred to as a "*distiller's beer"* or "*wash,"* which is typically 7-9% ethanol by volume[6].

The alcohol that was produced during fermentation must then be concentrated to yield the final product. This concentration is accomplished by distillation of the distiller's beer, separating compounds based on volatility. In a solution of different compounds, the vapor phase will be enriched in compounds with a lower vapor pressure. Distillation utilizes this phenomena by boiling the wash and condensing the vapor that is produced[4]. Distillation yields a liquid that is higher in the more volatile components, in this case ethanol and congeners, than the original solution. The simplest form of distillation apparatus is called a pot still, a single pot that contains the wash and the condensate is collected in a different pot[7]. The more common method of commercial distillation is to use a column still. A column still is comprised of a tall column with a series of plates distributed throughout and a temperature gradient is established in the column from hot at the bottom to cooler at the top.[2] Each of these plates act as locations of condensation and boiling, each repeated boiling will yield a vapor richer in the volatile components of the wash. The higher the number of plates in the still, the higher the concentration of low vapor pressure compounds, such as ethanol, in the final distillate. The pot still is still used to make Irish whiskey and is used in conjunction with a column still to produce American whiskey[1]. The

distillation process is divided into three phases (called cuts): the heads cut, the hearts cut, and the tails cut. The heads cut is the first portion of the distillation and is highest in low boiling point compounds such as acetone and ethyl acetate. The hearts cut is the middle portion of the distillation, containing ethyl esters and branched alcohols, that is collected and further processed. The tails are the end of the distillation, this cut has the lowest ethanol content as well as a high concentration of relatively high boiling point compounds such as fatty acids and phenylethyl alcohol[2].

Before the product can be called whiskey, it must be barrel aged. The exception being corn whiskey, which does not require aging prior to bottling and distribution[4]. For American whiskeys, this aging must take place in charred, unused oak barrels. Other whiskey varieties such as scotch or Irish whiskey may be aged in used barrels, often used bourbon barrels[1]. The processing of barrels typically involves toasting of the wood, and in the case of American whiskey charring. Toasting is a mild heating that causes caramelization to occur in the wood while charring is a more intense heat that creates a layer of burned wood inside the barrel as well a layer of toasted wood. The heads of a barrel are typically toasted while the staves are charred[4]. The two treatments have different effects on the composition of the distillate that is stored in the barrel. The toasting is thought to impart caramel and maple flavors while the charcoal acts to filter out compounds. From the wood itself, the distillate extracts wood derived compound such as vanillin or phenolics[7]. This process takes place over the course of years and temperature fluctuation appears to be vital for the extraction of barrel derived compounds[1].

These are the necessary steps for production of a whiskey (except for corn whiskey not needing barrel aging as sated previously), but there are additional steps which may occur during whiskey production. The most common of these is chill filtering, which is done by cooling the whiskey to separate oils that may be present[4]. This results in a whiskey that will not turn cloudy when cold or served on ice, which is often viewed as an undesirable characteristic.

Another of these variations is known as the Lincoln county process (LCP) or charcoal mellowing. The LCP is necessary for labelling as a Tennessee whiskey (with the special exception of Pritchard's distillery) and takes place between distillation and barrel aging of the spirit. The step is performed by exposing the fresh distillate to charcoal from the maple (*Acer sp.*) tree[8]. The process is named for Lincoln county Tennessee, the location of the original Jack Daniel's distillery. The LCP is the focus of the work presented here, because it is believed to yield a smoother tasting final product.

1.2 Whiskey Aroma Literature

In 1908, W.M. Dudley published an article investigating the Lincoln County process. His study found that when distillates made from rye or corn were treated with sugar maple charcoal they took on similar aroma characters. He additionally found that furfurol was completely removed and the fusel oils, aldehydes, and esters were removed to various degrees[8].

In 1993 Conner *et al* investigated the concentration of lignin-derived compounds in barrels with different levels of use, ranging from unused bourbon barrels to Scotch barrels that have been reused several times[9]. The study found that all barrels had the highest concentration of these compounds at a depth of 5 mm into the wood and the more used barrels had lower concentrations of compounds, such as vanillin and guaiacol, closer to the exterior of the barrel. This suggests that the compounds migrate towards the interior of the barrel with successive uses.

In 2000, Lee *et al* published an article investigating the sources of odorants responsible for sensory characteristics in Scotch whisky and designed a flavor wheel to be used as a tool for sensory analysis[10]. The article explained all of the sensory characteristics associated with Scotch whisky and correlated those with odorants present in the whiskey as well as where these odorants might originate during production.

In 2011, Harrison *et al* published an article examining the impact of still construction material on whiskey distillate aroma[11]. This was performed by producing whiskey distillate in a still constructed from stainless steel, one constructed from copper, and stills that were stainless steel except for one section. Quantitative descriptive analysis was used to determine differences between the products. The study found that using stainless steel equipment resulted in a new make whiskey that was higher in unpleasant smelling sulfurous and meaty aroma character. It was also found that the using a condenser or the wash pot constructed from copper in the still was the most effective at reducing these negative aroma characters.

In 2019 Arnold *et al.* published a study on the impact of corn variety and growing conditions on the aroma of the finished whiskey[12]. The study utilized lab scale whiskey production equipment to mash, ferment, and distill whiskey made from corn of different varieties as well as corn that was grown in different geographical regions of Texas. Their findings showed that there was a change in whiskey aroma as well as alcohol yield based on changes in corn variety and growing conditions. It was also found that benzaldehyde levels present in corn may act as an indicator of desirable sensory characteristics in the finished product.

There have been several studies identifying volatile compounds in whiskey, but very few studies were quantitative[13-15]. Lee *et al* used headspace analysis to identify 39 volatile compounds from several varieties of Scotch whisky (note the exclusion of the 'e' when referring to Scotch whisky) and used principle component analysis to discriminate between different qualities of whisky based on gas chromatogram peak areas[14]. Nishimura and Masuda analyzed the fusel oil composition of Scotch and Japanese whiskys by GC-MS. The fusel oil was fractionated into basic, phenolic, and lactonic fractions prior to analysis and 45 volatile compounds were identified in both whiskys[15]. Kahn *et al* analyzed distillate samples via GC-MS in order to identify volatile compounds. What distinguishes this study from the previous is that the analysis was done on distillate instead of aged whiskey and analysis of hearts and tails cuts was performed. This study identified 87 volatiles in whiskey distillate, 34 of them had previously not been reported in whiskey distillate[13]. Zhao *et al* compared the composition of six distilled beverages including

whiskey, rum, vodka, gin, brandy, and Chinese liquor. They identified a total of 138 volatile compounds, 25 of which were found to be in common between the six beverages[16].

In 2008 Poisson and Schieberle published a manuscript identifying odorants in bourbon whiskey followed by an additional study quantitating the key odorants[17, 18]. In the first study, they identified 45 odorants with Flavor Dilution (FD) factors from 1024 to 1 by application of aroma extract dilution analysis (AEDA) to the aged whiskey and 13 odorants in the headspace of the sample. The second study used stable isotope dilution assay (SIDA) to quantitate 31 odorants in bourbon whiskey resulting in 26 odorants with an odor activity value ≥1.

A 2008 dissertation by Matthew Vocke was done on the aroma of a bourbon whiskey throughout processing[18]. This study investigated the aroma of Bourbon whiskey throughout the production process by use of SAFE, AEDA, and SIDA to isolate, identify, and quantitate key odorants present in each step of beverage processing. They also took measurements of odorants throughout the barrel aging process to determine how the barrel aging process changes the composition of the whiskey.

A 2010 Master's thesis by Jacob Lahne characterized the aroma of a rye whiskey[19]. This was done by utilizing AEDA and Sample Dilution Assay (SDA) to identify odorants and SIDA to quantitate them. In the rye whiskey samples, 30 odorants were identified and quantitated, and OAVs were determined for 15 of the odorants.

Whiskey has been the subject of much study throughout the years. Several articles have been published with the goal of investigating the chemical composition of whiskey, some as early as 1908. With some exceptions, these studies have not been quantitative. The quantitative studies were on bourbon or rye whiskey. The bulk of the research into whiskey has been regarding the composition of the beverage with only a handful of studies correlating chemical and sensory data and even fewer studies have been published on the odorant composition of whiskeys. Very few studies have been performed investigating the effect that processing has on whiskey composition and sensory properties. The work presented here is building an analytical toolkit to aid manufacturers in understanding how their process can be adjusted to create products with their desired flavor profile.

2. Materials and Methods

2.1 Distillate and Charcoal

The whiskey distillate used in this study was produced at Sugarlands Distilling Company located in Gatlinburg, TN and was made from a mash of 65% corn, 20% wheat, 10% rye, and 5% malted 6-row barley. This mash was fermented for seven days prior to being distilled in a pot-column hybrid still with a whiskey helmet, four plates, and a dephlegmater. During distillation the heads and tails cuts were discarded while the hearts cut was saved yielding a distillate of 64% alcohol by volume. The batch of distillate was split into two groups, one for analysis as before LCP distillate (bLCP) and one for charcoal treatment and subsequent analysis (aLCP)

Charcoal used for filtration was produced by wetting wood from the sugar maple tree with new make whiskey distillate and setting the wood on fire. The flame was maintained at a temperature appropriate for charcoal formation with a spray of water. Once pyrolysis of the wood was complete, the newly formed charcoal was allowed to cool before being ground to pieces roughly an inch in size and smaller particles were removed with a number 8 sieve. Prior to use, charcoal (100.8 g) was washed with 1.6 liters of 60:40 alcohol:water and 400 milliliters of distillate prior to treatment of distillate. Washes were performed by adding 400 milliliters of solution to charcoal, agitating by hand, and decanting the wash solution.

2.2 Charcoal Treatment of Distillate

Whiskey distillate was treated with sugar maple charcoal in a model system designed to be representative of industrial charcoal mellowing processes. Distillate (400 mL) was exposed to charcoal (100.8 g) for 24 hours in a glass jar without stirring. At the end of the 24 hours, the distillate was decanted and filtered in a Buchner funnel with number 41 filter paper.

2.3 Chemicals

2.3.1 Reference Odorants

When possible, odorants used for reference standards were obtained from commercial vendors as stated below. The odorant 3,4-dimethyl-2-pentylfuran was not commercially available and was synthesized in house to use as a reference standard.

The following odorants were purchased from Sigma-Aldrich (St. Louis, MO, USA): 1,1-diethoxyethane, ethyl propanoate, ethyl isobutyrate, butane-2,3-dione, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate , 2-methylpropan-1-ol, 3-methylbutyl acetate, 3-methylbutan-1-ol, ethyl hexanoate, 1-octen-3-one, dimethyl trisulfide, ethyl octanoate, 2-isopropyl-3-methoxypyrazine, acetic acid, 3-(methylsulfanyl)propanal, (2*E*)-non-2-enal, linalool, 2-methylpropanoic acid, (2*E,6Z*)-nona-2,6-dienal, butanoic acid, 2-acetylthiazole, 2-phenylacetaldehyde, 3-methylbutanoic acid, (2*E,4E*)-nona-2,4-dienal, ethyl phenylacetate, (*E*)-β-damascenone, (2*E,4E*)-deca-2,4-dienal, 2-phenylethylacetate, hexanoic acid, 2-methoxyphenol, ethyl 3-phenylpropanoate, 2-phenylethanol, γ-nonalactone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 4-allyl-2-methoxyphenol, 4-ethyl phenol, 2′-aminoacetophenone, 2,6-dimethoxyphenol, decanoic

acid, (*E*)-isoeugenol, 1*H*-indole, 2-phenylacetic acid, and 4-hydroxy-3-methoxybenzaldehyde.

The odorant 2′-aminoacetophenone was purchased from Fisher Scientific (Pittsburgh, PA, USA).

The odorant 3-methylnonane-2,4-dione was purchased from Penta Manufacturing (Livingston, NJ, USA).

The odorant 2-acetyl-1-pyrroline was purchased from aromaLAB (Planegg, Germany).

2.3.2 Isotopically Labelled Odorants

As with the reference odorants, isotopically labelled standards were commercially purchased when possible. Three odorants, ($^2H_7$)-2-methylpropanol, ($^2H_5$)-2-phenylethyl acetate, and ($^2H_3$)-2′-aminoacetophenone were not available for purchase and were therefore synthesized in house.

The following odorants were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada): ($^2H_4$)-1,1-diethoxyethane, ($^2H_6$)-butane-2,3-dione, ($^2H_3$)-ethyl butanoate, ($^2H_9$)-ethyl 2-methylbutanoate, ($^2H_3$)-3-methylbutyl acetate, ($^2H_4$)-3-methylbutan-1-ol, ($^2H_{11}$)-ethyl hexanoate, ($^2H_{15}$)-ethyl octanoate, ($^2H_3$)-linalool, ($^2H_7$)-2-methylpropanoic acid, ($^2H_7$)-butanoic acid, ($^2H_9$)-3-methylbutanoic acid, ($^2H_4$)-2-methoxyphenol, ($^2H_5$)-2-phenylethanol, and ($^2H_3$)-4-hydroxy-3-methoxybenzaldehyde.

The following odorants were purchased from aromaLAB (Planegg, Germany): ($^{2}H_{2}$)-ethyl 2-methylpropanoate, ($^{2}H_{2}$)-ethyl 3-methylbutanoate, ($^{13}C_{5}$)-2-acetyl-1-pyrroline, ($^{2}H_{2}$)-(2*E*)-non-2-enal, ($^{13}C_{2}$)-2-acetylthiazole, ($^{2}H_{5}$)-(2*E,4E*)-nona-2,4-dienal, ($^{2}H_{4}$)-*(E)*-β-damascenone, and ($^{2}H_{4}$)- (2*E,4E*)-deca-2,4-dienal.

The following odorants were purchased from Sigma Aldrich (St. Louis, MO, USA): ($^{13}C_{1}$)-acetic acid and ($^{13}C_{1}$)-decanoic acid.

The following odorant was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA): ($^{2}H_{5}$)-1*H*-indole.

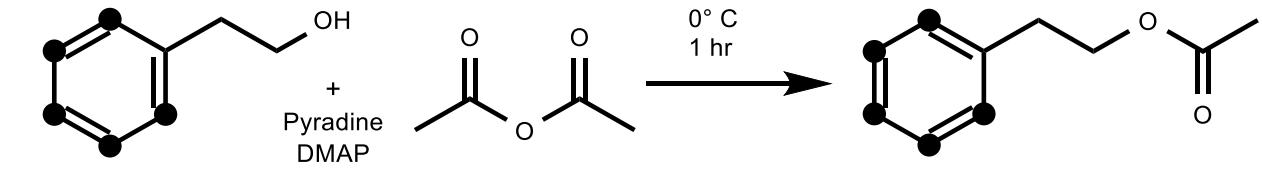
2.3.3 Synthesis of ($^{2}H_{5}$)-2-phenylethyl acetate

($^{2}H_{5}$)-2-phenylethyl acetate was synthesized by the acetylation of ($^{2}H_{5}$)-2-phenylethanol as shown in figure 1. ($^{2}H_{5}$)-Phenylethanol (3.5 mmol) was combined with 5 mL of anhydrous pyridine in a roundbottom flask. A septum was placed on the flask and the atmosphere was purged with N_{2} gas. To the flask, acetic anhydride (8.75 mmol, 826 µL) was added dropwise while maintaining a temperature of 0°C. Next, (dimethylamino)pyridine (0.25 mmol, 30.5 mg) was added and the reaction mixture was stirred for one hour at 0°C. The reaction mixture was then poured onto crushed ice and acidified to pH 2 with 2 M HCl. The mixture was extracted with pentane, dried over

anhydrous Na_2SO_4, and isolated under reduced pressure ($1x10^{-3}$ mbar). This procedure produced ~512.5 mg of ($^2H_5$)-2-phenylethyl acetete, an 86% yield.

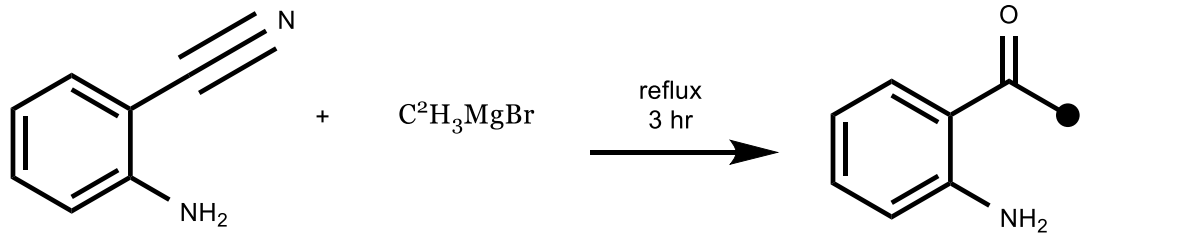
2.3.4 Synthesis of ($^2H_3$)-2′-aminoacetophenone

($^2H_3$)-2′-aminoacetophenone was synthesized by Grignard addition of deuterated methanol to 2-aminobenzonitrile as shown in figure 2. To make the Grignard reagent, 171.8 mg of powdered Mg was placed in a double neck round-bottom flask fitted with a condenser. Septa were placed in the second neck as well as at the top of the condenser. The atmosphere in the round-bottom was purged with N_2 gas and 10 mL of dry diethyl ether was added. To the flask, ($^2H_3$)-iodomethane (1 g, 7 mmol) was added and refluxed until Mg was dissolved. The reaction was cooled to room temperature and a mixture of 2-aminobenzonitrile (134 mg, 1.1 mmol) in 10 mL of absolute toluene was added. The mixture was refluxed for 3 hours while mixing and was subsequently cooled in an ice bath. To the mixture, 5 mL of chilled 7.7 M HCl was added dropwise, and the reaction mixture was stirred for 30 minutes followed by the addition of 7 mL of 6 M NaOH. The aqueous layer was extracted three times with 10 mL (3x10 mL) of diethyl ether and the combined organic layers were washed with water. The organic portion was dried over anhydrous Na_2SO_4 and the product was isolated under reduced pressure ($1x10^{-3}$ mbar). This procedure produced ~167.5 mg of ($^2H_3$)-2′-aminoacetophenone, for a yield of 86%.



($^2H_5$)-2-phenylethanol acetic anhydride ($^2H_5$)-phenylethyl acetate

Figure 1 Reaction scheme for synthesis of ($^2H_5$)-2-phenylethyl acetate



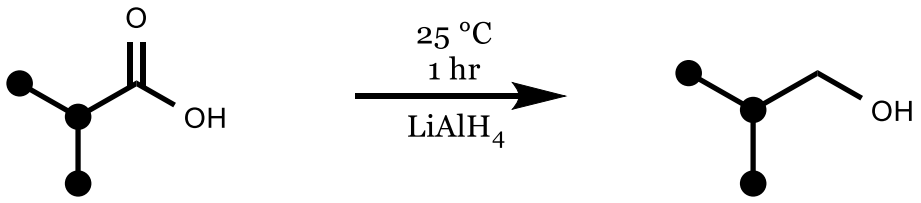
($^2H_3$)-2'-aminoacetophenone ($^2H_3$)-2'-aminoacetophenone

Figure 2 Reaction scheme for synthesis of ($^2H_3$)-2′-aminoacetophenone

2.3.5 Synthesis of ($^2H_7$)-2-methylpropan-1-ol

($^2H_7$)-2-methylpropan-1-ol was synthesized by reduction of ($^2H_7$)-2-methylpropionic acid with lithium aluminum hydride as show in figure 3. Lithium aluminum hydride (26.3 mmol, 997 mg) and 15 mL of anhydrous diethyl ether were added to a round-bottom flask with a septum and the atmosphere was purged with N_2 gas. To the round-bottom flask, a mixture of ($^2H_7$)-2-methylpropionic acid (250 mg, 2.63 mmol) in 5 mL of anhydrous diethyl ether was slowly added. The reaction was stirred at room temperature for one hour. Deionized water was added, resulting in gas and precipitate production, until there was no further gas produced. Next, 2 M sulfuric acid was added until the precipitate was

dissolved. The organic phase was collected, and the aqueous phase was extracted with 2x10 mL of diethyl ether. The organic layers were combined and washed with 2x10mL of saturated $NaHCO_3$ solution followed by 2x10 mL of saturated NaCl solution. The product was isolated under reduced pressure (1x10^{-3} mbar). This procedure produced ~141.3 mg of ($^2H_7$)-2-methylpropan-1-ol, for a yield of 68%.



($^2H_7$)-2-methylpropanoic acid ($^2H_7$)-2-methylpropan-1-ol

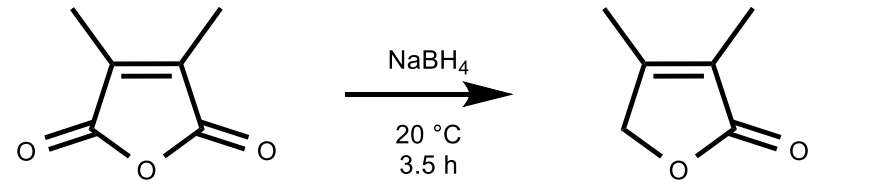
Figure 3 Reaction scheme for synthesis of ($^2H_7$)-2-methylpropan-1-ol

2.3.6 Synthesis of 3,4-dimethyl-2-pentylfuran

3,4-dimethyl-2-pentylfuran was synthesized by first synthesizing 3,4-dimethyl-2-(5H)-furanone from dimethyl maleic anhydride followed by Grignard addition to yield the final product as shown in figure 4. $NaBH_4$ (5 mmol, 189 mg) was dissolved in 1% NaOH and added to a mixture of dimethyl maleic anhydride while maintaining a temperature below 30°C. The reaction mixture was stirred for 2.5 hours at 20°C and had 50% sulfuric acid added (12 mmol, 590 mg). The mixture was heated for one hour at 90°C and allowed to cool before diethyl ether was added. The aqueous phase was saturated with NH_4Cl and the organic phase was separated and dried over Na_2SO_4. The solvent was distilled off, yielding 3,4-dimethyl-2-(5H)-furanone as crystals.

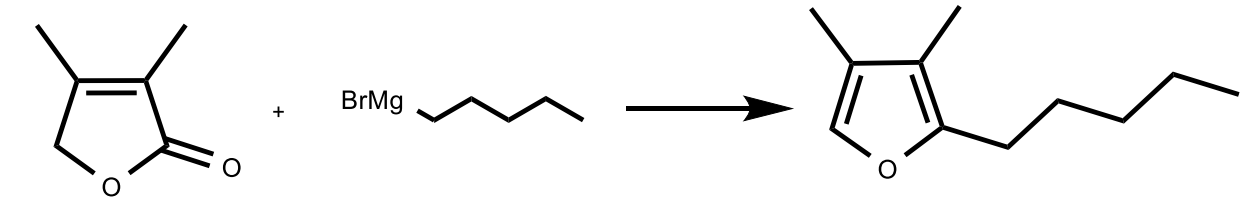
To synthesize the final product, the crystals from the previous step were dissolved in 15 mL of anhydrous diethyl ether and added to a two-neck round-bottom flask fitted with a condenser. The atmosphere was purged with N_2 gas and 3 mL of 2.0 M pentyl magnesium bromide was added to the flask. The reaction was refluxed with stirring for three hours and cooled to 10 °C. 10% HCl (4.25 mmol, 1.76 mL) was added to the reaction and a white precipitate formed. The reaction mixture was stirred for an additional 30 minutes at 20°C and the organic phase was collected. The organic phase was washed with 2x20 mL of 10% NaOH solution, dried over anhydrous Na_2SO_4, and isolated under reduced pressure ($1x10^{-3}$ mbar). This procedure produced 127 mg of 3,4-dimethyl-2-petnylfuran, for a yield of 16%.

Step One



dimethyl maleic anhydride 3,4-dimethyl-2-(5H)-furanone

Step Two



3,4-dimethyl-2-(5H)-furanone pentyl magnesium bromide 3,4-dimethyl-2-pentylfuran

Figure 4. Reaction scheme for synthesis of 3,4-dimethyl-2-pentylfuran

2.4 Identification of Key Whiskey Odorants

2.4.1 Extraction of Volatiles

Whiskey distillate samples (25 mL) were combined with an equal volume of saturated NaCl solution. The sample was then combined with 50 mL of freshly distilled diethyl ether in a separatory funnel and shaken by hand for five minutes. The organic phase was set aside, and the aqueous phase was extracted twice more in the same manner, again setting aside the organic phase. The collected organic phases were then dried over anhydrous Na_2SO_4.

2.4.2 Solvent Assisted Flavor Evaporation (SAFE)

The SAFE apparatus is shown in figure 5; this technique utilizes high vacuum to facilitate low temperature distillation of samples to prevent thermal degradation of odorants. In short, the system is under high vacuum ($1x10^{-3}$ mbar) and the distillation chamber is maintained at 40°C while the collection vessel is submerged in liquid N_2. The sample was placed in the dropping funnel of the SAFE and the valve was opened to allow the sample to slowly flow into the distillation chamber. The volatiles in the sample then evaporated and traveled into the collecting flask where they condensed.

The sample was then allowed to thaw at room temperature and was dried over anhydrous Na_2SO_4. The dry sample was then condensed to approximately 2 mL by a Vigereux column and a water bath maintained at 43 °C to remove the solvent. The 2 mL of sample was then further condensed to 200 µL using a gentle stream of N_2 gas.

2.4.3 Comparative Aroma Extract Dilution Analysis (cAEDA)

In order to identify the impactful odorants present in treated and untreated whiskey distillate, the SAFE isolates of each sample were analyzed via Gas Chromatography-Olfactometry (GC-O) as described below. Samples were then diluted 1:1 and analyzed again by GC-O. This process of dilution and GC-O was repeated until no further odorants were detected at the GC-O sniffing port. As odorants were detected, the odor quality was noted on the chromatogram and the highest dilution at which a given odorant could be detected at was assigned as that odorant's flavor dilution (FD) factor.

2.4.4 Gas Chromatography-Olfactometry (GC-O)

For GC-O, an Agilent Technologies Gas Chromatograph 6890 series with flame ionization detector was used. One μL of the sample was manually injected using a 10 μL syringe. The carrier gas was He at a flow rate of 1.5 mL/min. The initial temperature was 35°C, and this was maintained for one minute before being increased at 60°C/min to 60°C. Followed by an increase to 240°C at 6°C/min. The temperature was then held at 240°C for 10 minutes. Chromatographic separation was done by DB-5 and HP-FFAP capillary columns 30 meters in length, with a 0.32 mm inner diameter and 0.25 μL film thickness (30 m × 0.32 mm × 0.25 μm) (Agilent). At the end of the column was a Y-shaped glass splitter that directed the effluent into two 50 cm long piece of uncoated fused silica capillaries; one of which went to the flame ionization detector and the other going to a custom 80 mm × 25 mm I.D. cone sniffing port heated to 250 °C. The flame ionization detector was kept at

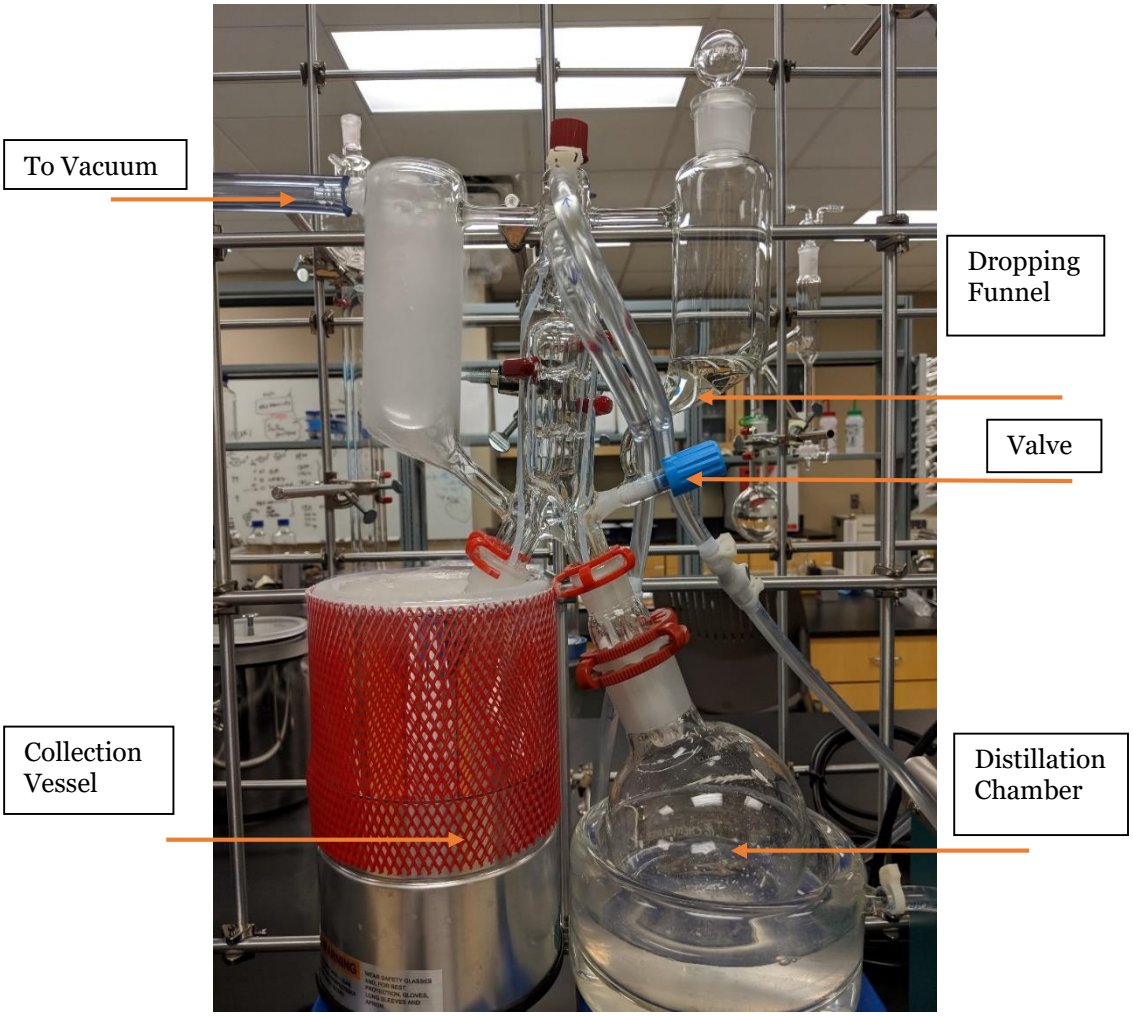


Figure 5 Diagram of Solvent Assisted Flavor Evaporation (SAFE) apparatu

250 °C, H_2 and air were flowing at 40.0 mL/min and 400 mL/min respectively with He as the make-up gas at 45 mL/min. The schematic of this can be seen in figure 6. During the GC-O run, an operator monitored the sniffing port and noted any aromas detected.

2.4.5 Gas-Chromatography Mass-spectrometry (GC-MS)

Gas chromatography-mass spectrometry was done by using an Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with either a DB-5 or an HP-FFAP of the same dimensions (30 m × 0.32 mm × 0.25 μm) column (Agilent) for separation of volatiles as well as an Agilent 5977 mass spectrometer for detection of odorants. SAFE isolates of bLCP and aLCP samples were injected on-column using an autosampler outfitted with a 10 μL syringe programmed to inject 1 μL of sample. The carrier gas, He, was maintained at a flow rate of 1 mL/min. The initial oven temperature was 35°C, this temperature was maintained for one minute after injection before being increased at a rate of 60°C/min until a temperature of 60°C had been achieved. The oven temperature was the heated at 6°C/min to 250°C, this final temperature was held for five minutes. The gas chromatograph and mass spectrometer were coupled by a transfer line that was maintained at 250°C. The mass spectrometer was operated in electron ionization mode at 70 eV and scanning in the range of 50-350 m/z.

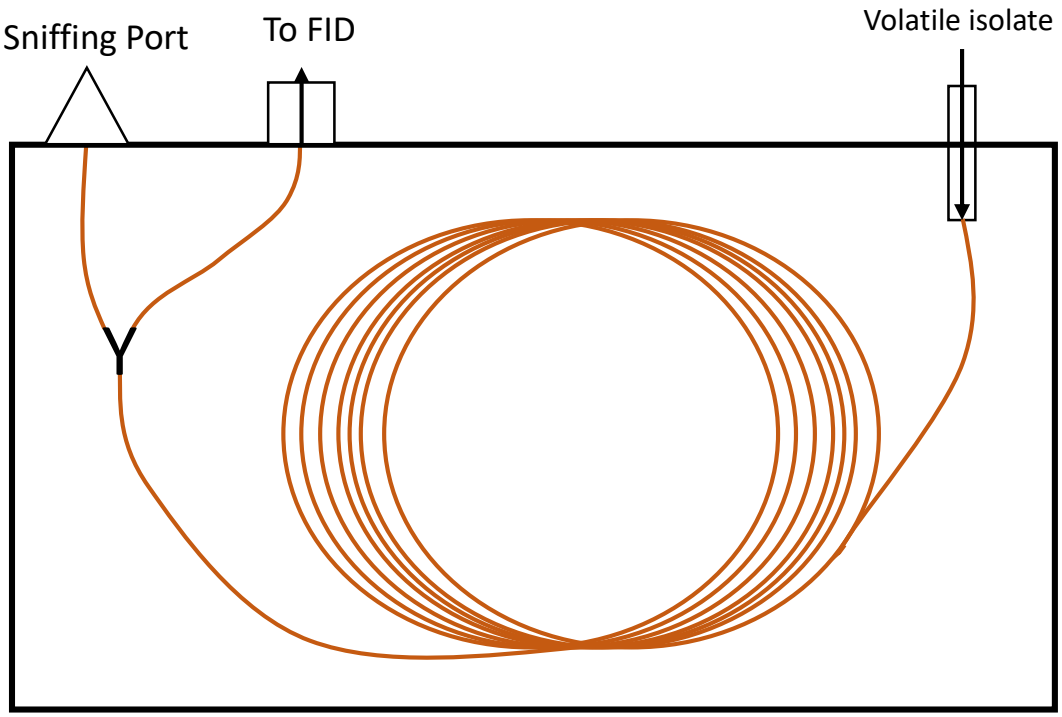


Figure 6 Diagram of Gas chromatography-Olfactometry (GC-O) instrument

2.4.6 Identification of Odorants

Odorants were identified based on three parameters: aroma quality upon detection in GC-O, retention index (RI) on two different capillary columns, and finally by comparing mass spectra in the sample to reference standards of pure odorant.

Retention index was measured by analyzing a mixture of alkanes with increasing chain length (C9-C26) on columns used for volatile separation and comparing retention times of hydrocarbons to odorants of interest. Retention index was calculated using the following equation:

$$RI = 100 \times \left[n + (N - n) \frac{t_a - t_n}{t_N - t_n} \right]$$

Where RI is retention index, n is the number of carbons in the hydrocarbon that elutes prior to the analyte, N is the number of carbons in the hydrocarbon that elutes after the analyte, t_a is the retention time of the analyte, t_n is the retention time of the hydrocarbon that elutes prior to the analyte, and t_N is the retention time of the hydrocarbon that elutes after the analyte.

2.5 Quantitation of Key Whiskey Odorants

2.5.1 Stable Isotope Dilution Assay (SIDA)

In order to accurately quantitate changes in odorants that were identified by AEDA, deuterium or carbon-13 labelled analogues of odorants of interest were obtained for use as internal standards, structures of labelled standards are displayed in figures 7 and 8. When possible, pure odorants of a known mass were dissolved in pentane and

concentration was calculated using weight of odorant and weight of solvent. When a labelled standard was received in a solution of unknown concentration or impurities were present, standards were quantified by use of GC-FID as described below.

Labelled standard was added to distillate prior to extraction and sample preparation and isolation of volatiles was carried out as described in the identification of key whiskey odorants section. SAFE isolates were then analyzed via GC-MS and extracted ion chromatograms (EICs) were generated for characteristic ions of analyte as well as the analogous ion in the labelled standard. Based on peak areas in EICs, amount of standard added, response factors determined as described below, and amount of sample used the concentration in μg/L of odorants was determined using the equation:

$$\mu g/L = \frac{c * v * UL}{V * L} * RF$$

Where μg/L is the concentration of analyte in the distillate sample, c is the concentration of the labelled standard in ng/mL, v is the volume of labelled standard added in μL, UL is peak area of the analyte from the EIC, L is the peak area of the labelled standard from the EIC, V is the volume of sample analyzed, and RF is the response factor that the analyte and labelled standard. Table 1 shows the extracted ions and response factors for each of the quantitated odorants and labelled standards.

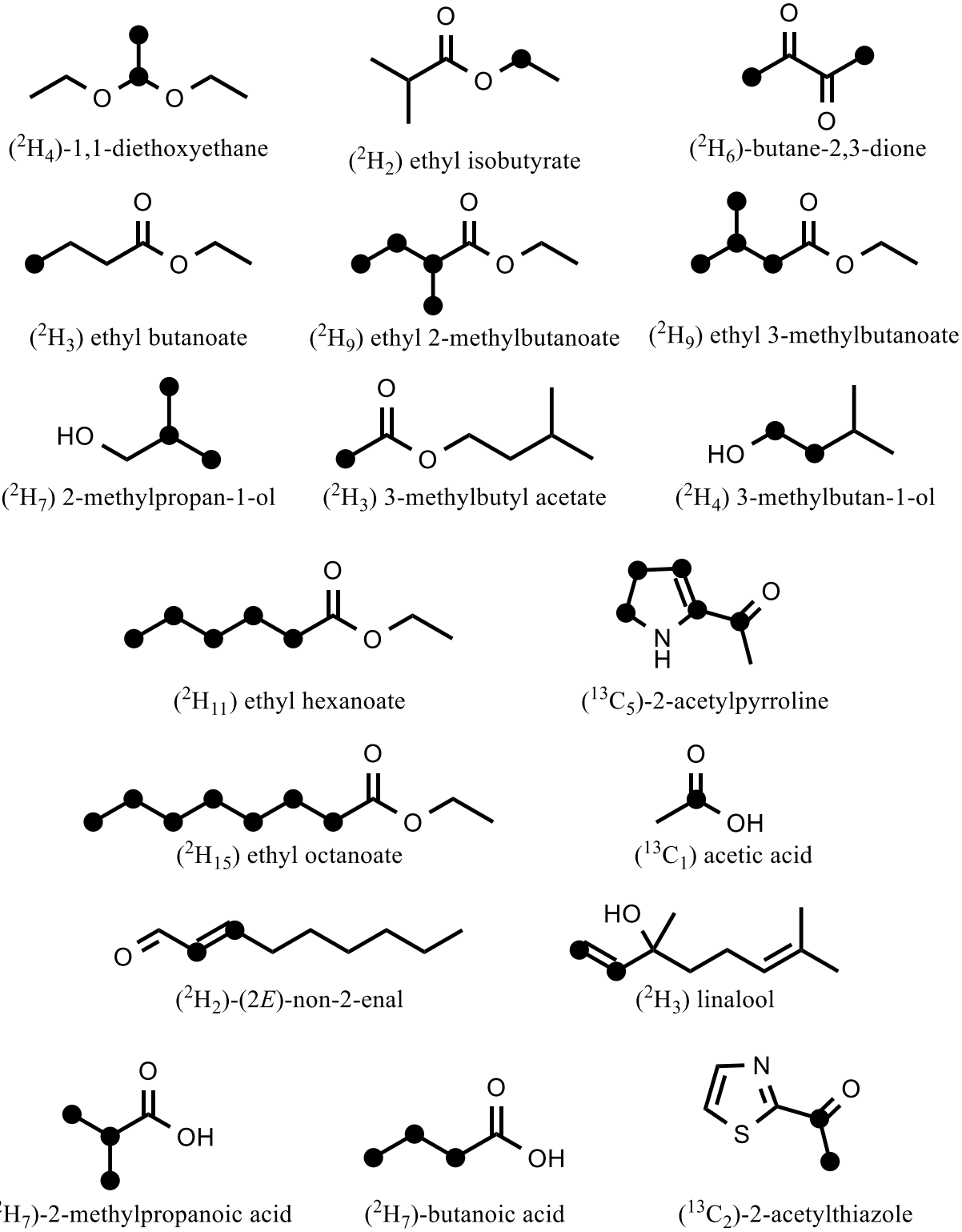


Figure 7 Structures of isotopically labelled odorants used for quantitation

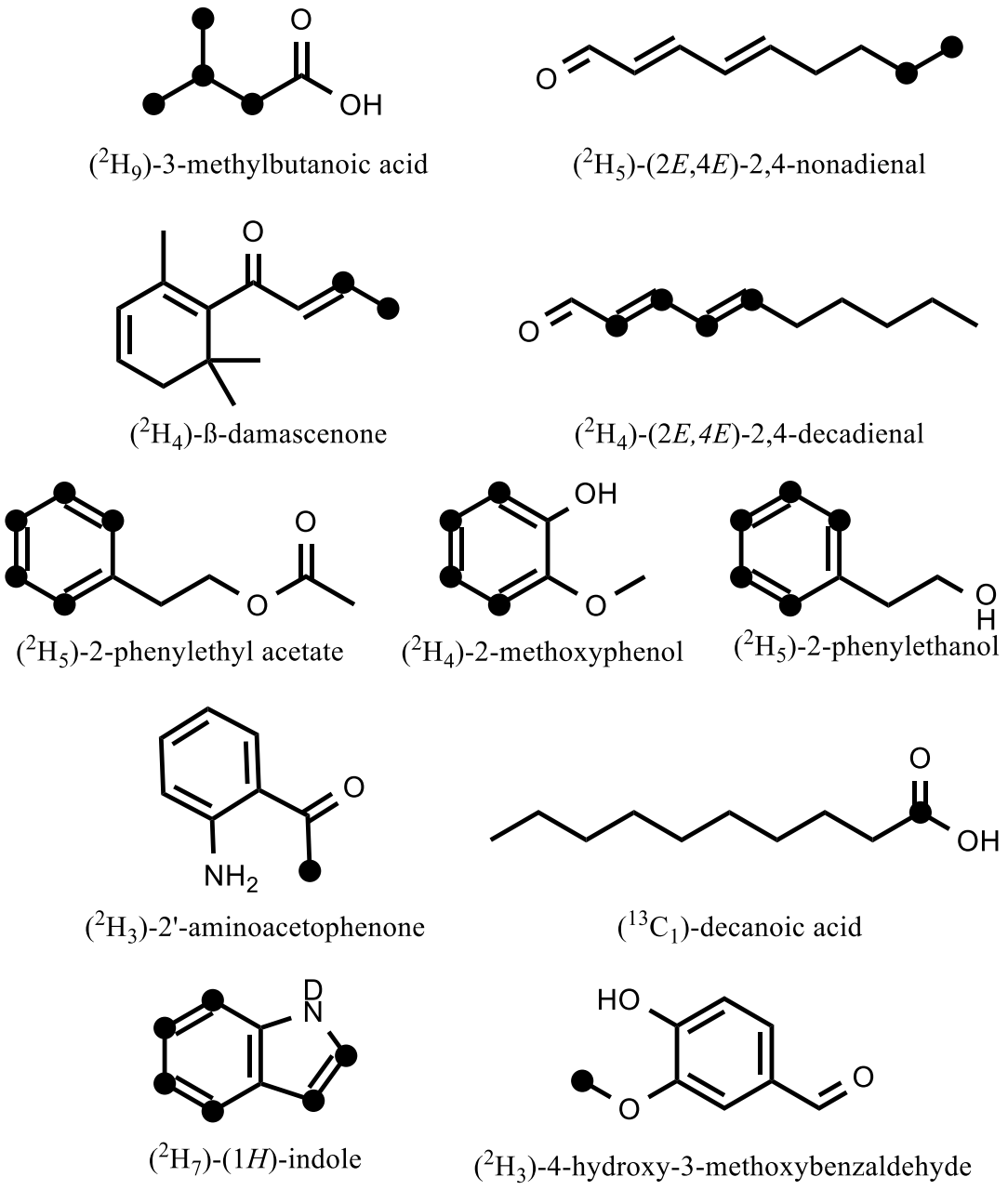


Figure 8 Structures of isotopically labelled odorants used for quantitation cont.

2.5.2 Quantitation of Labelled Standards by Gas Chromatography Flame Ionization Detector (GC-FID)

The concentration of labelled standards used for quantitation were determined by constructing a calibration curve via gas chromatography-flame ionization detector. Calibration curves were generated by running different concentrations of unlabeled reference odorants or a compound with the equivalent number of carbons. The instrument used was a 6890 series GC system (Hewlitt Packard, Palo Alto, CA) with a DB-5 capillary column (30 m x 250 um x .25 um). The inlet was operated in split mode 3:1 and was maintained at 250°C, the FID was also maintained at 250°C. For separation, He was used as the carrier gas at a rate of 1 mL/min and the oven started at 70°C and increased to 200°C at 8 °C/min. The temperature then ramped 3 °C/min to 250°C and held or 15 minutes.

2.5.3 Determination of Response Factors

For each pair of analyte and labelled standard, response factors were determined to account for differences in instrument response for each odorant and its labelled standard. This was done by preparing a mixture of similar concentrations of analyte and labelled standard and analyzing the mixture via GC-MS as described above. Response factors were then

Table 1 List of odorants quantified with isotope analogue, ions used for quantitation, and response factors

Odorant	Labelled analogue	Ion (m/z)		RF
		analyte	standard	
1,1-diethoxyethane	($^{2}H_4$)-1,1-diethoxyethane	103	104	1.06
ethyl isobutyrate	($^{2}H_2$) ethyl isobutyrate	116	118	0.98
butane-2,3-dione	($^{2}H_6$)-butane-2,3-dione	86	92	1.3
ethyl butanoate	($^{2}H_3$) ethyl butanoate	116	119	0.96
ethyl 2-methylbutanoate	($^{2}H_9$) ethyl 2-methylbutanoate	102	107	0.86
ethyl 3-methylbutanoate	($^{2}H_9$) ethyl 3-methylbutanoate	115	117	0.58
2-methylpropan-1-ol	($^{2}H_7$)-2-methylpropan-1-ol	74	81	1.66
3-methylbutyl acetate	($^{2}H_3$)-3-methylbutyl acetate	87	90	1.21
3-methylbutan-1-ol	($^{2}H_4$)-3-methylbutan-1-ol	55	59	1.22
ethyl hexanoate	($^{2}H_{11}$) ethyl hexanoate	99	110	0.83
2-acetyl-1-pyrroline	($^{13}C_5$)-2-acetyl-1-pyrroline	111	116	0.69
ethyl octanoate	($^{2}H_{15}$) ethyl octanoate	127	142	0.8
acetic acid	($^{13}C_1$) acetic acid	60	61	1.13
(2*E*)-non-2-enal	($^{2}H_2$)-(2*E*)-non-2-enal	111	113	0.79
linalool	($^{2}H_3$) linalool	121	124	0.85
2-methylpropanoic acid	($^{2}H_7$)-2-methylpropanoic acid	88	95	1.14
butanoic acid	($^{2}H_7$)-butanoic acid	73	77	0.89
2-acetylthiazole	($^{13}C_2$)-2-acetylthiazole	127	129	1.13
3-methylbutanoic acid	($^{2}H_9$)-2-methylbutanoic acid	60	63	1.02
(2E,4E)-nona-2,4-dienal	($^{2}H_5$)-(2*E,4E*)-nona-2,4-dienal	138	143	1.81
(E)-β-damascenone	($^{2}H_4$)-β-damascenone	190	194	0.92
(2*E,4E*)-deca-2,4-dienal	($^{2}H_4$)-(2*E,4E*)-deca-2,4-dienal	152	156	0.92
2-phenylethyl acetate	($^{2}H_5$)-phenylethyl acetate	104	109	1.05
2-methoxyphenol	($^{2}H_4$)-2-methoxyphenol	109	113	0.95
2-phenylethanol	($^{2}H_5$)-2-phenylethanol	91	96	0.99
2′-aminoacetophenone	($^{2}H_3$)-2′-aminoacetophenone	135	138	0.87
decanoic acid	($^{13}C_1$)-decanoic acid	129	130	0.76
1*H*-indole	($^{2}H_7$)-(1*H*)-indole	117	122	0.34
4-hydroxy-3-methoxybenzaldehyde	($^{2}H_3$)-4-hydroxy-3-methoxy-benzaldehyde	152	155	0.22

determined by extracting the appropriate ions and the response factor was given by:

$$RF = \frac{C_{UL}A_L}{C_L A_{UL}}$$

Where C_{UL} is the concentration of the unlabeled odorant, A_L is the peak area of the labelled ion, C_L is the concentration of the labelled odorant, and A_{UL} is the peak area of the unlabeled ion.

2.5.3 Determination of Odor Thresholds

Orthonasal odor thresholds in a 60:40 ethanol:water matrix were obtained from literature when available. Odor thresholds were determined in house for 1H-indole, 2-acetyl-1-pyrroline, 2-methylpropanoic acid, 2-acetylthiazole, and 2′-aminoacetophenone according to ASTM methods[20]. A solution of the odorant was prepared in 60:40 ethanol:water at a concentration at which all panelists could detect the odorant. This concentrated solution was then serially diluted 1:1. Panelists (n=10) were presented with a series of triangle tests comprised of three scintillation vials (7 mL vials with 1 mL of solution), one sample vial and two blanks with just the carrier solution. The triangle tests were presented starting with the lowest concentration of odorant with each subsequent test having double the odorant concentration. Panelists were determined to have reached detection threshold at first sample after which they no longer had any incorrect results in the triangle tests. Odor thresholds were determined for each panelist by taking the geometric mean of the highest concentration with an incorrect result and the lowest concentration at which they could detect the odorant. The odor threshold for the group was determined by the arithmetic mean of each panelist's threshold.

2.6 Sensory Evaluation of Distillate Samples

In order to determine the effects that the Lincoln county process had on fresh whiskey distillate, olfactory profile analysis was performed on bLCP and aLCP samples. This was done by placing 2 milliliters of sample into unmarked 20 mL scintillation vials. Panelists were presented with the two samples as well as vials that contained odorants for use as aroma references: 2-phenylethanol (rose), ethyl 2-methylbutanoate (fruity), (2*E,4E*)-deca-2,4-ienal (fatty), butanoic acid (rancid), 3-methylbutanal (malty), and 2-acetyl-1-pyrroline (popcorn). Panelists were asked to smell the reference standards followed by the sample and rate the intensity of the given odor quality in the sample on a scale of 0-3 in 0.5 point increments where 0 indicates the descriptor was not observable and 3 indicates the descriptor was strongly observable. Data was averaged for each descriptor and a radar plot was generated in Microsoft® Excel Version16.21 for Office 365 (Microsoft Corporation, Redman, Wa). Analysis of the data was done by way of one-way analysis of variance (ANOVA) to compare the aroma of the samples and Tukey's test to compare the attributes. This was done in JMP Pro 14.0.0 software (SAS Institute, Cary, NC) with a significance level of 0.05.

Table 2 List of odorants used as reference standards in sensory testing

Odorant	Descriptor
3-methylbutanal	malty
2-phenylethanol	floral
(2*E,4E)*-deca-2,4-dienal	fatty
butanoic acid	rancid
2-acetyl-1-pyrroline	popcorn
ethyl 2-methylbutanoate	fruity

3. Results and Discussion

3.1 Sensory Comparison of Treated and Untreated Distillate

In order to determine the effect charcoal mellowing has on whiskey distillate aroma, trained panelists assessed the intensity of six descriptors in bLCP and aLCP samples (figure 9). The aLCP sample was described as significantly lower in the fatty, rancid, popcorn, and malty characters. There was no significant difference found in the fruity and floral characters of the two distillate samples.

3.2 Identification of Odorants

Key odorants in two whiskey distillate samples were identified by first extracting volatiles in organic solvent and isolating said volatiles from non-volatiles by SAFE. The SAFE isolate was then concentrated to approximately 200 μL. A strip of filter paper dipped into the aroma concentrate had a smell like that of the original distillate sample, indicating that the aroma isolation was successful. Aroma isolates were analyzed by GC-O, resulting in the 49 areas where aromas were detected. Of these 49 aromas, 40 have been previously reported in whiskey samples[16, 18, 21] . Eight of the nine remaining odorants were identified without issue, 2-methylpropanoic acid (**22**, sweaty), 2-acetylthiazole (**25**, tortilla), 3-methylnonane-2,4-dione (**29**, hay-like), 2′-aminoacetophenone (**43**, foxy), decanoic acid (**45**, rancid), (*E*)-isoeugenol (**46**, clove), ethyl 3-phenylpropanoate (**36,** clove) and 1H-indole (**47**, animal). The remaining odorant was detected as an anise-like aroma at FFAP RI 1408 and DB-5 RI 1191 with an FD of 4 in bLCP and aLCP samples. This odorant was tentatively identified as 3,4-dimethyl-2-pentylfuran, previously

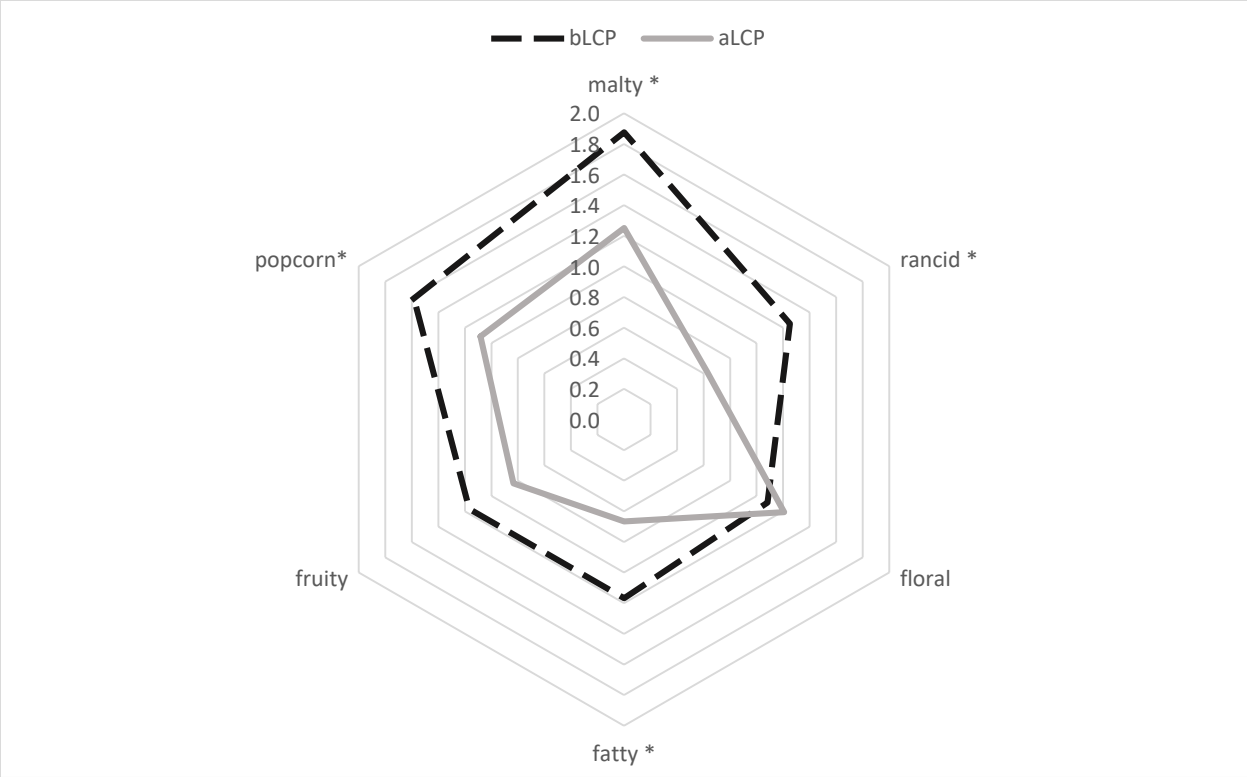


Figure 9 Sensory comparison of bLCP and aLCP samples. Descriptors noted with * were found to be significantly different at α=0.05

reported in cocoa[22], based only on RIs. No MS signal for the odorant could be obtained in the SAFE isolate due to it co-eluting with acetic acid, so the SAFE isolate was fractionated by SPE to eliminate interfering compounds and a MS spectrum was obtained. Unfortunately, a reference standard was not commercially available, and as a result the odorant was synthesized in-house and compared to the signal from the SPE fraction of the SAFE isolate, resulting in a positive identification of the odorant as 3,4-dimethyl-2-pentylfuran. In conclusion, 49 odorants were identified in whiskey distillate samples, nine of which (**15**, **22**, **25**, **29**, **36**, **43**, **45**, **46**, and **47**) are to the best of our knowledge being reported in whiskey distillate for the first time (figure 10).

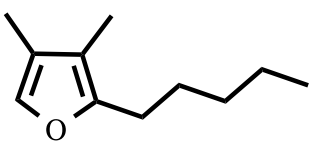
3.3 Comparative Aroma Extract Dilution Analysis (cAEDA)

In order to determine the importance of the 49 odorants found in the whiskey distillates, serial 1:1 dilutions of the SAFE isolates were performed and analyzed via GC-O and FD factors were assigned to each odorant. The results of AEDA can be seen in Table 1 and more easily visualized in the FD chromatograms presented in figure 11 where the x-axis is the retention index and the y-axis represents the FD factor of the odorant.

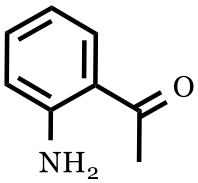
The odorant with the highest FD factor in the bLCP sample was the malty smelling 3-methylbutan-1-ol (**10**), with an FD factor of 4096. The next highest FD factor was that of rose smelling 2-phenylethanol (**37**), with an FD factor of 2048. The odorants with an FD factor of 1024 were (2*E*)-non-2-enal (**20**), and (2*E,4E*)-deca-2,4-dienal (**32**). (2*E*)-non-2-enal has a green aroma and (2*E,4E*)-deca-2,4-dienal has a fatty aroma. The structures of these odorants are shown in figure 12.

Table 3 List of odorants identified in distillate samples

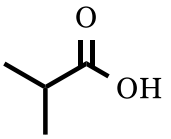
no.	odorant	odor quality	RI on		FD factor		ref.
			FFAP	DB-5	bLCP	aLCP	
1	1,1-diethoxyethane	fruity	900	729	4	1	[16, 18, 21]
2	ethyl propanoate	fruity	952	730	256	256	[16, 18, 21]
3	ethyl isobutyrate	fruity	958	756	16	16	[16, 18, 21]
4	butane-2,3-dione	butter	985	<600	16	1	[18]
5	ethyl butanoate	fruity	1039	801	64	64	[18, 21]
6	ethyl 2-methylbutanoate	fruity	1054	848	256	256	[16, 18, 21]
7	ethyl 3-methylbutanoate	fruity	1072	855	64	64	[16, 18, 21]
8	2-methylpropan-1-ol	malty	1091	648	64	16	[16, 18, 21]
9	3-methylbutyl acetate	fruity	1120	879	64	64	[16, 18, 21]
10	2 and 3-methylbutan-1-ol	malty	1215	735	4096	2048	[16, 18, 21]
11	ethyl hexanoate	fruity	1237	998	64	64	[16, 18, 21]
12	1-octen-3-one	mushroom	1285	980	16	4	[21]
13	2-acetyl-1-pyrroline	popcorn	1350	920	4	1	[21]
14	dimethyl trisulfide	sulfurous	1365	968	16	4	[16]
15	3,4-dimethyl-2-pentylfuran	anise	1408	1191	4	4	
16	ethyl octanoate	fruity	1420	1197	16	1	[18]
17	2-isopropyl-3-methoxypyrazine	earthy	1428	1091	16	4	[17, 21]
18	acetic acid	vinegar	1439	600	4	<1	[18]
19	3-(methylsulfanyl)propanal	potato	1443	902	16	16	[18]
20	(2*E*)-non-2-enal	green	1530	1161	1024	256	[18, 21]
21	linalool	floral	1550	1096	4	4	[16]
22	2-methylpropanoic acid	sweaty	1565	762	1	1	
23	(2*E,6Z*)-nona-2,6-dienal	green	1578	1153	16	4	[18]
24	butanoic acid	sweaty	1610	820	64	<1	[17]
25	2-acetylthiazole	roasty, tortilla	1624	1018	4	1	
26	2-phenylacetaldehyde	floral, honey	1640	1045	4	1	[17]
27	2- and 3-methylbutanoic acid	sweaty, rancid	1660	885	256	<1	[17, 21]
28	(2*E,4E*)-nona-2,4-dienal	fatty	1697	1212	64	4	[18, 21]
29	3-methylnonane-2,4-dione	hay-like	1715	1246	16	16	
30	ethyl phenylacetate	floral	1724	1244	1	1	[16, 18, 21]
31	(*E*)-β-damascenone	cooked apple	1809	1259	4	4	[16, 18, 21]
32	(2*E,4E*)-deca-2,4-dienal	fatty	1811	1318	1024	16	[18, 21]
33	2-phenylethylacetate	floral	1814	1265	4	1	[18, 21]
34	hexanoic acid	rancid	1840	973	1	1	[16]
35	2-methoxyphenol	smoky	1860	1187	64	64	[17]
36	ethyl 3-phenylpropanoate	clove	1888	1390	16	16	
37	2-phenylethanol	rose	1909	1117	2048	1024	[16, 18, 21]
38	trans-4,5-epoxy-(*E*)-2-decenal	metallic	1989	1380	16	4	[21]
39	γ-nonalactone	coconut	2020	1361	16	4	[18, 21]
40	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel	2040	1080	16	4	[18, 21]
41	4-allyl-2-methoxyphenol	clove	2142	1359	4	4	[18]
42	4-ethyl phenol	phenolic	2168	1165	16	4	[18]
43	2′-aminoacetophenone	foxy	2222	1300	64	1	
44	2,6-dimethoxyphenol	smoky	2271	1349	16	16	[18]
45	decanoic acid	rancid	2280	1405	1	1	
46	(*E*)-isoeugenol	clove	2323	1451	1	1	
47	1*H*-indole	animal	2446	1288	4	4	
48	2-phenylacetic acid	honey	2558	1274	4	4	[18, 21]
49	4-hydroxy-3-methoxybenzaldehyde	vanilla	2600	1411	16	16	[18, 21]



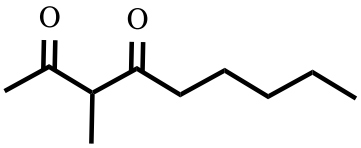
(**15**, anise-like)



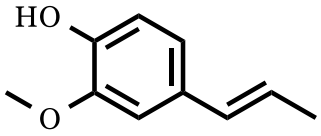
(**43**, foxy)



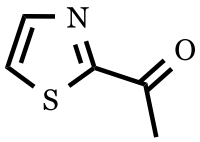
(**22**, sweaty)



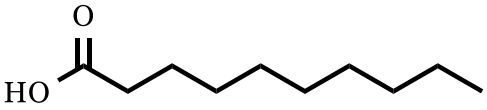
(**29,** hay-like)



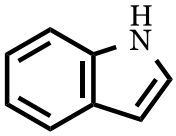
(**46**, clove)



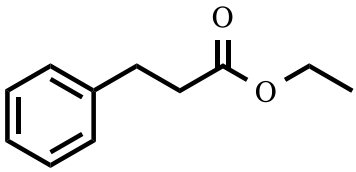
(**25**, tortilla)



(**45**, rancid)



(**47**, animal)



(**36**, clove)

Figure 10 Structures of odorants being reported in whiskey distillate for the first time

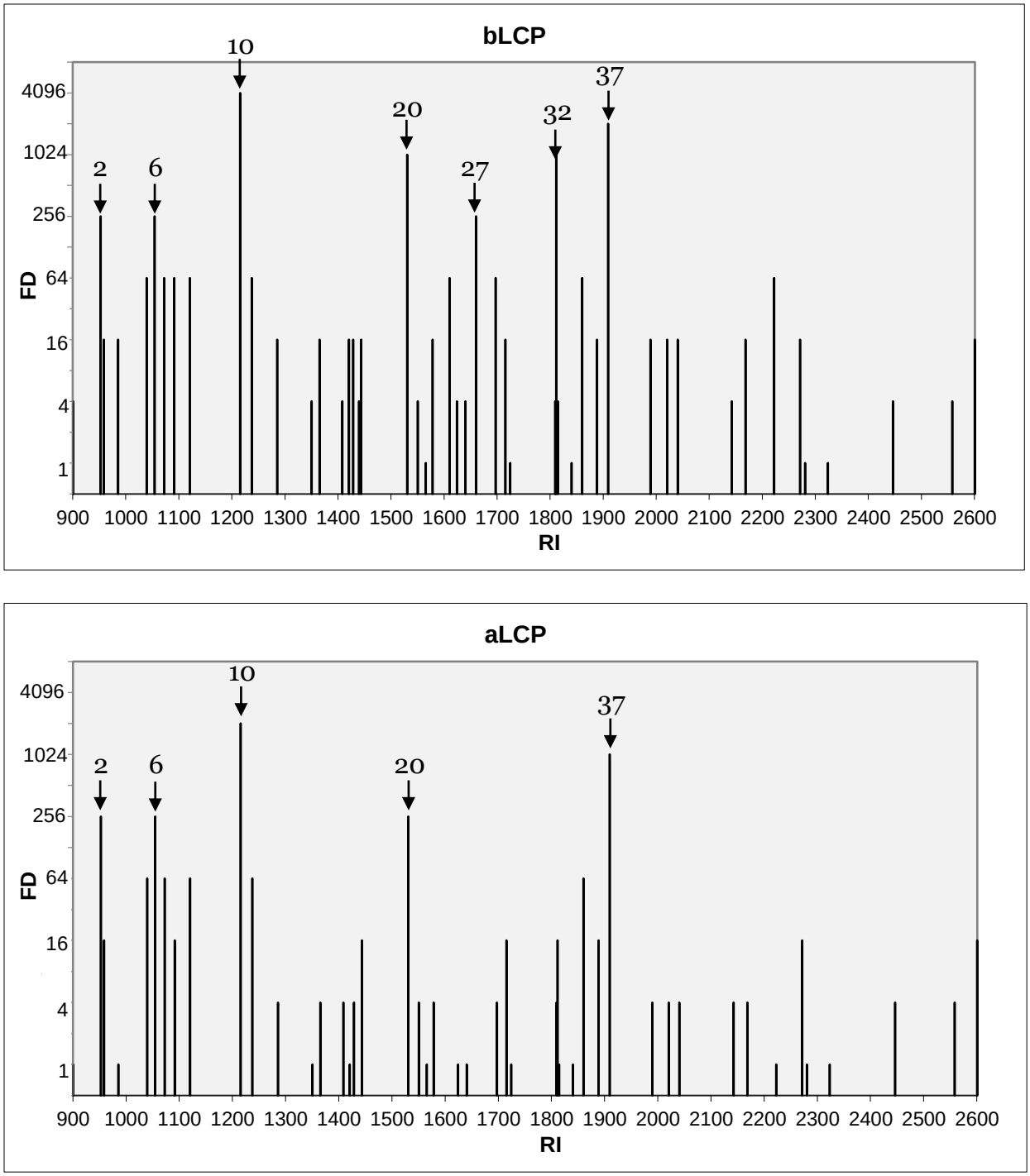


Figure 11 FD chromatograms of bLCP (top) and aLCP (bottom) distillate samples. Odorants with FD factor ≥256 are numbered.

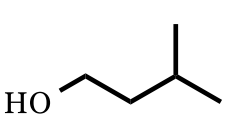
There were three odorants with an FD factor of 256 (Figure 13), these were the fruity smelling esters, ethyl propanoate (**2**) and ethyl 2-methylbutanoate (**6**), as well as the sweaty smelling 2- and 3-methylbutanoic acid (**27**).

Nine odorants were assigned FD 64 in the bLCP sample, the structures of these odorants can be seen in figure 14. These odorants were the fruity smelling esters, ethyl butanoate (**5**), ethyl 3-methylbutanoate (**7**), 3-methylbutal acetate (**9**), and ethyl hexanoate (**11**). Additionally, 2-methylpropan-1-ol (**8**), butanoic acid (**24**), (2*E,4E*)-nona-2,4-dienal (**28**), 2-methoxyphenol (**35**), and 2′-aminoacetophenone (**43**) were FD 64. 2-methylpropan-1-ol is described as a malty aroma, while butanoic acid is rancid, (2*E,4E*)-nona-2,4-dienal is fatty, 2-methoxyphenol has a smoky aroma, and 2′-aminoacetophenone is described as foxy.

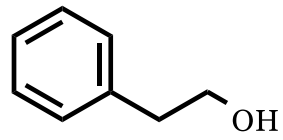
The FD with the largest number of odorants in the bLCP sample was FD 16 with 16 odorants (figures 15 and 16). Namely they were, ethyl isobutyrate (**3,** fruity), butane-2,3-dione (**4,** butter), 1-octen-3-one (**12,** mushroom), dimethyl trisulfide (**14,**

Table 4 List of odorants with FD factor ≥ 1024 in bLCP sample

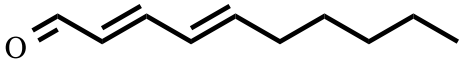
no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
10	2 and 3-methylbutan-1-ol	malty	1215	735	4096
20	(2*E*)-non-2-enal	green	1530	1161	1024
32	(2*E,4E*)-deca-2,4-dienal	fatty	1811	1318	1024
37	2-phenylethanol	rose	1909	1117	2048



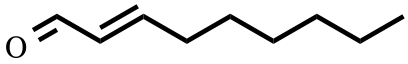
10, malty



37, rose



32, fatty

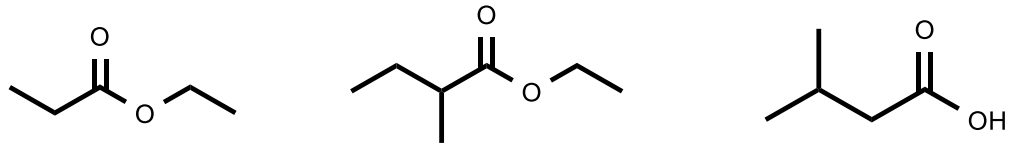


20, green

Figure 12 Structure of odorants with FD factors ≥ 1024 in bLCP sample

Table 5 List of odorants with FD factor 256 in bLCP sample

no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
2	ethyl propanoate	fruity	952	730	256
6	ethyl 2-methylbutanoate	fruity	1054	848	256
27	2- and 3-methylbutanoic acid	sweaty, rancid	1660	885	256

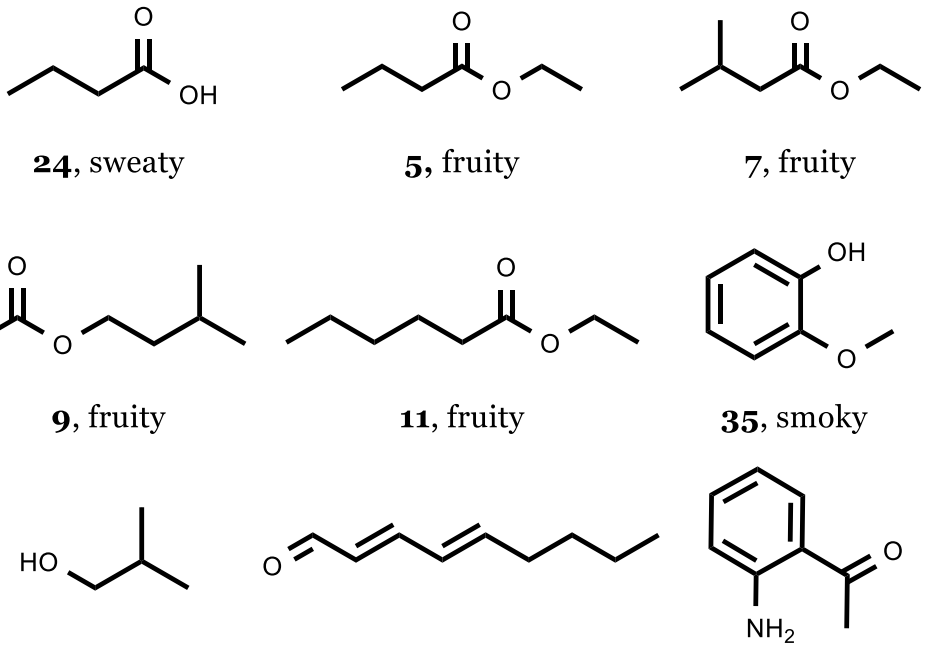


2, fruity **6**, fruity **27**, sweaty

Figure 13 Structures of odorants with FD factor 256 in bLCP sample

Table 6 List of odorants with FD factor 64 in bLCP sample

no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
5	ethyl butanoate	fruity	1039	801	64
7	ethyl 3-methylbutanoate	fruity	1072	855	64
8	2-methylpropan-1-ol	malty	1091	648	64
9	3-methylbutyl acetate	fruity	1120	879	64
11	ethyl hexanoate	fruity	1237	998	64
24	butanoic acid	sweaty	1610	820	64
28	(2*E*,4*E*)-nona-2,4-dienal	fatty	1697	1212	64
35	2-methoxyphenol	smoky	1860	1187	64
43	2′-aminoacetophenone	foxy	2222	1300	64



24, sweaty **5,** fruity **7**, fruity

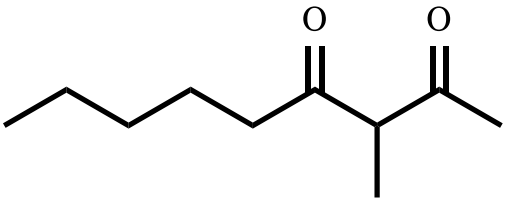
9, fruity **11**, fruity **35**, smoky

8, malty **28**, fatty **43**, foxy

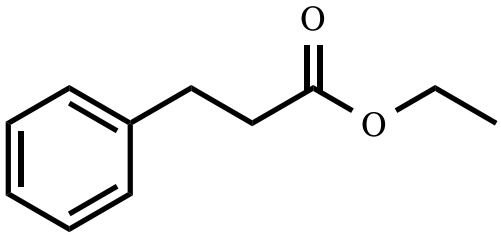
Figure 14 Structures of odorants with FD factor 64 in bLCP sample

Table 7 List of odorants with FD factor 16 in bLCP sample

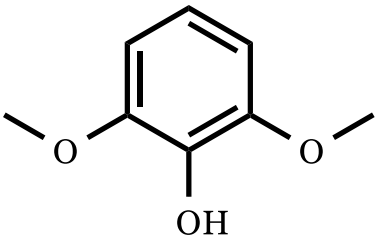
no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
3	ethyl isobutyrate	fruity	958	756	16
4	butane-2,3-dione	butter	985	<600	16
12	1-octen-3-one	mushroom	1285	980	16
14	dimethyl trisulfide	sulfurous	1365	968	16
16	ethyl octanoate	fruity	1420	1197	16
17	2-isopropyl-3-methoxypyrazine	earthy	1428	1091	16
19	3-(methylsulfanyl)propanal	potato	1443	902	16
23	(2*E,6Z*)- nona-2,6-dienal	green	1578	1153	16
29	3-methylnonane-2,4-dione	hay-like	1715	1246	16
36	ethyl 3-phenylpropanoate	clove	1888	1390	16
38	trans-4,5-epoxy-(*E*)-2-decenal	metallic	1989	1380	16
39	γ-nonalactone	coconut	2020	1361	16
40	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel	2040	1080	16
42	4-ethyl phenol	phenolic	2168	1165	16
44	2,6-dimethoxyphenol	smoky	2271	1349	16
49	4-hydroxy-3-methoxybenzaldehyde	vanilla	2600	1411	16



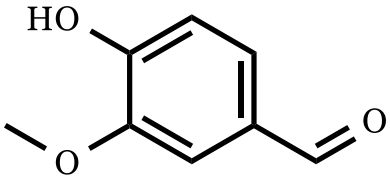
29, hay-like



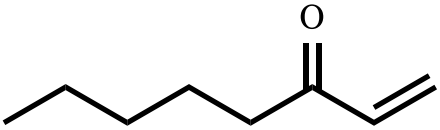
36, clove



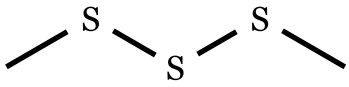
44, smoky



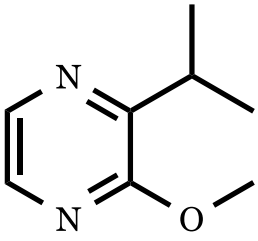
49, vanilla



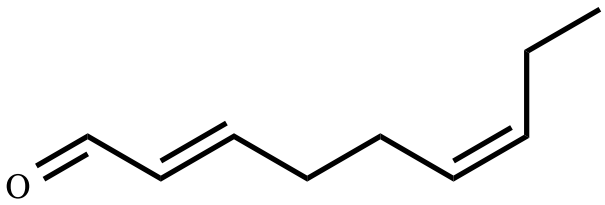
12, mushroom



14, sulfurous

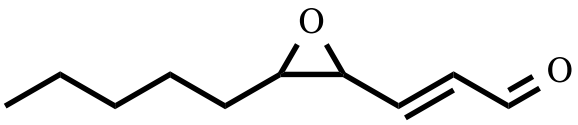


17, earthy

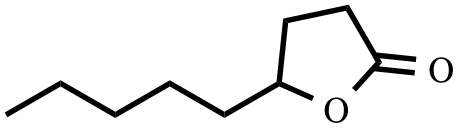


23, cucumber

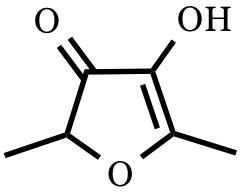
Figure 15 Structures of odorants with FD factor 16 in bLCP sample



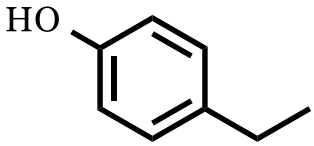
38, metallic



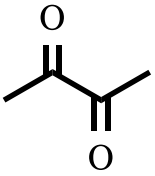
39, coconut



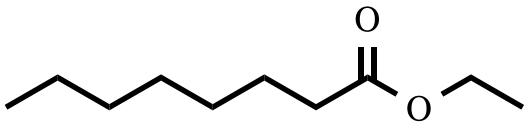
40, caramel



42, phenolic



4, butter



16, fruity

Figure 16 Structures of odorants with FD factor 16 in bLCP sample cont.

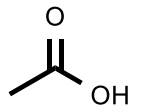
sulfurous), ethyl octanoate (**16,** fruity), 2-isopropyl-3-methoxypyrazine (**17,** earthy 3-(methylsulfanyl)propanal (**19,** potato), (2*E,6Z*)-nona-2,6-dienal (**23,** cucumber), 3-methylnonane-2,4-dione (**29,** hay-like), ethyl 3-phenylpropanoate (**36**, clove), trans-4,5-epoxy-(*E*)-2-decenal (**38,** metallic), γ-nonalactone (**39,** coconut), 4-hydroxy-2,5-dimethylfuran-3-(2H)-one (**40,** caramel), 4-ethylphenol (**42**, phenolic), 2,6-dimethoxyphenol (**44**, smoke), and 4-hydroxy-3-methoxybenzaldehyde (**49**, vanilla).

Of the 49 total odorants identified in the whiskey distillate, 12 had an FD factor of 4 in the bLCP sample (figure 17). These odorants were: 1,1-diethoxyethane (**1**, fruity), 2-acetyl-1-pyrroline (**13**, popcorn), 3,4-dimethyl-2-pentlyfuran (**15**, anise-like), acetic acid (**18,** vinegar), linalool (**21**, floral), 2-acetylthiazole (**25**, tortilla), (*E*)-β-damascenone (**31**, cooked apple), 2-phenylacetaldehyde (**26**, honey), 4-allyl-2-methoxyphenol (**41**, clove), 1*H*-indole (**47**, animal), and 2-phenylacetic acid (**48**, honey).

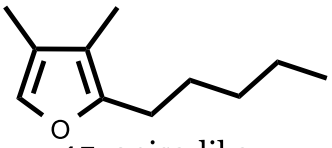
There were five odorants that were not detected beyond an FD factor of 1 in the bLCP sample, as seen in figure 18. These odorants were 2-methylpropanoic acid (**22**, sweaty), ethyl phenylacetate (**30**, floral), decanoic acid (**45**, rancid), hexanoic acid (**34**, rancid), and (*E*)-isoeugenol (**46**, clove).

Table 8 List of odorants with FD factor 4 in bLCP sample

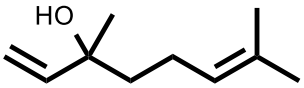
no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
1	1,1-diethoxyethane	fruity	900	729	4
13	2-acetyl-1-pyrroline	popcorn	1350	920	4
15	3,4-dimethyl-2-pentylfuran	anise-like	1408	1191	4
18	acetic acid	vinegar	1439	600	4
21	linalool	floral	1550	1096	4
25	2-acetylthiazole	roasty, tortilla	1624	1018	4
26	2-phenylacetaldehyde	floral, honey	1640	1045	4
31	(*E*)-β-damascenone	cooked apple	1809	1259	4
33	2-phenylethylacetate	floral	1814	1265	4
41	4-allyl-2-methoxyphenol	clove	2142	1359	4
47	1*H*-indole	animal	2446	1288	4
48	2-phenylacetic acid	honey	2558	1274	4



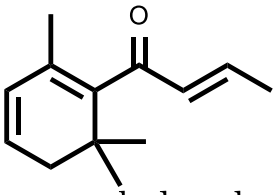
18, vinegar



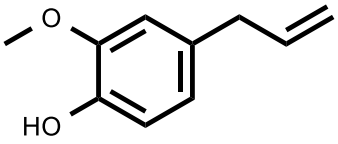
15, anise-like



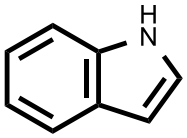
21, floral



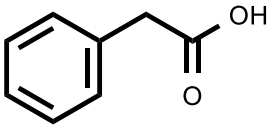
31, cooked-apple



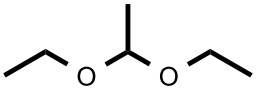
41, clove



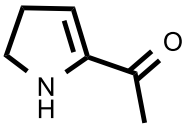
47, animal



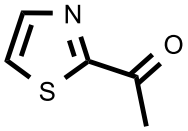
48, honey



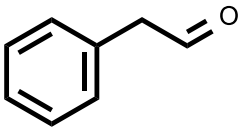
1, fruity



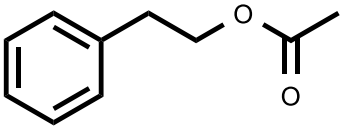
13, popcorn



25, tortilla



26, honey

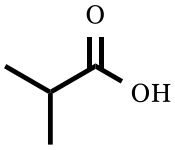


33, floral

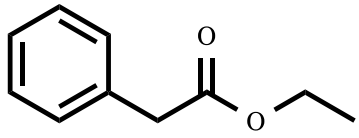
Figure 17 Structures of odorants with FD factor 4 in bLCP sample

Table 9 List odorants with FD factor 1 in bLCP sample

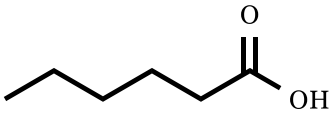
no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
22	2-methylpropanoic acid	sweaty	1565	762	1
30	ethyl phenylacetate	floral	1724	1244	1
34	hexanoic acid	rancid	1840	973	1
45	decanoic acid	rancid	2280	1405	1
46	(*E*)-isoeugenol	clove	2323	1451	1



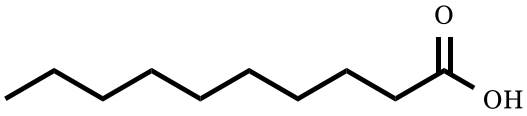
22, sweaty



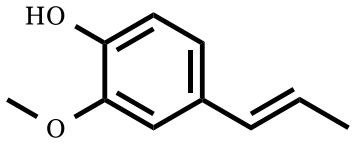
30, floral



34, rancid



45, rancid



46, clove

Figure 18 Structures of odorants with FD factor 1 in bLCP sample

In the aLCP samples, the odorants with the highest FD factors (figure 19) were 3-methylbutan-1-ol (**10**, malty) with an FD factor of 2048 and 2-phenylethanol (**37**, rose) with an FD factor of 1024.

Three odorants had an FD factor of 256 in the aLCP sample (figure 20). These odorants were: (2*E)*-non-2-enal (**20**, green), ethyl propanoate (**2**, fruity), and ethyl 2-methylbutanoate (**6**, fruity).

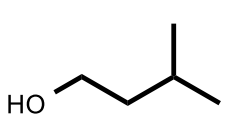
Five odorants had an FD factor of 64 in the aLCP sample (figure 21). These odorants were ethyl butanoate (**5**, fruity), ethyl 3-methylbutanoate (**7**, fruity), 3-methylbutyl acetate (**9**, fruity), ethyl hexanoate (**11**, fruity), and 2-methoxyphenol (**35**, smoky).

Eight odorants had an FD factor of 16 in the aLCP sample (figure 22). These odorants were: (2*E,4E*)-deca-2,4-dienal (**32**, fatty), 2-methylpropan-1-ol (**8**, malty), ethyl isobutyrate (**3**, fruity), 3-(methylsulfanyl)propanal (**19**, potato), 3-methylnonane-2,4-dione (**29**, hay-like), ethyl 3-phenylpropanoate (**36**, clove), 2,6-dimethoxyphenol (**44**, smoky), and 4-hydroxy-3-methoxybenzaldehyde (**49**, vanilla).

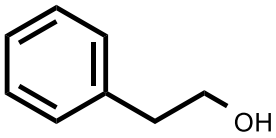
There were 15 odorants with an FD factor of 4 in the aLCP sample (figures 23 and 24). These odorants were: (2*E,4E*)-nona-2,4-dienal (**28**, fatty), 1-octen-3-one (**12**, mushroom), dimethyl trisulfide (**14**, sulfurous), 2-isopropyl-3-methoxypyrazine (**17**, earthy),

Table 10 List of odorants with FD factor ≥ 1024 in aLCP sample

no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
10	2 and 3-methylbutan-1-ol	malty	1215	735	2048
37	2-phenylethanol	rose	1909	1117	1024



10, malty

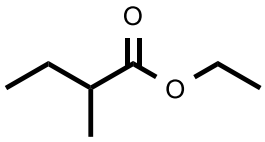


37, rose

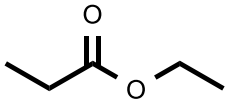
Figure 19 Structures of odorants with FD factor ≥ 1024 in aLCP sample

Table 11 List of odorants with FD factor 256 in aLCP sample

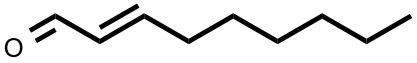
no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
2	ethyl propanoate	fruity	952	730	256
6	ethyl 2-methylbutanoate	fruity	1054	848	256
20	(2*E*)-non-2-enal	green	1530	1161	256



6, fruity



2, fruity

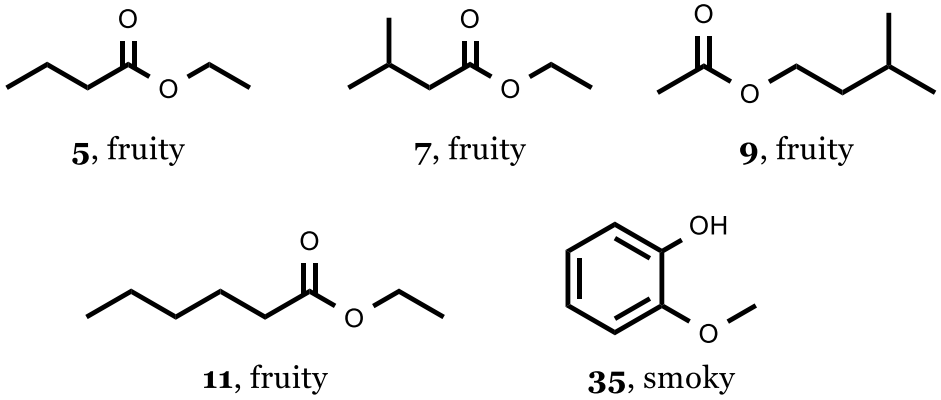


20, green

Figure 20 Structures of odorants with FD factor 256 in aLCP sample

Table 12 List of odorants with FD factor 64 in aLCP sample

no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
5	ethyl butanoate	fruity	1039	801	64
7	ethyl 3-methylbutanoate	fruity	1072	855	64
9	3-methylbutyl acetate	fruity	1120	879	64
11	ethyl hexanoate	fruity	1237	998	64
35	2-methoxyphenol	smoky	1860	1187	64



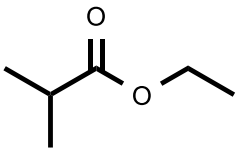
5, fruity　　7, fruity　　**9**, fruity

11, fruity　　**35**, smoky

Figure 21 Structures of odorants with FD factor 64 in aLCP sample

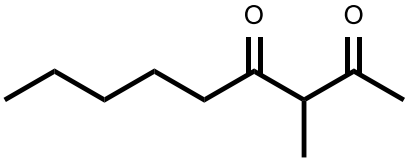
Table 13 List of odorants with FD factor 16 in aLCP sample

no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
3	ethyl isobutyrate	fruity	958	756	16
8	2-methylpropan-1-ol	malty	1091	648	16
19	3-(methylsulfanyl)propanal	potato	1443	902	16
29	3-methylnonane-2,4-dione	hay-like	1715	1246	16
36	ethyl 3-phenylpropanoate	clove	1888	1390	16
44	2,6-dimethoxyphenol	smoky	2271	1349	16
49	4-hydroxy-3-methoxybenzaldehyde	vanilla	2600	1411	16

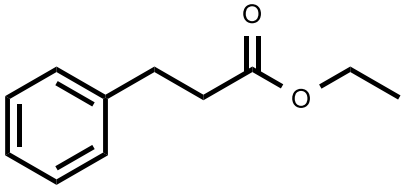


3, fruity

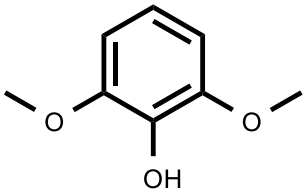
19, potato



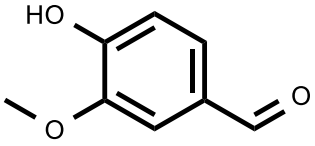
29, hay-like



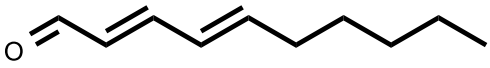
36, clove



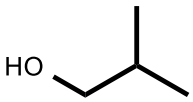
44, smoky



49, vanilla



32, fatty

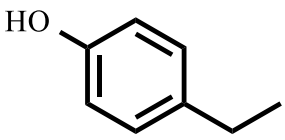


8, malty

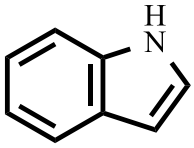
Figure 22 Structures of odorants with FD factor 16 in aLCP sample

Table 14 List of odorants with FD factor 4 in aLCP sample

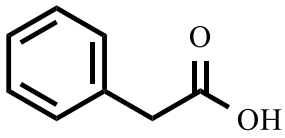
no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
12	1-octen-3-one	mushroom	1285	980	4
14	dimethyl trisulfide	sulfurous	1365	968	4
15	3,4-dimethyl-2-pentylfuran	anise-like	1408	1191	4
17	2-isopropyl-3-methoxypyrazine	earthy	1428	1091	4
21	linalool	floral	1550	1096	4
23	(2*E,6Z*)-nona-2,6-dienal	green	1578	1153	4
31	(*E*)-β-damascenone	cooked apple	1809	1259	4
38	trans-4,5-epoxy-(*E*)-2-decenal	metallic	1989	1380	4
39	γ-nonalactone	coconut	2020	1361	4
40	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel	2040	1080	4
41	4-allyl-2-methoxyphenol	clove	2142	1359	4
42	4-ethyl phenol	phenolic	2168	1165	4
47	1*H*-indole	animal	2446	1288	4
48	2-phenylacetic acid	honey	2558	1274	4



42, penolic

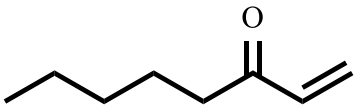


47, animal

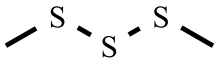


48, honey

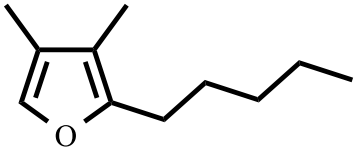
Figure 23 Structures of odorants with FD factor 4 in aLCP sample



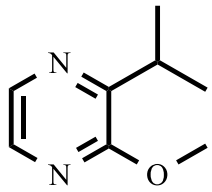
12, mushroom



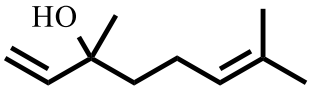
14, sulfurous



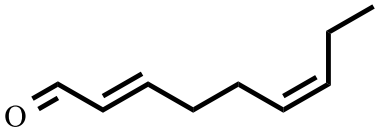
15, anise-lke



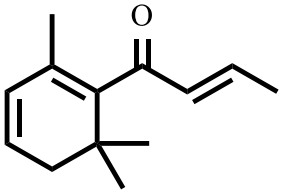
17, earthy



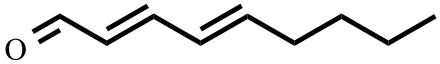
21, floral



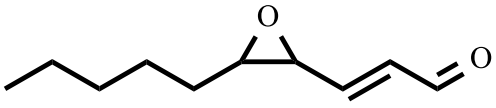
23, green



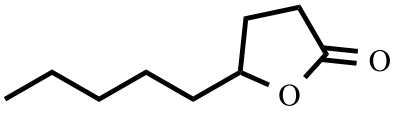
31, cooked apple



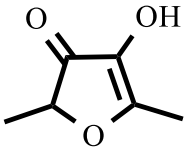
32, fatty



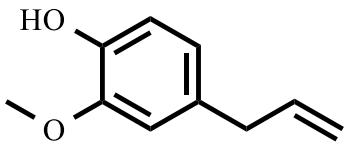
38, metallic



39, coconut



40, caramel



41, clove

Figure 24 Structures of odorants with FD factor 4 in aLCP sample cont.

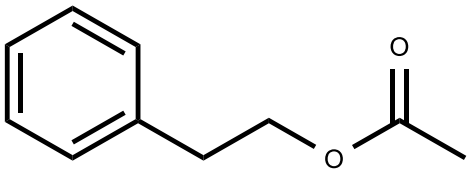
(2*E,6Z*)-nona-2,6-dienal (**23**, green), trans-4,5-epoxy-(*E*)-2-decenal (**38**, metallic), γ-nonalactone (**39**, coconut), 4-hydroxy-2,5-dimethylfuran-3(2H)-one (**40**, caramel), 4-ethyl phenol (**42**, phenolic), 3,4-dimethyl-2-pentylfuran (**15**, anise-like), linalool (**21**, floral), (*E*)-β-damascenone (**31**, cooked apple), 4-allyl-2-methoxyphenol (**41**, clove), 1*H*-indole (**47**, animal), 2-phenylacetic acid (**48**, honey).

There were 12 odorants with an FD factor of 1 in the aLCP sample (figures 25 and 26). These odorants were: 2′-aminoacetophenone (**43**, foxy), butane-2,3-dione (**4**, butter), ethyl octanoate (**16**, fruity), 1,1-diethyoxyethane (**1**, fruity), 2-acetyl-1-pyrroline (**13**, popcorn), 2-acetylthiazole (**25**, tortilla), 2-phenylacetaldehyde (**26**, honey), 2-phenylethyl acetate (**33**, floral), 2-methylpropanoic acid (**22**, sweaty), ethyl phenylacetate (**30**, floral), hexanoic acid (**34**, rancid), decanoic acid (**45**, rancid), and (*E*)-isoeugenol (**46**, clove).

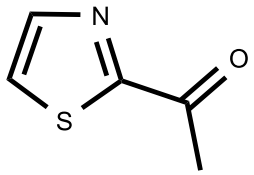
When the FD factors of odorants in the untreated distillate sample are compared to the FD factors of odorants in the distillate that has been treated in a model LCP system, 24 odorants decreased in FD factor while 25 odorants showed now change in FD factor after treatment and no odorants had an observed increase in FD factor. This indicates that this model LCP system is selective in the odorants that are removed from the distillate and that the LCP does not impart any odorants to distillate, only removes them.

Table 15 List of odorants with FD factor 1 in aLCP sample

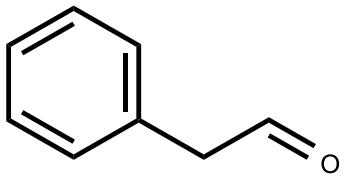
no.	odorant	odor quality	RI on		FD factor
			FFAP	DB-5	
1	1,1-diethoxyethane	fruity	900	729	1
4	butane-2,3-dione	butter	985	<600	1
13	2-acetyl-1-pyrroline	popcorn	1350	920	1
16	ethyl octanoate	fruity	1420	1197	1
22	2-methylpropanoic acid	sweaty	1565	762	1
25	2-acetylthiazole	roasty, tortilla	1624	1018	1
26	2-phenylacetaldehyde	floral, honey	1640	1045	1
30	ethyl phenylacetate	floral	1724	1244	1
33	2-phenylethylacetate	floral	1814	1265	1
34	hexanoic acid	rancid	1840	973	1
43	2′-aminoacetophenone	foxy	2222	1300	1
45	decanoic acid	rancid	2280	1405	1
46	(*E*)-isoeugenol	clove	2323	1451	1



33, floral

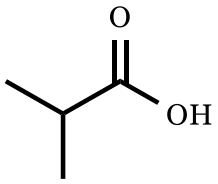


25, tortilla

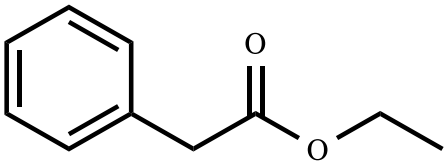


33, honey

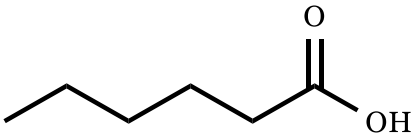
Figure 25 Structures of odorants with FD factor 1 in aLCP sample



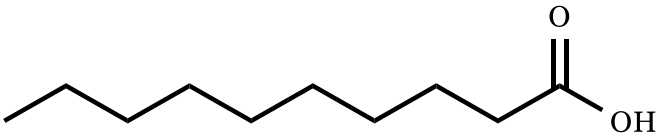
22, sweaty



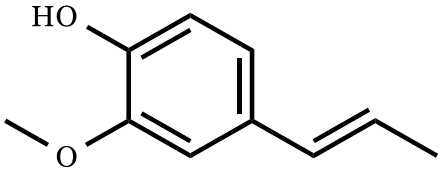
30, floral



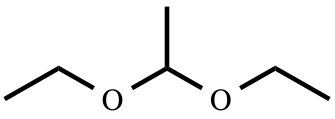
34, rancid



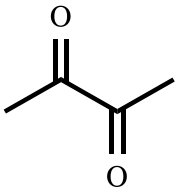
45, rancid



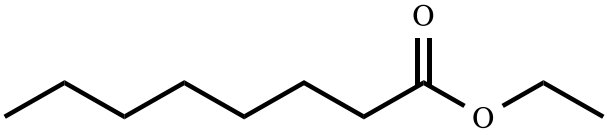
46, clove



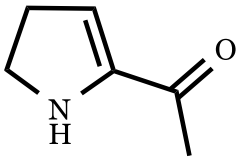
1, fruity



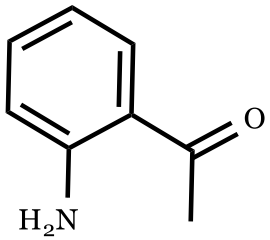
4, butter



16, fruity



13, popcorn



43, foxy

Figure 26 Structures of odorants with FD factor 1 in aLCP sample

This change in FD appears to be similar for odorants with similar chemical structures. For example, the branched alcohols, 3-methylbutan-1-ol (**10**, maty) and 2-methylpropan-1-ol (**8**, malty), decreased from FD factors 4096 and 64 in the bLCP sample to 2048 and 16 in the aLCP respectively. The fatty, green, and metallic smelling aldehydes all decreased in FD factor as a result of LCP treatment. (2*E,4E*)-Deca-2,4-dienal (**32**, fatty) decreased from FD factor 1024 to FD factor 16, (2*E,4E*)-nona-2,4-dienal went factor 64 to FD factor 4, (2*E*)-non-2-enal (**20**, green) went from FD factor 1024 to FD factor 256, and trans-4,5-epoxy-(*E*)-2-decenal (**38**, metallic) decreased from FD factor 16 to FD factor 4. The sweaty and vinegar smelling organic acids 2- and 3-methybutanoic acid (**27**, sweaty), butanoic acid (**24**, sweaty), and acetic acid (**18**, vinegar) were FD factors 256, 64, and 4 in the bLCP sample and all decreased to FD <1 in the charcoal mellowed sample; the structurally similar odorant 2-methylpropanoic acid (**22**, sweaty) was found at FD factor 1 in both samples.

Additionally, the corn-derived odorants 2-acetyl-1-pyrroline (**13**, popcorn), 2-acetylthiazole (**25**, tortilla), and 2′-aminoacetophenone (**43**, foxy) decreased from FD factors 16, 4, and 64 respectively in the bLCP sample to FD factor 1 in the aLCP sample. In contrast, the fruity esters, ethyl propanoate (**2**, fruity), ethyl isobutyrate (**3**, fruity), ethyl butanoate (**5**, fruity), ethyl 2-methylbutanoate (**6**, fruity), ethyl 3-methyl butanoate (**7**, fruity), 3-methylbutyl acetate (**9**, fruity), and ethyl hexanoate (**11**, fruity) were all observed at the same FD factors in both samples, indicating that the LCP had a minimal impact on this class of odorants.

3.4 Quantitation of Key Odorants in Treated and Untreated Distillate

In order to determine the effect of the LCP on the concentration of each odorant, stable isotope dilution assays (SIDA) were conducted on twenty-nine odorants and odor activity values (OAV) were calculated for each odorant. The results of this are shown in table 14.

Odorants determined to have high OAVs in the untreated distillate (bLCP) were: butane-2,3-dione (**4**, OAV 765), (*E*)-β-damascenone (**31**, OAV 520), (2*E*)-non-2-enal (**20**, OAV 483), and (2*E,4E*)-deca-2,4-dienal (**32**, OAV 129) indicating that these odorants might have a large contribution to the aroma of the distillate. On the other hand, 2-methylpropanoic acid (**22**), butanoic acid (**24**), decanoic acid (**45**), 1*H*-indole (**47**), and acetic acid (**18**) were all found to be OAV < 1, suggesting they have minimal contribution to the overall aroma of the distillate.

Odorants found to have the highest OAVs in the treated distillate (aLCP) were butane-2,3-dione (**4**, OAV 343), (*E*)-β-damascenone (**31**, OAV 340), and (2*E*)-non-2-enal (**20**, OAV 217). Interestingly, 3-methylbutanoic acid (**27**), (1*H*)-indole (**47**), 2-methylpropanoic acid (**22**), butanoic acid (**24**), decanoic acid (**45**), 2-acetyl-1-pyrroline (**13**), 2′-aminoacetophenone (**43**), (2*E,4E*)-nona-2,4-dienal (**28**), and acetic acid (**18**) were all be OAV < 1, suggesting they have minimal impact on the overall aroma.

The branched alcohols 2-methylpropan-1-ol (**8**, malty, OAV 2 to OAV 1) and 2- and 3-methylbutan-1-ol (**10**, malty, OAV 30 to 20), also known as fusel alcohols, decreased in concentration by 29% and 34% respectively. The esters, ethyl 2-methylpropanoate (**3**,

fruity, OAV 11 to 8), ethyl butanoate (**5**, fruity, OAV 5 to 2), ethyl 2-methylbutanoate (**6**, fruity, OAV 50 to 44), ethyl 3-methylbutanoate (**7**, fruity, OAV 70 to 31), 3-methylbutyl acetate (**9**, fruity, OAV 15 to 10), and ethyl hexanoate (**11**, fruity, OAV 56 to 34) were also found to have a decrease between 13% and 40%. Despite this decrease in concentration, and decreases in the FDs of the alcohols, the OAVs for these odorants do not decrease to a level that is much different from the original OAV. The relatively small change in the ethyl esters is consistent with the lack of a difference in fruity character between the two distillate samples. Interestingly, there was a difference in the malty character between the two distillate samples despite the relatively small change in the branched alcohols. This could indicate that a small change in these odorants can have an impact on the sensory properties of a sample.

The aldehydes, (2*E,4E*)-nona-2,4-dienal (**28**, fatty, OAV 5 to <1), and (2*E,4E*)-deca-2,4-dienal (**32**, fatty, OAV 129 to 15) showed some of the largest decreases as a result of LCP treatment with 89% and 88% decreases respectively. (2*E,4E*)-Nona-2,4-dienal decreased in concentration below 1 OAV as a result of model LCP treatment. This decrease is reflected by changes in FD factor and OAV as well as the sensory data, which shows a significant difference in the fatty character of the samples.

The corn derived odorants, 2-acetyl-1-pyrroline (**13**, popcorn, OAV 2 to <1), 2-acetylthiazole (**25**, tortilla, OAV 3 to 1), and 2′-aminoacetophenone (**43**, foxy, OAV 2 to <1) were another group of odorants that showed a relatively large decrease as a result of LCP treatment, decreasing by 90%, 73%, and 70% respectively. This is in line with the fact

that all of these odorants decrease to FD 1 in the aLCP samples and the decrease in OAV of two of these odorants below 1 OAV. This is also in line with the significant decrease in popcorn character between the two distillate samples seen in the sensory experiments.

The organic acids 3-methylbutanoic acid (**27**, rancid), butanoic acid (**24**, rancid), acetic acid (**18**, vinegar) all decreased below a level detectable by GC-MS after model LCP treatment. These odorants were also not detected via GC-O in the aLCP sample. This is not especially noteworthy for the aroma of the distillate samples as all of these odorants were OAV <1 in the bLCP as well as the aLCP with the exception of 3-methylbutanoic acid. The decrease in 3-methylbutanoic acid to OAV <1 could be responsible in the significant decrease in rancid character as a result of LCP treatment seen in the sensory experiments. While this change in organic acids may not be impactful on the aroma of the distillate, they have been shown to react with ethanol during barrel aging to produce the corresponding ethyl ester, which could impart a fruity character to the finished whiskey[23]. Therefore, optimization of the LCP treatment to retain organic acids may result in more fruity esters after barrel aging, but further studies are required to support this hypothesis.

3.5 Sensory Comparison of Aroma Simulation Models

To confirm the data obtained from quantitative measurements of whiskey odorants, aroma simulation models of the bLCP and aLCP samples were compounded by adding odorants at levels corresponding to data obtained from SIDA to a 60:40 water:ethanol matrix. These aroma models were then compared to the original distillates by quantitative descriptive analysis. The results of these sensory studies can be seen in Figures 27 and 28.

Table 16. Concentrations of odorants in bLCP and aLCP samples

Odorant	Odor Threshold (μg/L)	bLCP		aLCP		Decrease
		Concentration (μg/L)	OAV	Concentration (μg/L)	OAV	
butane-2,3-dione[23]	2.8	2141	765	960	343	0.55
(*E*)-β-damascenone[17]	0.1	52	520	34	340	0.35
(2*E*)-non-2-enal [17]	0.6	290	483	130	217	0.55
(2*E,4E*)-deca-2,4-dienal [17]	1.1	142	129	17	15	0.88
1,1-diethoxyethane [17]	719	58198	81	40306	56	0.31
ethyl hexanoate [17]	30	1679	56	1015	34	0.40
ethyl-2-methylbutanoate [17]	0.2	10	50	8.7	44	0.13
4-hydroxy-3-methoxybenzaldehyde [17]	22	996	45	857	39	0.14
ethyl 3-methylbutanoate [17]	1.6	70	44	49	31	0.30
3-methylbutanoic acid [23]	78	3304	42	nd	<1	1.00
ethyl octanoate [17]	147	4887	33	3260	22	0.33
3-methylbutan-1-ol [17]	56100	1708253	30	1128915	20	0.34
3-methylbutyl acetate [17]	245	3752	15	2515	10	0.33
ethyl 2-methyl propanoate [17]	4.5	49	11	34	8	0.31
2-phenylethyl acetate [17]	108	1024	9	134	1	0.87
2-phenylethanol [17]	2600	15667	6	3470	1	0.78
(2*E,4E*)-nona-2,4-dienal [17]	2.6	14.2	5	1.5	<1	0.89
ethyl butanoate [17]	9.5	43	5	21	2	0.51
2-acetylthiazole	48	166	3	50	1	0.70
linalool [23]	24	69	3	30	1	0.57
2′-aminoacetophenone	2.6	6.4	2	1.7	<1	0.73
2-acetlypyrroline	8.7	22	2	2.2	<1	0.90
2-methoxyphenol [17]	9.2	16	2	13	1	0.19
2-methylpropan-1-ol [23]	160000	271633	2	192965	1	0.29
1*H*-indole	242	23	<1	7	<1	0.70
2-methylpropanoic acid	51	2836	<1	1662	<1	0.41
butanoic acid [23]	6900	4019	<1	nd	<1	1.00
decanoic acid[23]	2800	936	<1	750	<1	0.20
acetic acid [23]	230000	3104	<1	nd	<1	1.00

For both the bLCP and the aLCP samples, there was no statistical difference found between the distillate and the aroma simulation models. This indicates that the quantitation was accurate, but omission experiments are required to determine the minimum number of these odorants that are necessary to mimic whiskey distillate aroma.

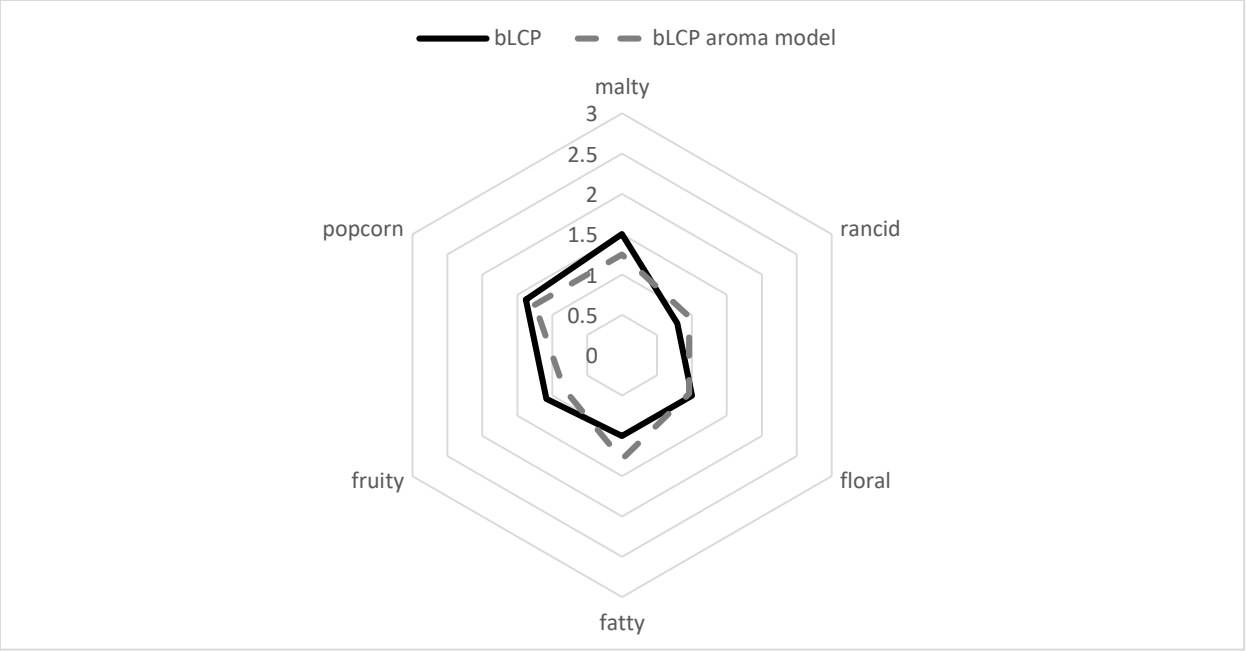


Figure 27 Sensory comparison of bLCP distillate sample and aroma simulation model

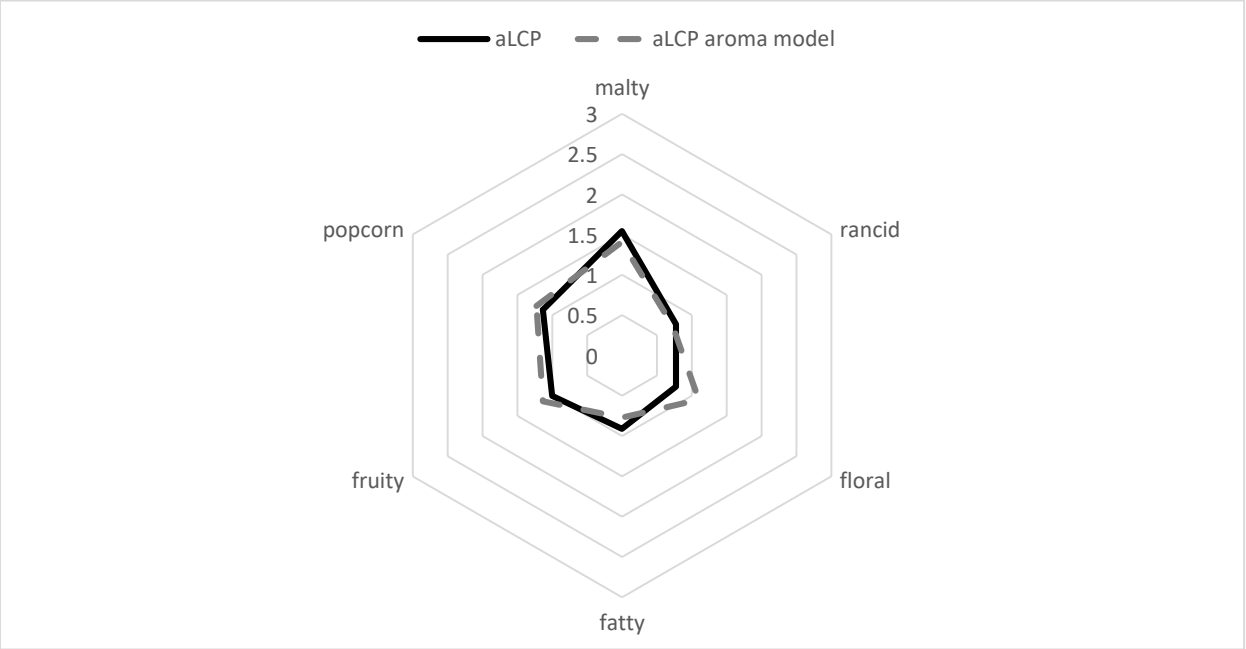


Figure 28 Sensory comparison of aLCP distillate sample and aroma simulation model

Conclusion

Fresh Tennessee whiskey distillate was treated with sugar maple charcoal for 24 hours in an experimental system modeling the Lincoln County Process (LCP). The intent was to gain a fundamental knowledge of the selectivity in odorant removal and retention and determine the impact of the process on final odorant concentrations and sensory properties of the treated distillate. In sensory comparison of the treated (aLCP) and untreated (bLCP) distillate, the treated distillate was found to have lower intensity of the malty, rancid, fatty, and popcorn character compared to the fresh distillate. The volatile isolates prepared from the two distillate samples were then subjected a cAEDA. Forty-nine odorants were identified in the distillates, nine of which are reported here for the first time in whiskey distillate. Quantitative studies were performed on twenty-nine odorants. LCP treatment caused a decrease in concentration for all quantitated odorants, with 13% being the smallest measured change and the largest being >99%. Lipid derived aldehydes, including (2*E,4E*)-nona-2,4-dienal and (2*E,4E*)-deca-2,4-dienal, and corn-derived odorants, including 2-acetyl-1-pyrroline, 2-acetylthiazole, and 2′-aminoacetophenone showed a decrease between 55% and 90%. The organic acids, namely butanoic acid, acetic acid, and 3-methylbutanoic acid decreased below the limit of detection by GC-MS. Four odorants, 3-methylbutanoic acid (rancid), (2*E,4E*)-nona-2,4-dienal (fatty), 2-acteyl-1-pyrroline (popcorn), and 2-acetylthiazole (tortilla) dropped below odor detection threshold in the treated distillate, meaning that the LCP could result in distillate with lower intensities of the aroma qualities associated with these odorants and this is consistent with the sensory comparison of the two distillates. In this model

system, the LCP process showed a selectivity for removing organic acids and aldehydes. This study lays the groundwork for studies investigating changes in LCP processing conditions such as treatment time, charcoal particle size, treatment temperature, distillate to charcoal ratio, and charcoal longevity.

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Appendix

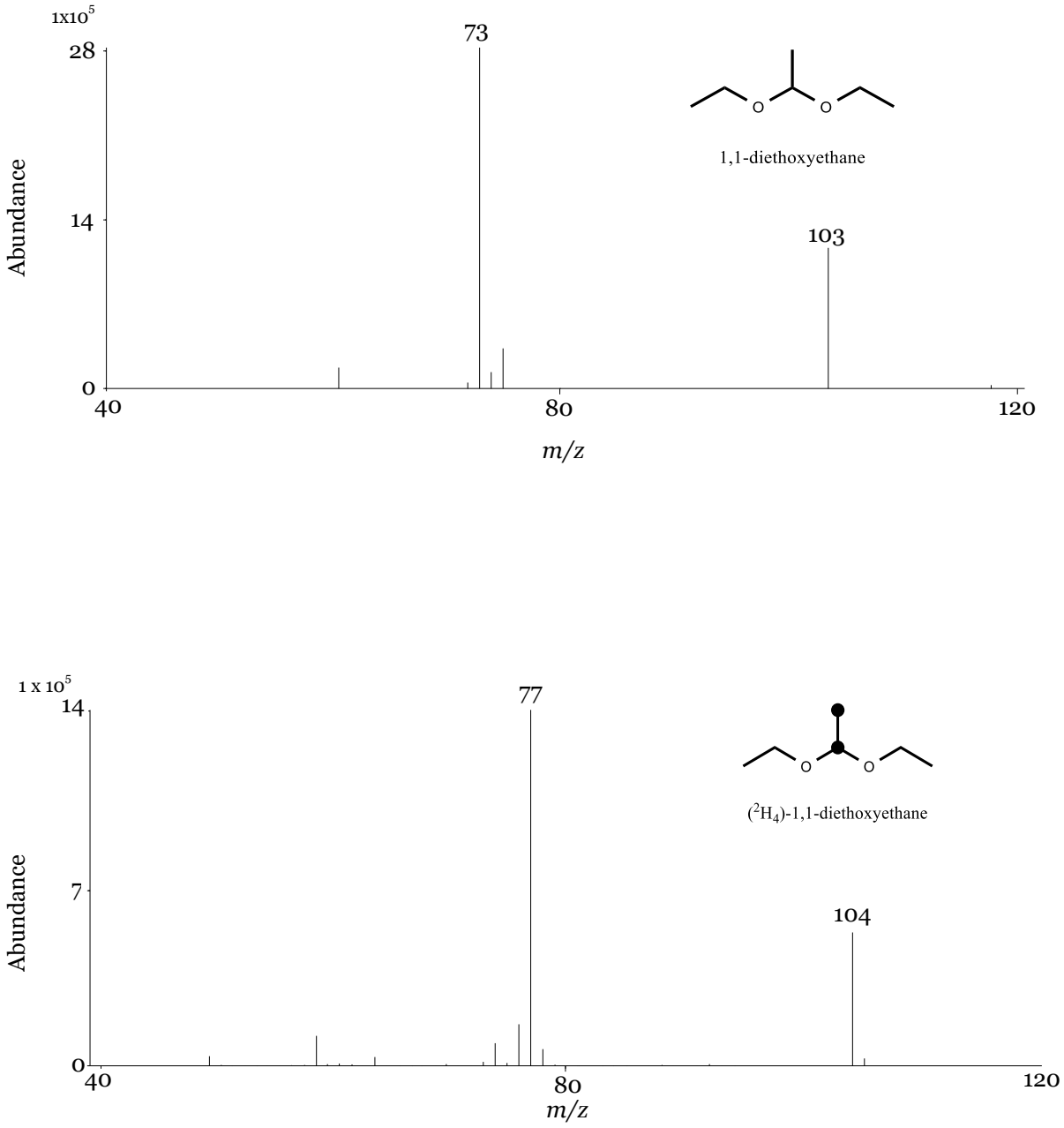


Figure 29 Mass spectra of 1,1-diethoxyethane and isotope analogue

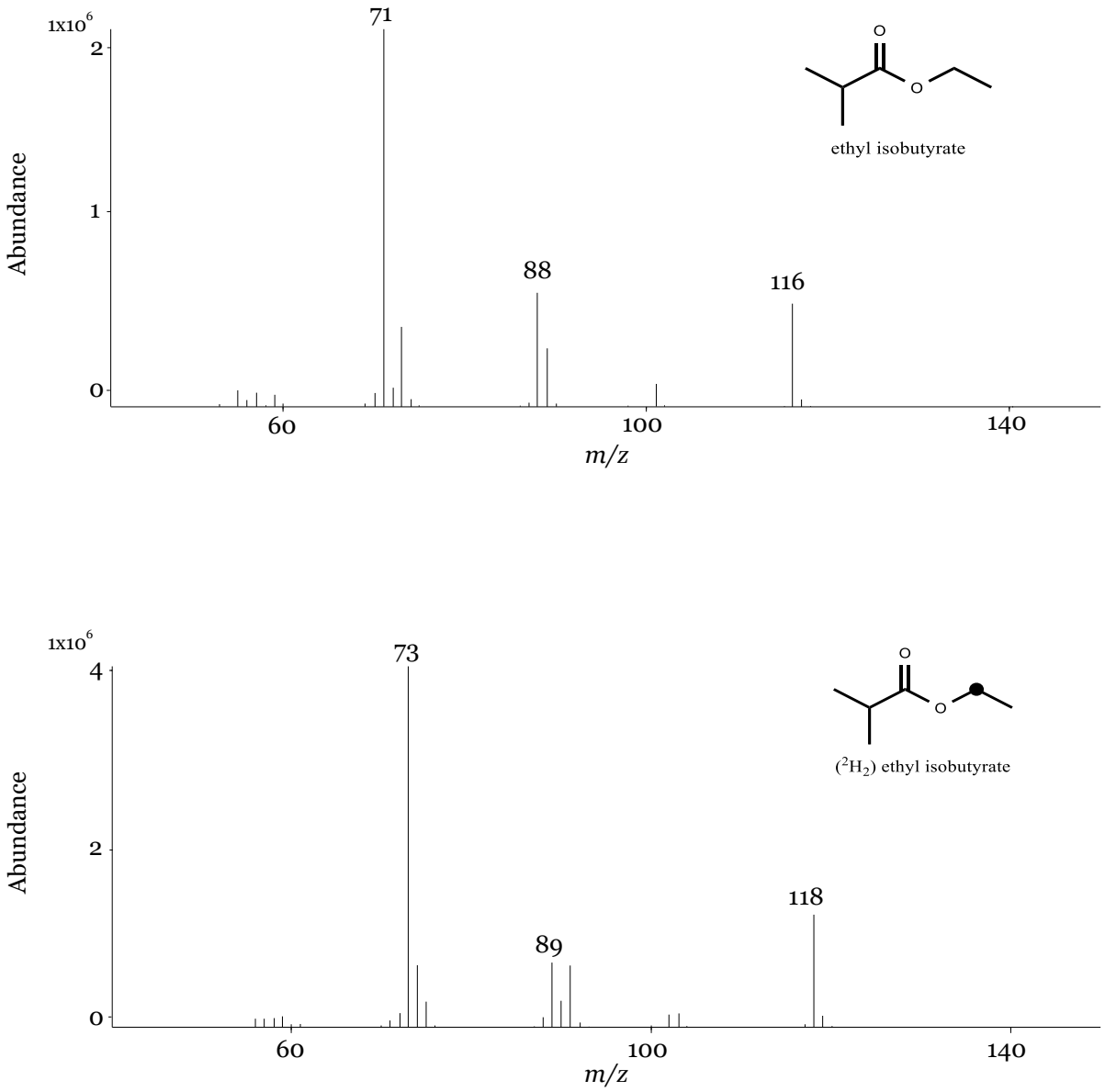


Figure 30 Mass spectra of ethyl isobutyrate and isotope analogue

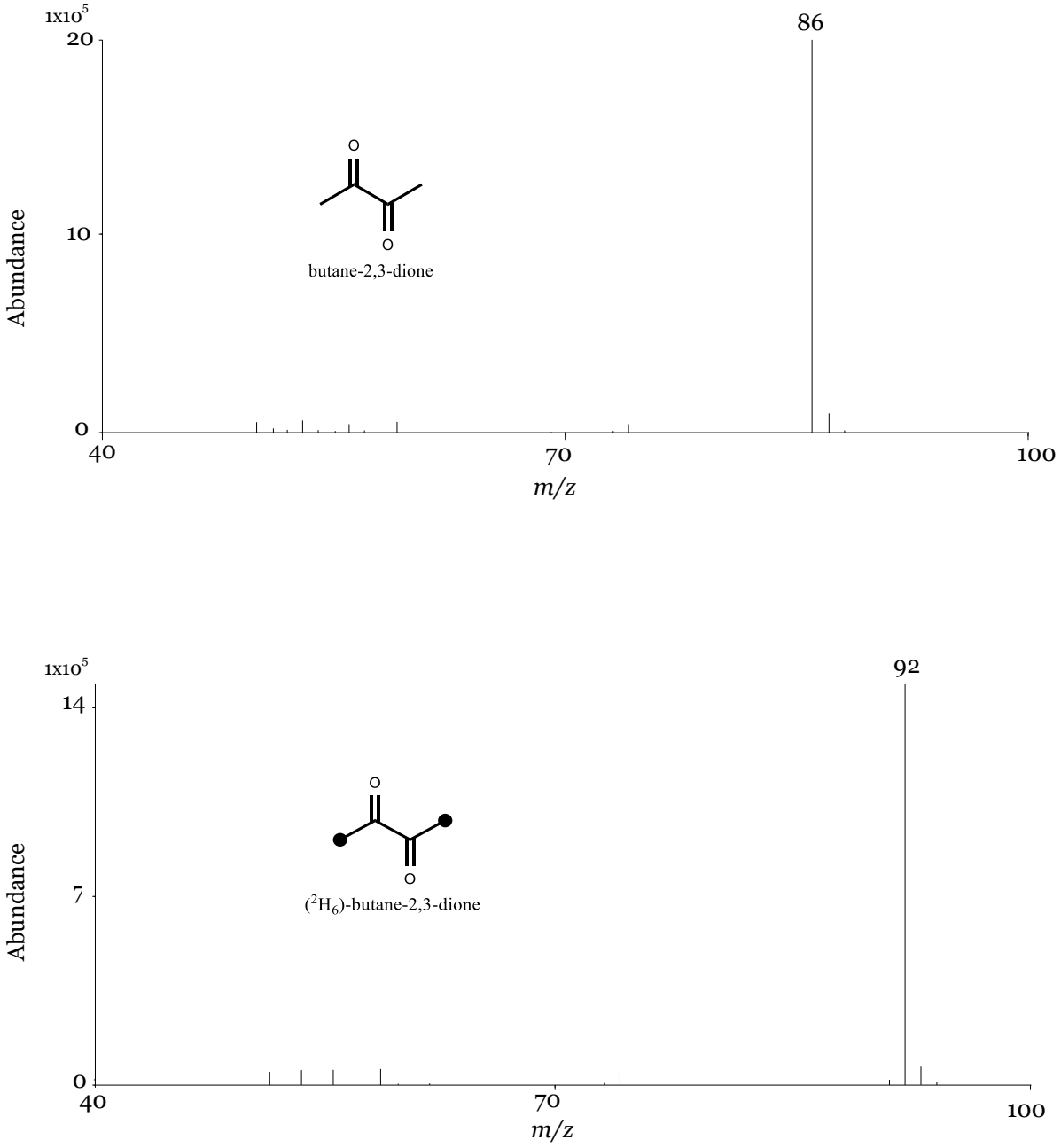


Figure 31 Mass spectra of butane-2,3-dione and isotope analogue

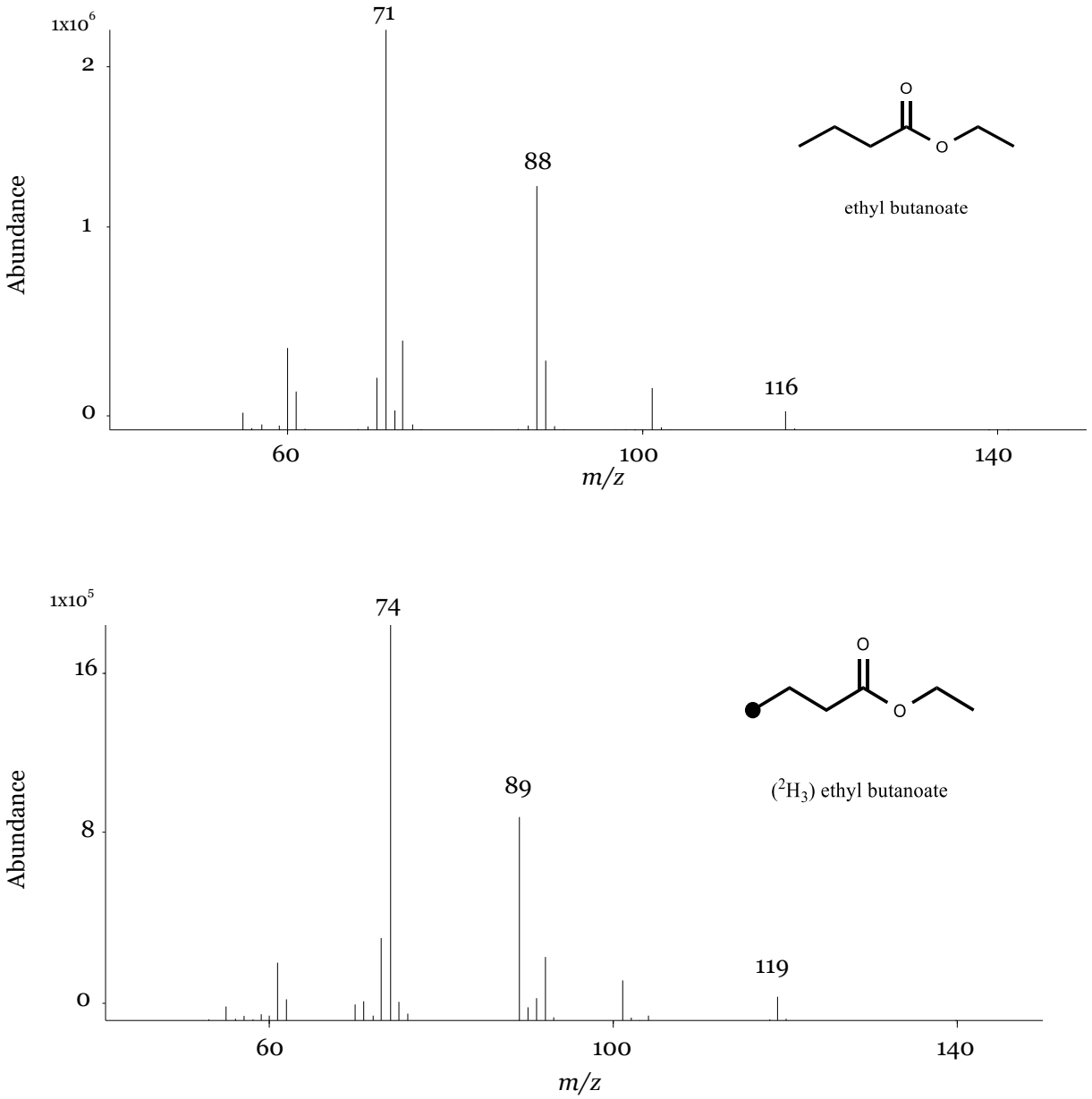


Figure 32 Mass spectra of ethyl butanoate and isotope analogue

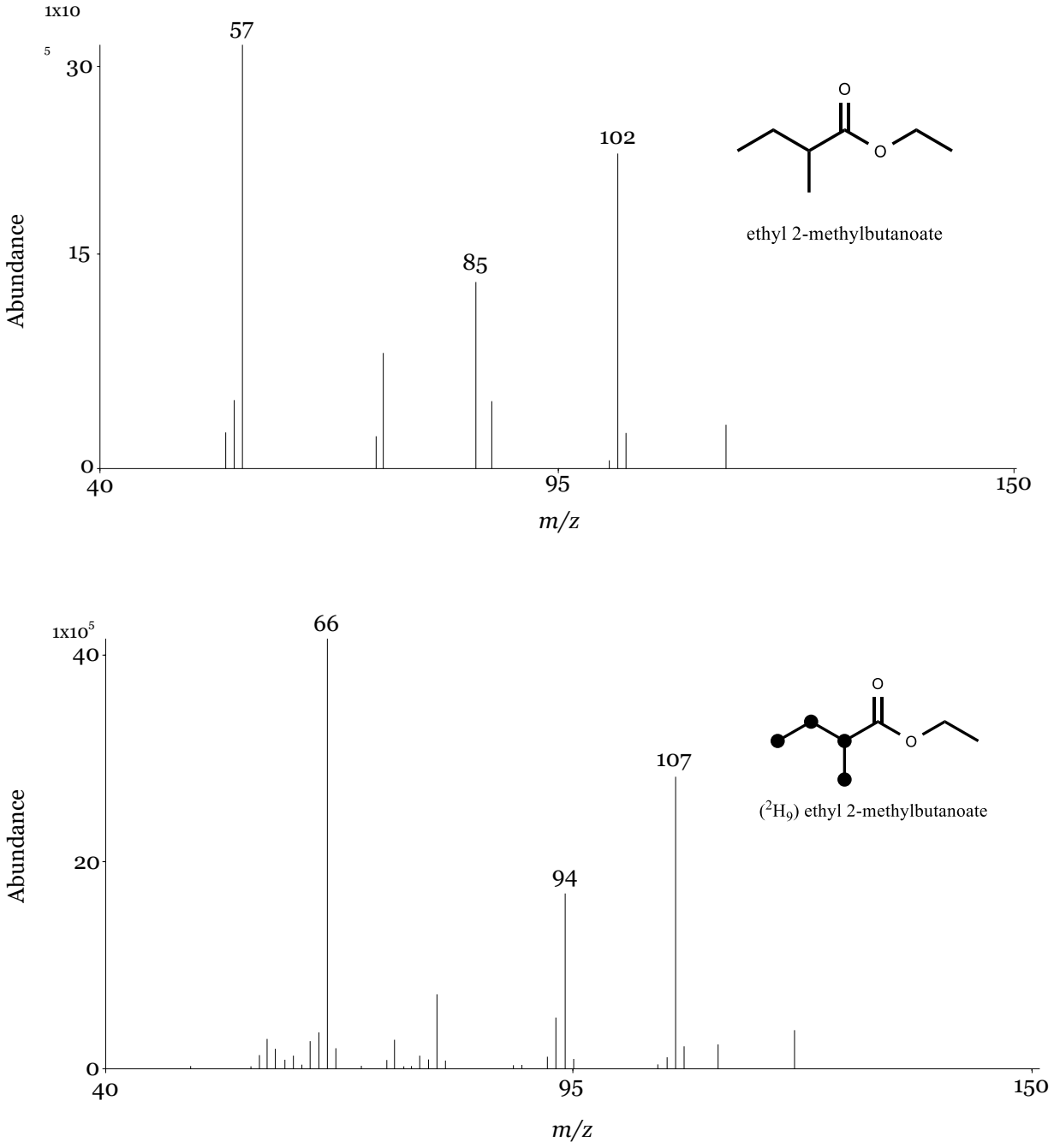


Figure 33 Mass spectra of ethyl 2-methylbutanoate and isotope analogue

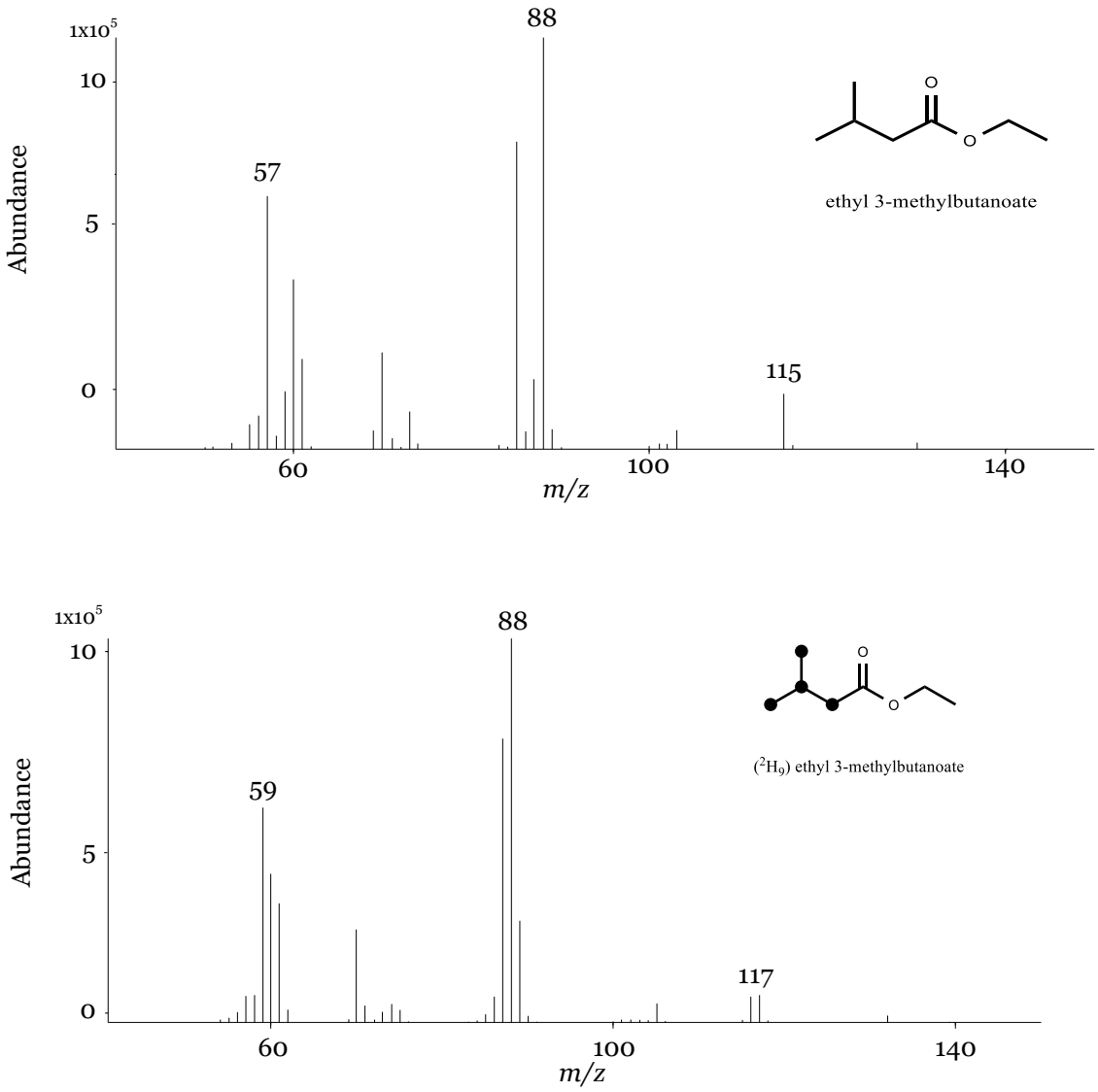


Figure 34 Mass spectra of ethyl 3-methylbutanoate and isotope analogue

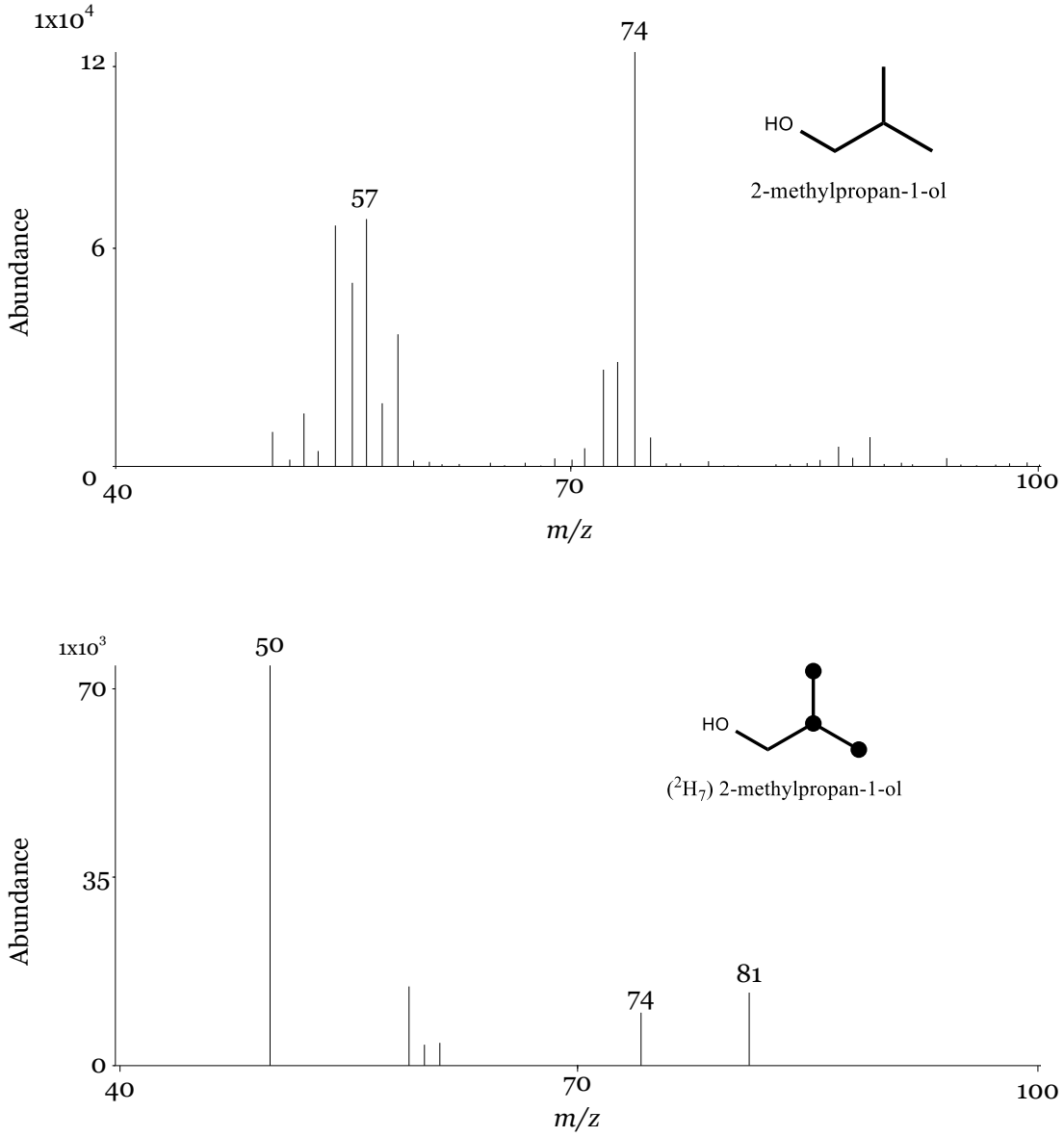


Figure 35 Mass spectra of 2-methylpropan-1-ol and isotope analogue

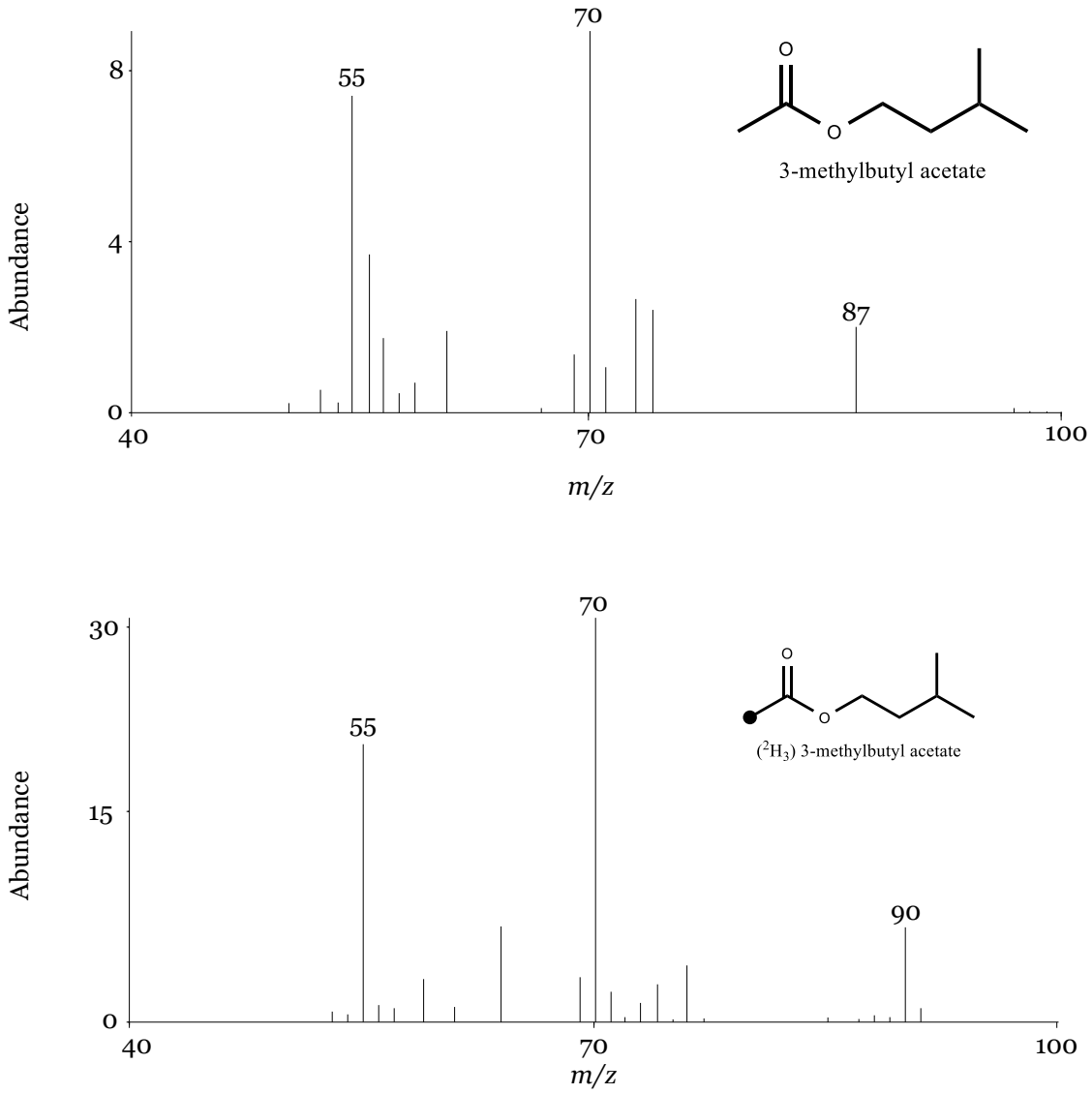


Figure 36 Mass spectra of 3-methylbutyl acetate and isotope analogue

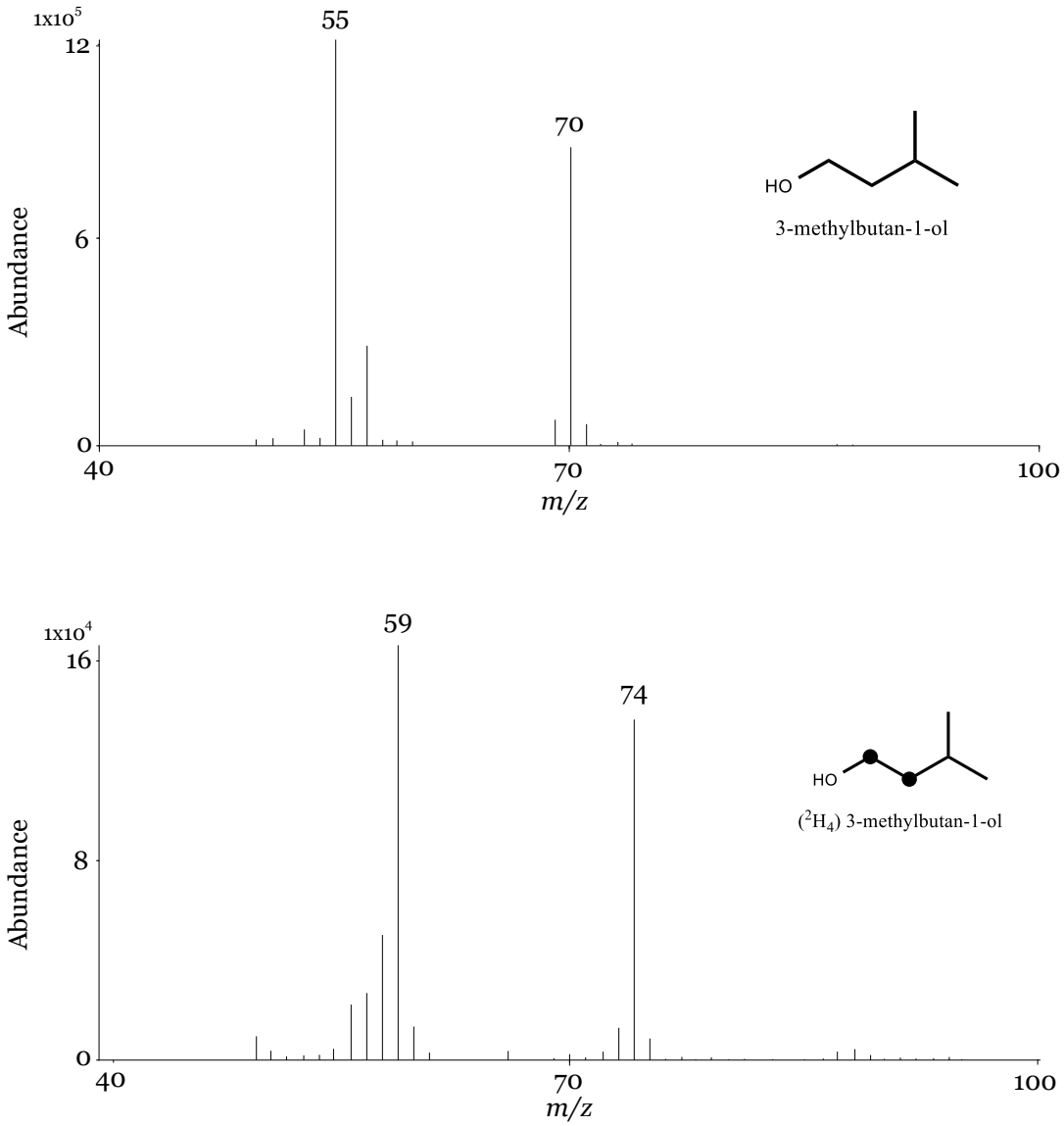


Figure 37 Mass spectra of 3-methylbutan-1-ol and isotope analogue

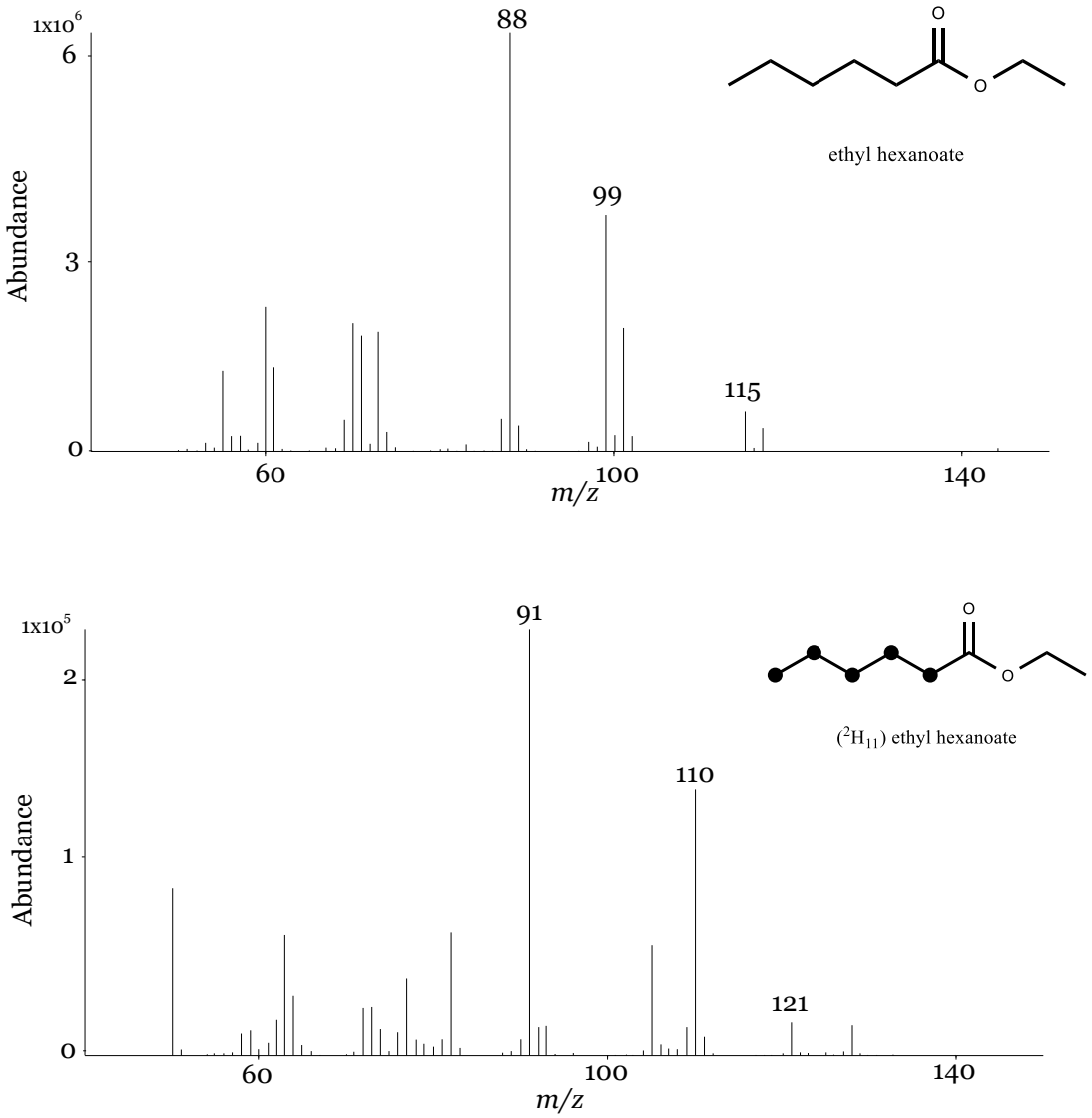


Figure 38 Mass spectra of ethyl hexanoate and isotope analogue

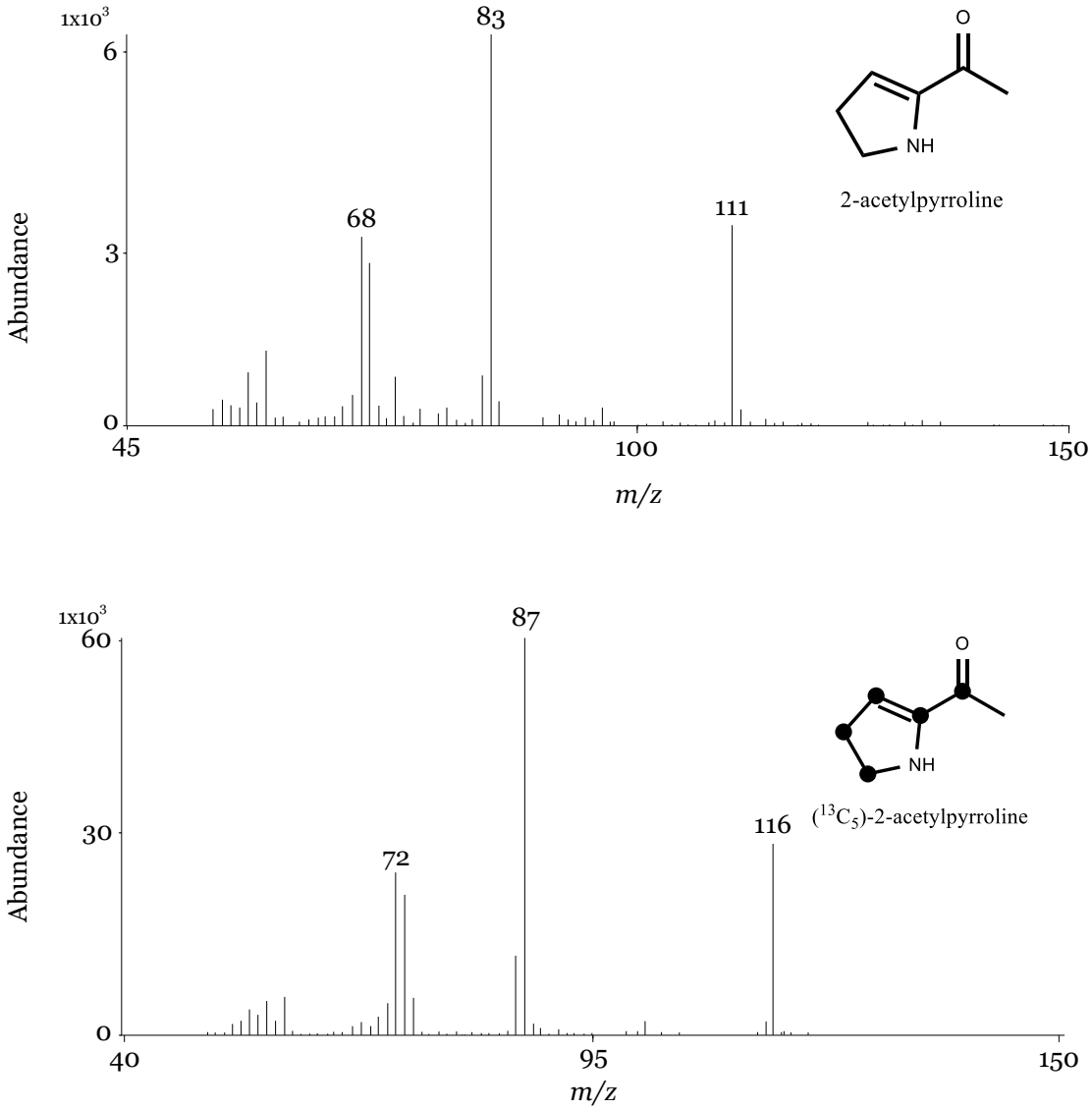


Figure 39 Mass spectra of 2-acetyl-1-pyrroline and isotope analogue

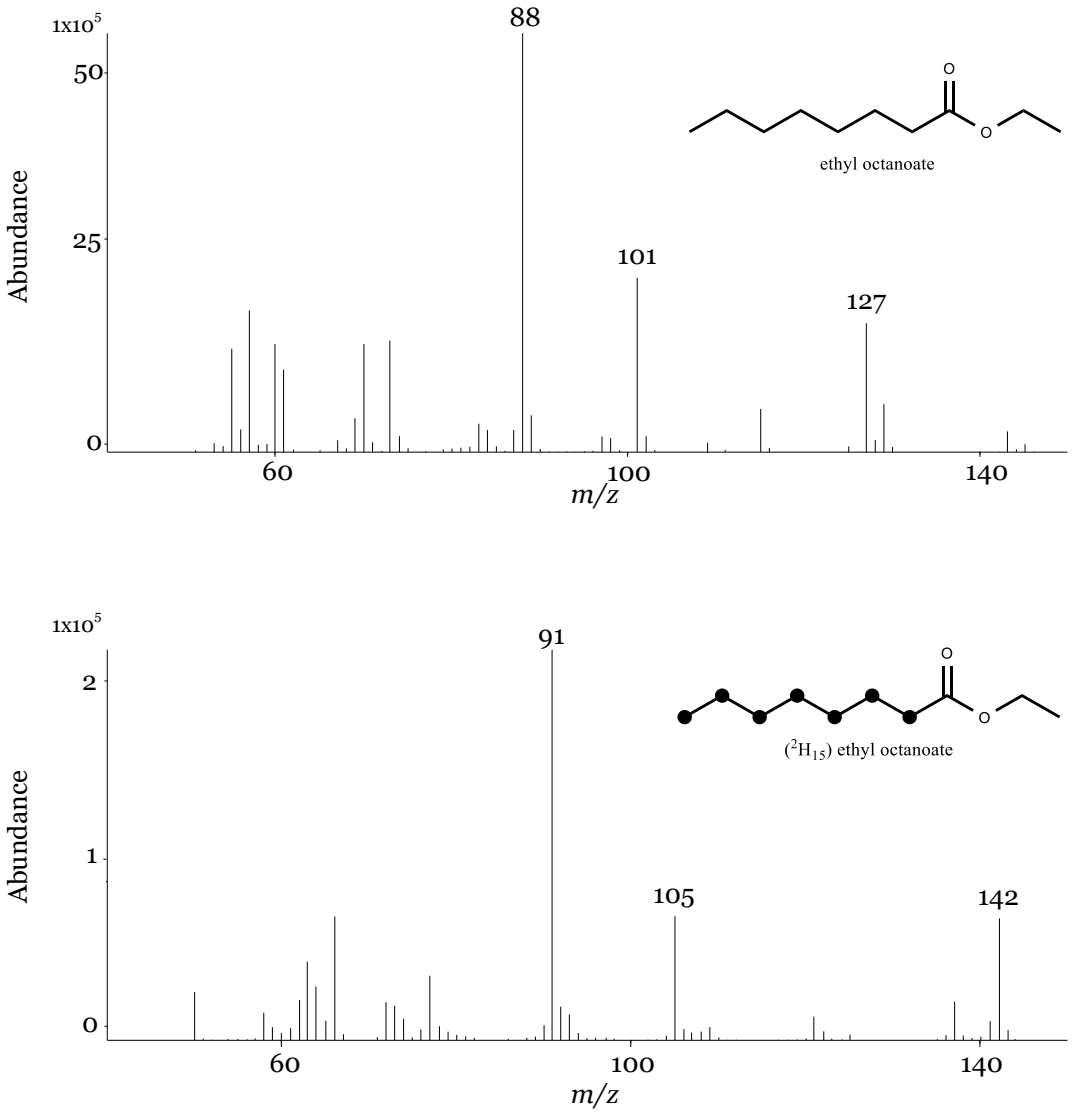


Figure 40 Mass spectra of ethyl octanoate and isotope analogue

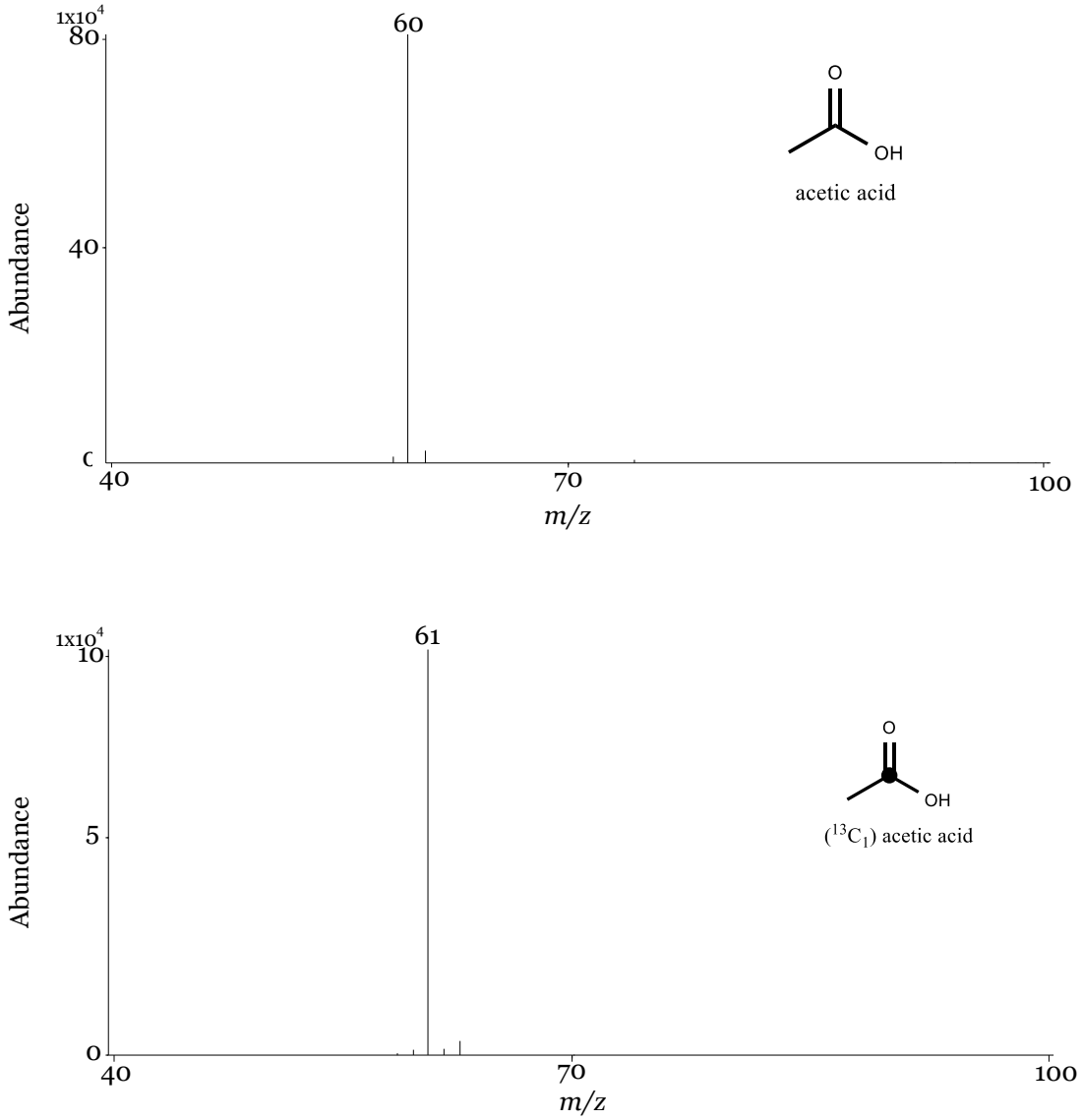


Figure 41 Mass spectra of acetic acid and isotope analogue

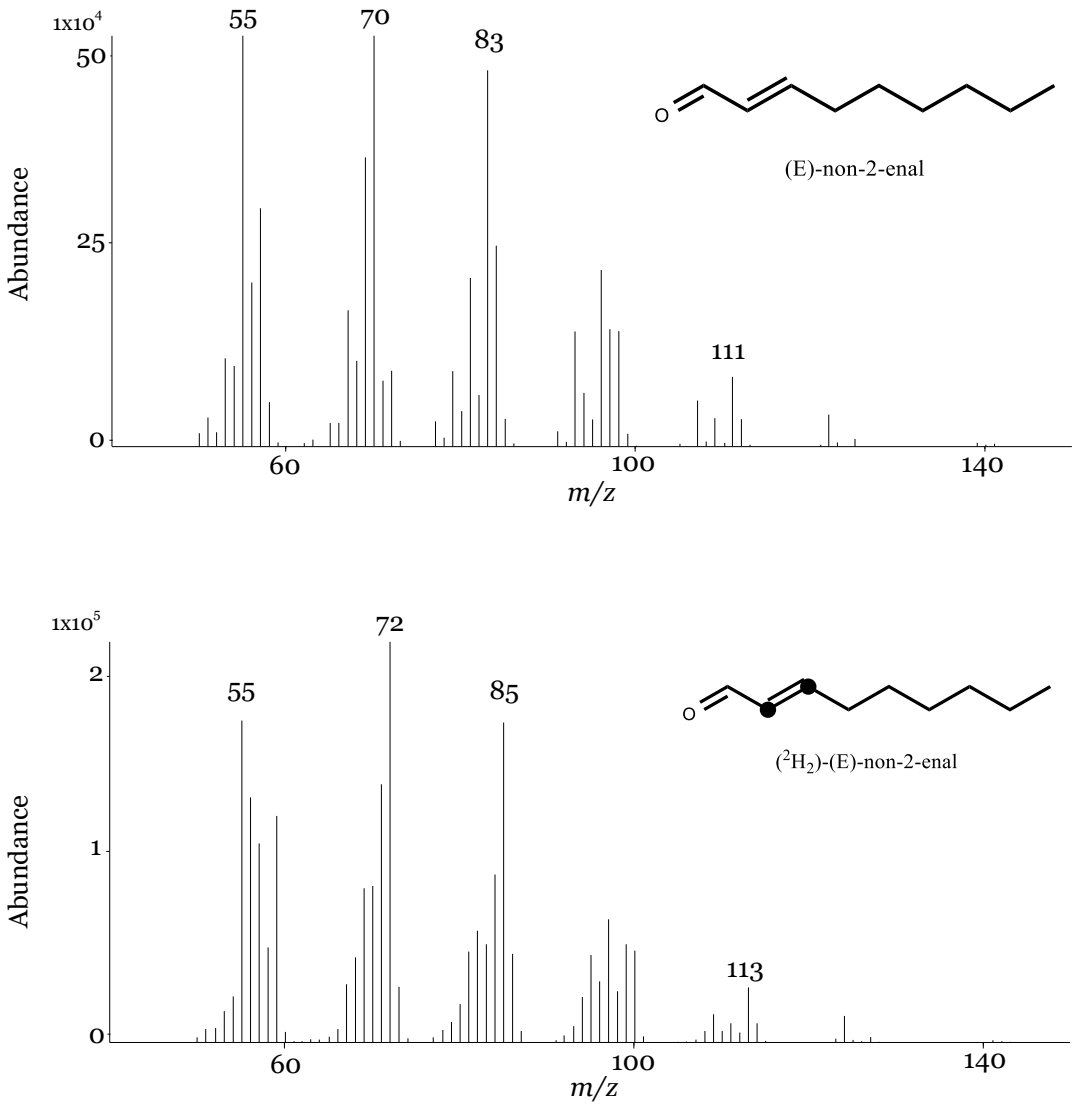


Figure 42 Mass spectra of (2*E*)-non-2-enal and isotope analogue

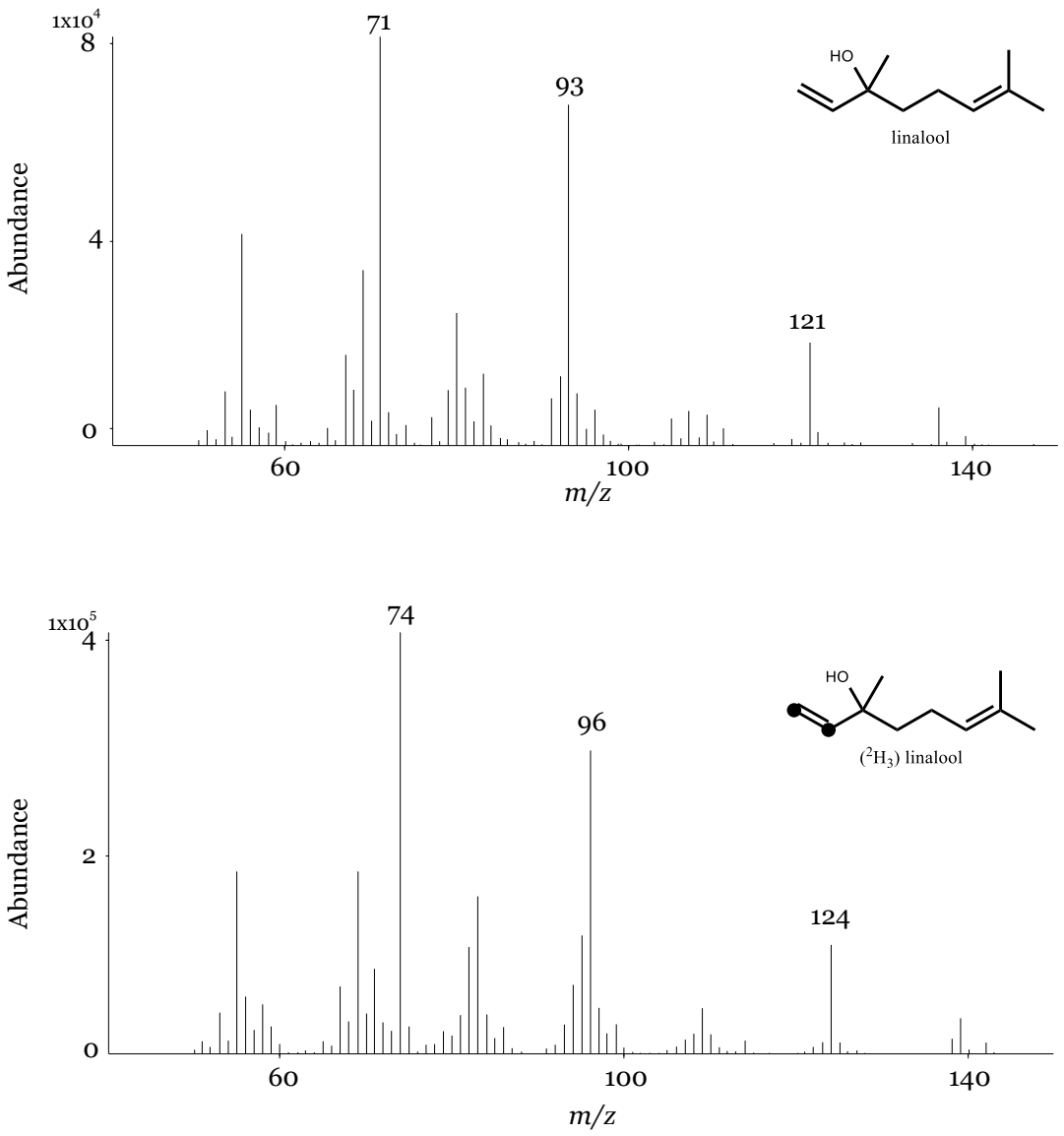


Figure 43 Mass spectra of linalool and isotope analogue

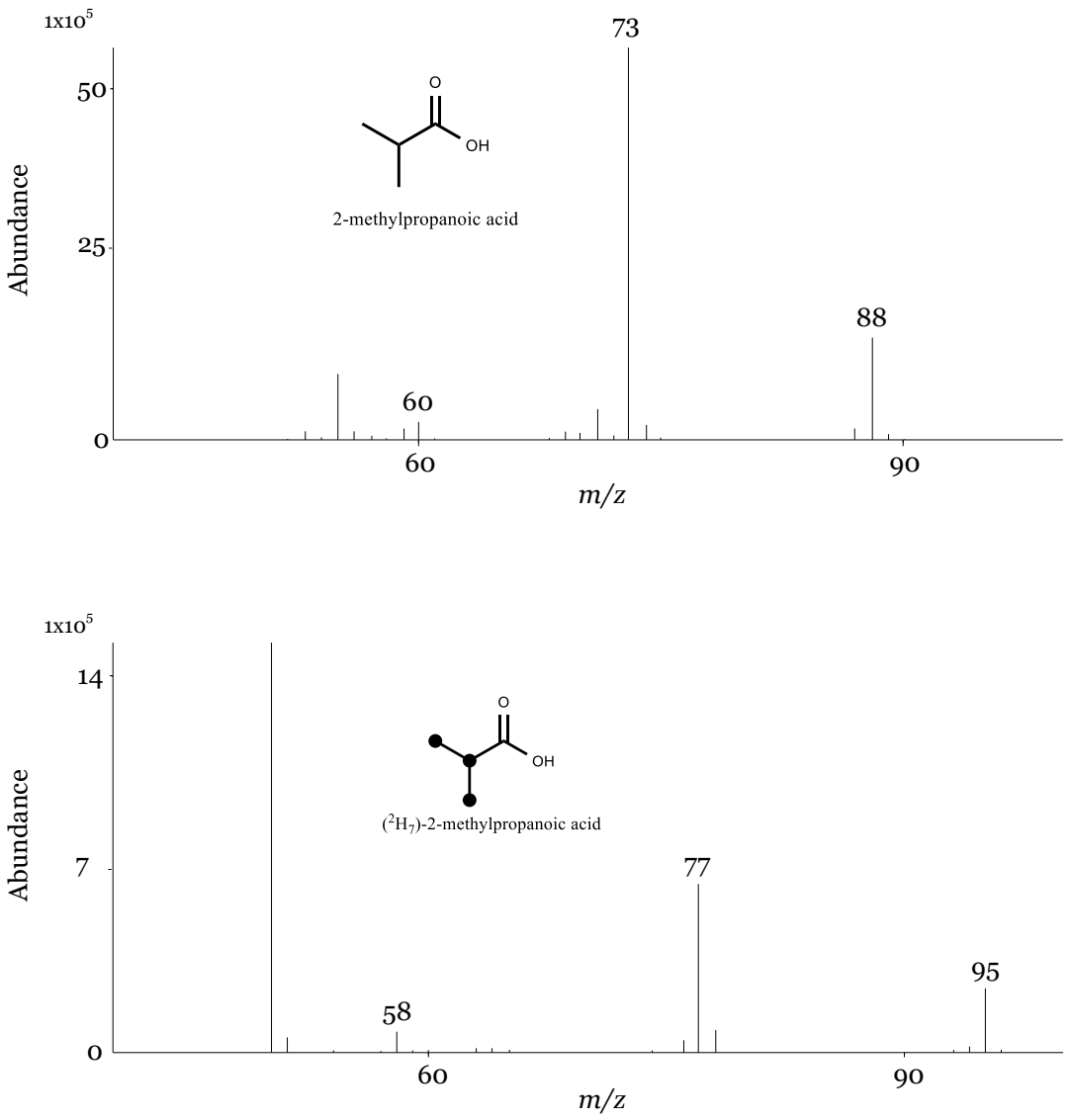


Figure 44 Mass spectra of 2-methylpropanoic acid and isotope analogue

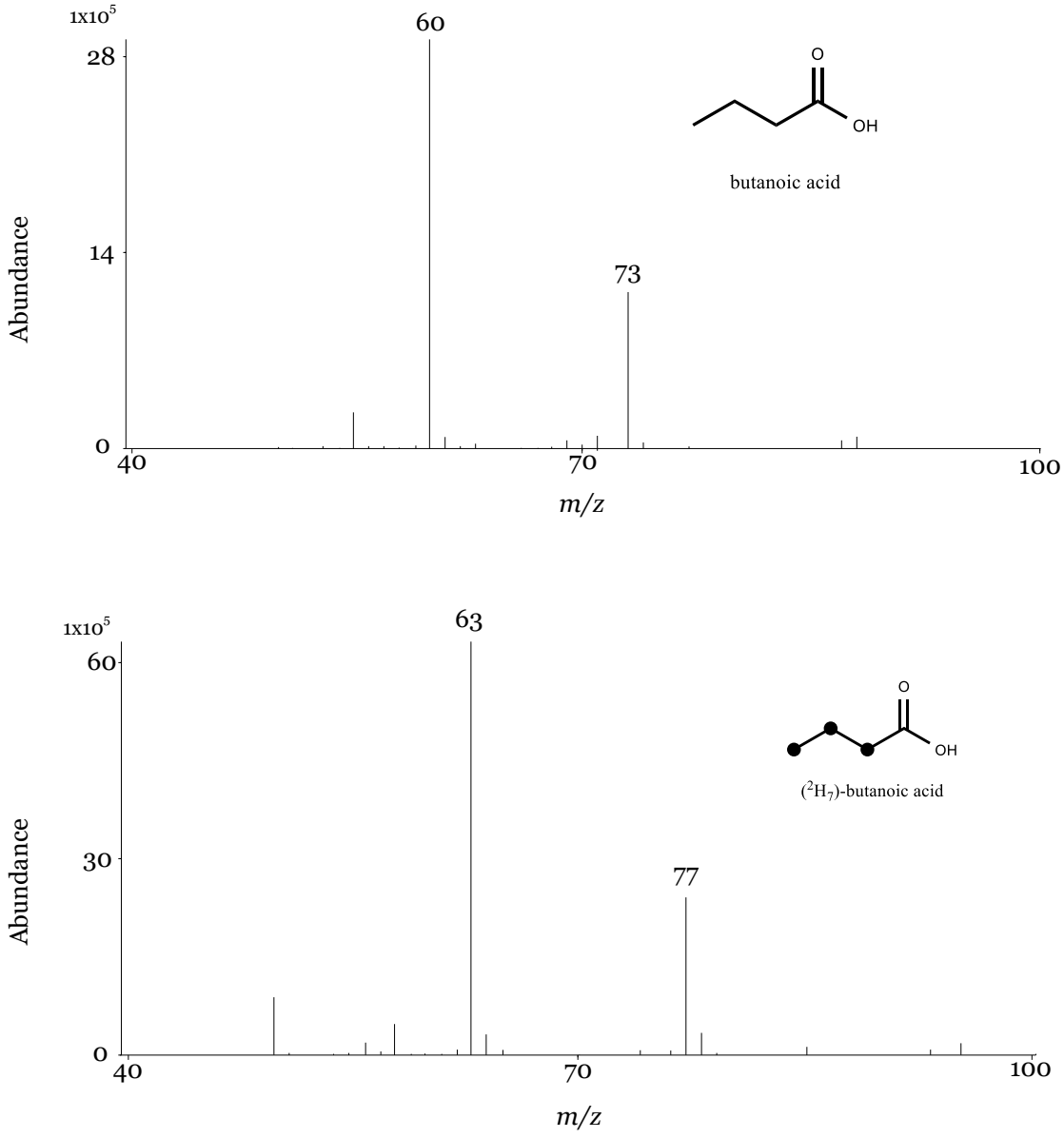


Figure 45 Mass spectra of butanoic acid and isotope analogue

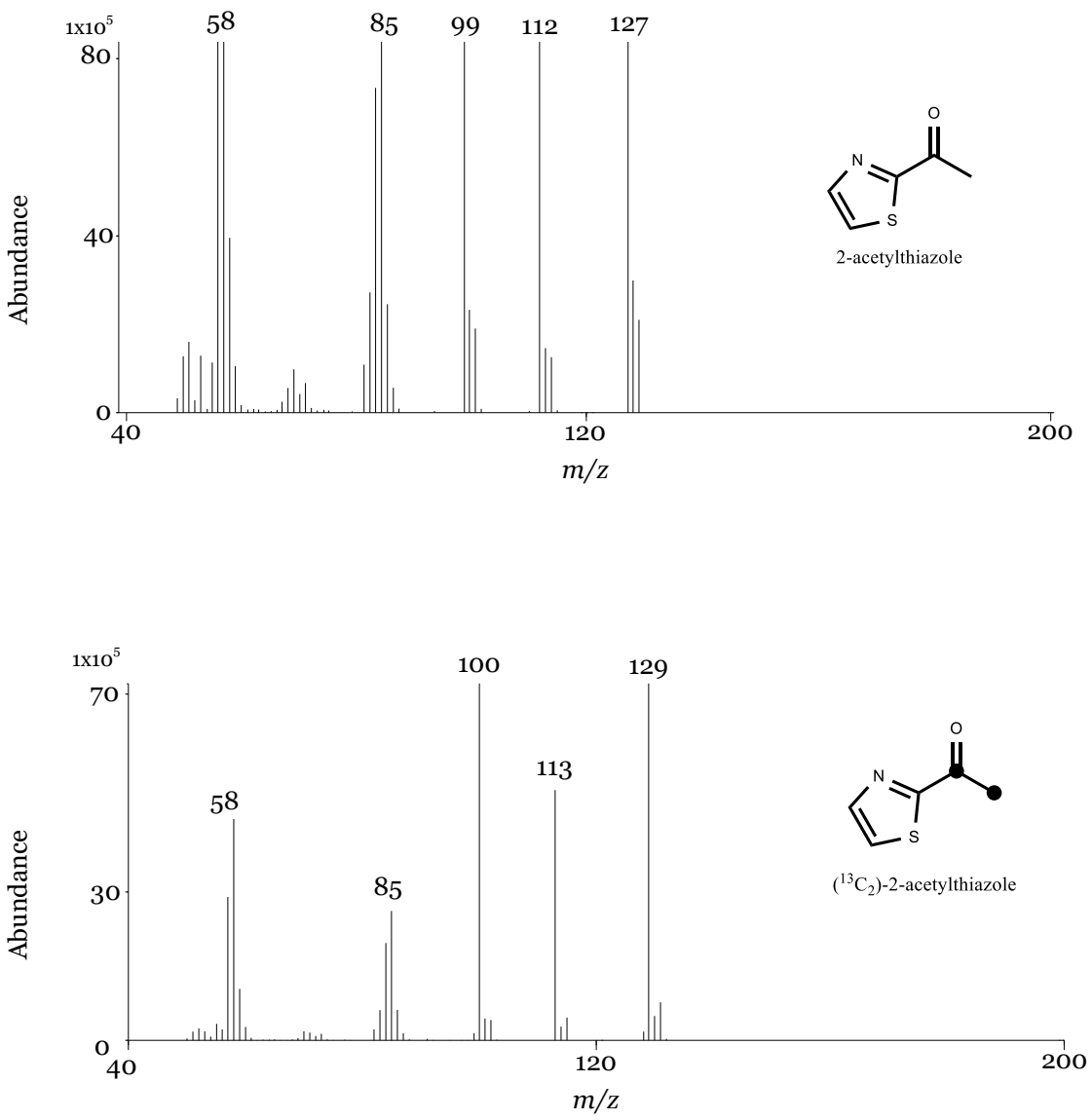


Figure 46 Mass spectra of 2-acetylthiazole and isotope analogue

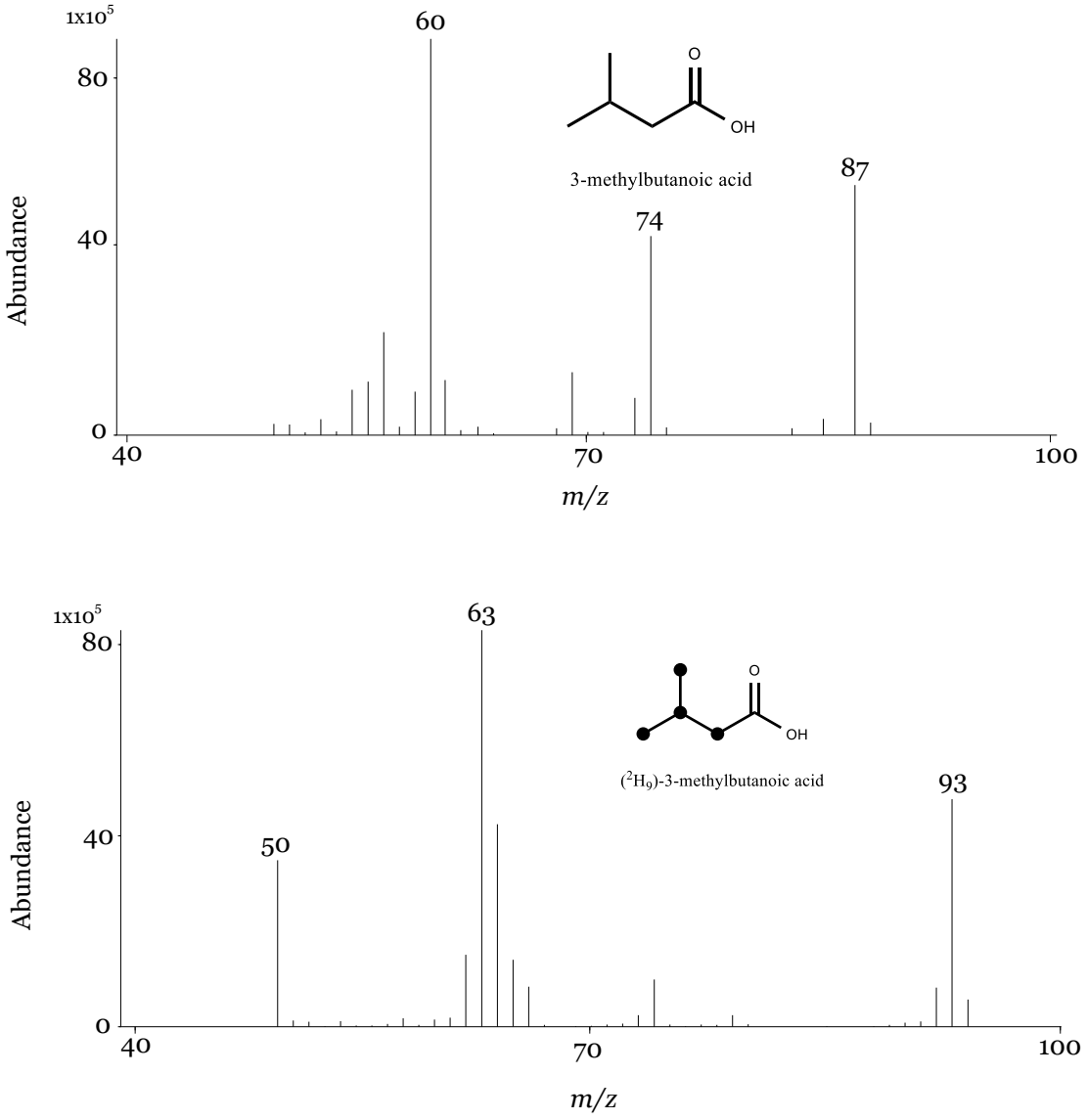


Figure 47 Mass spectra of 3-methylbutanoic acid and isotope analogue

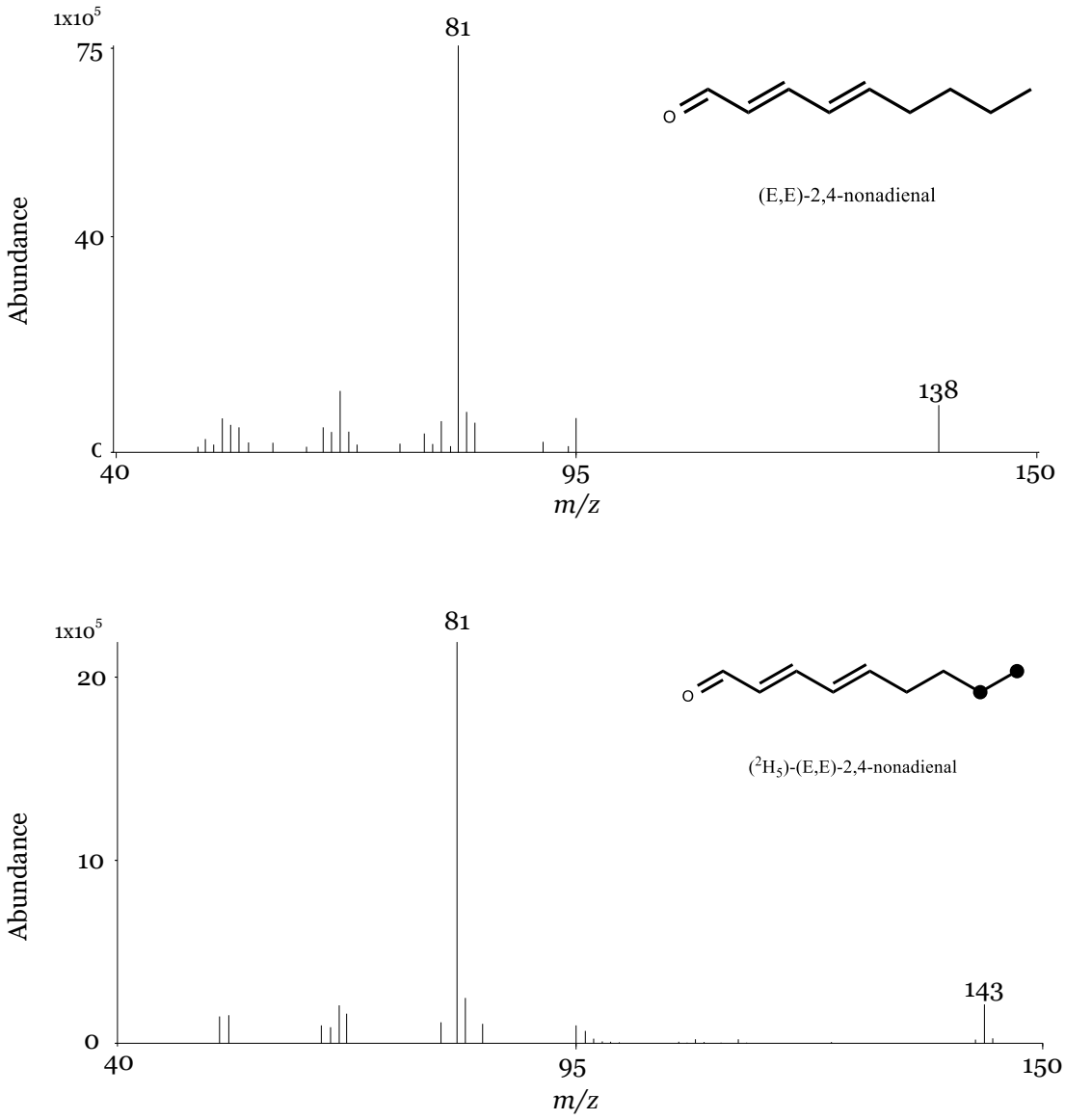


Figure 48 Mass spectra of (2*E,4E)*-nona-2,4-dienal and isotope analogue

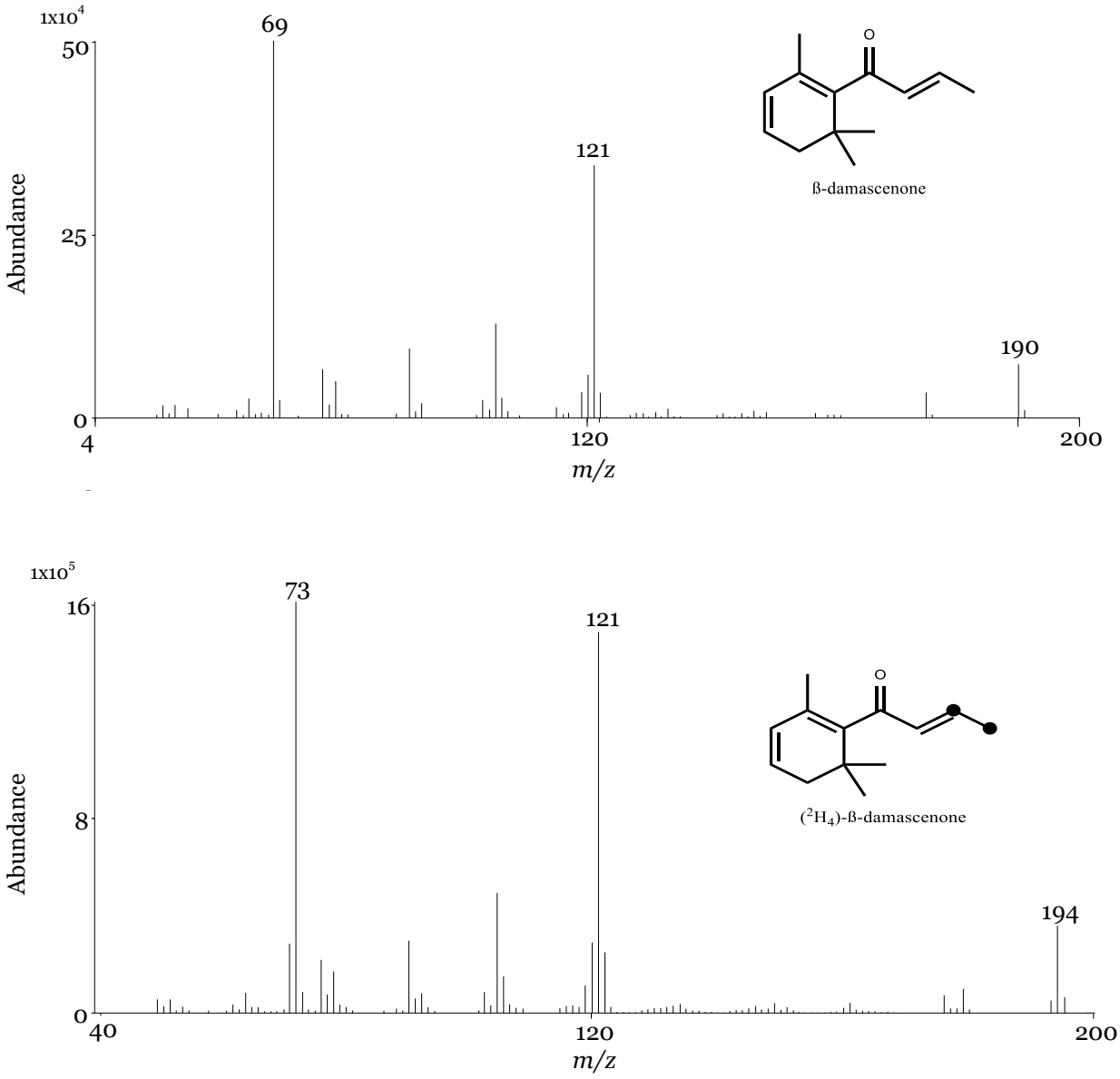


Figure 49 Mass spectra of β-damascenone and isotope analogue

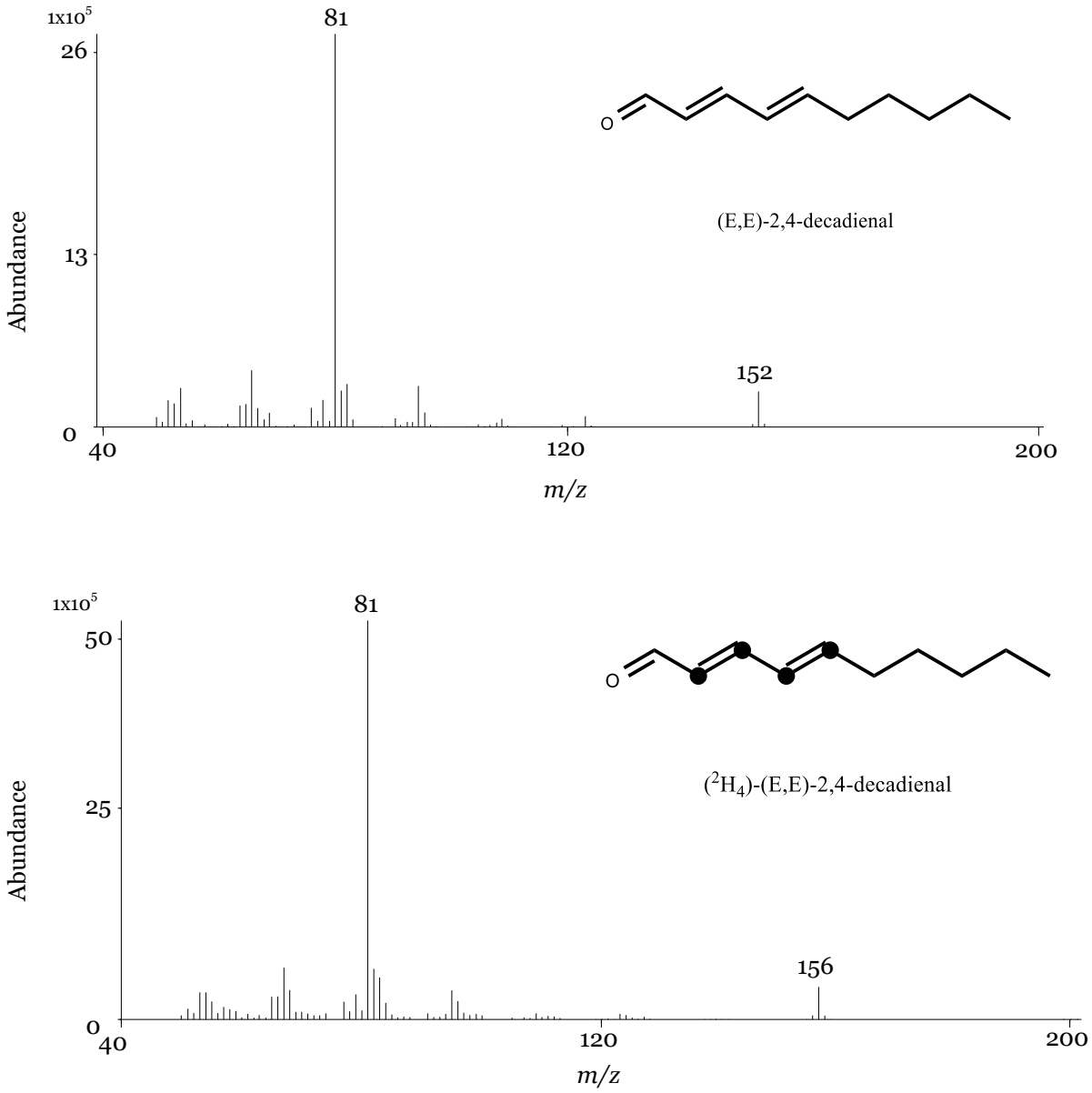


Figure 50 Mass spectra of (2*E,4E*)-deca-2,4-dienal and isotope analogue

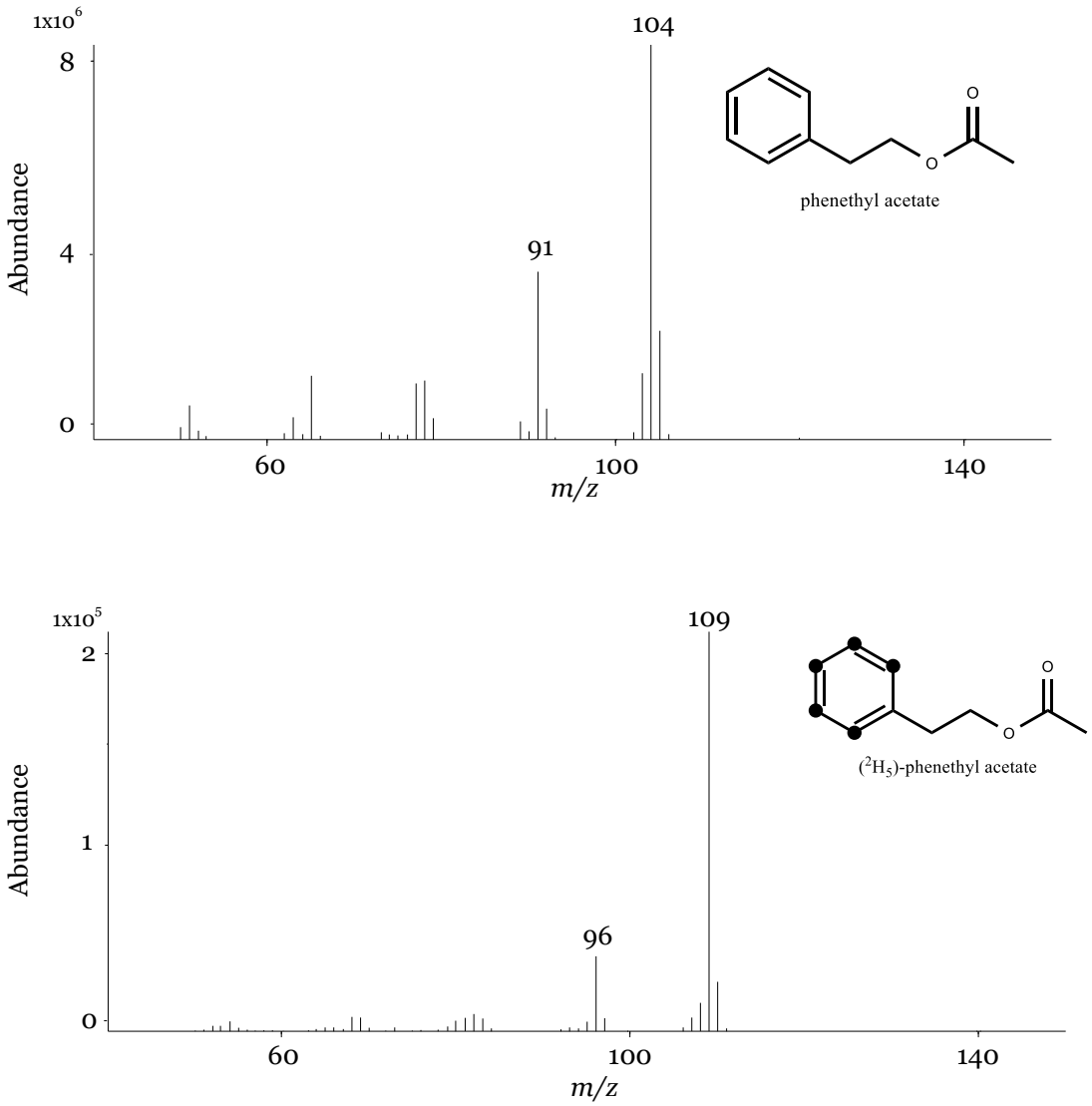


Figure 51 Mass spectra of 2-phenylethyl acetate and isotope analogue

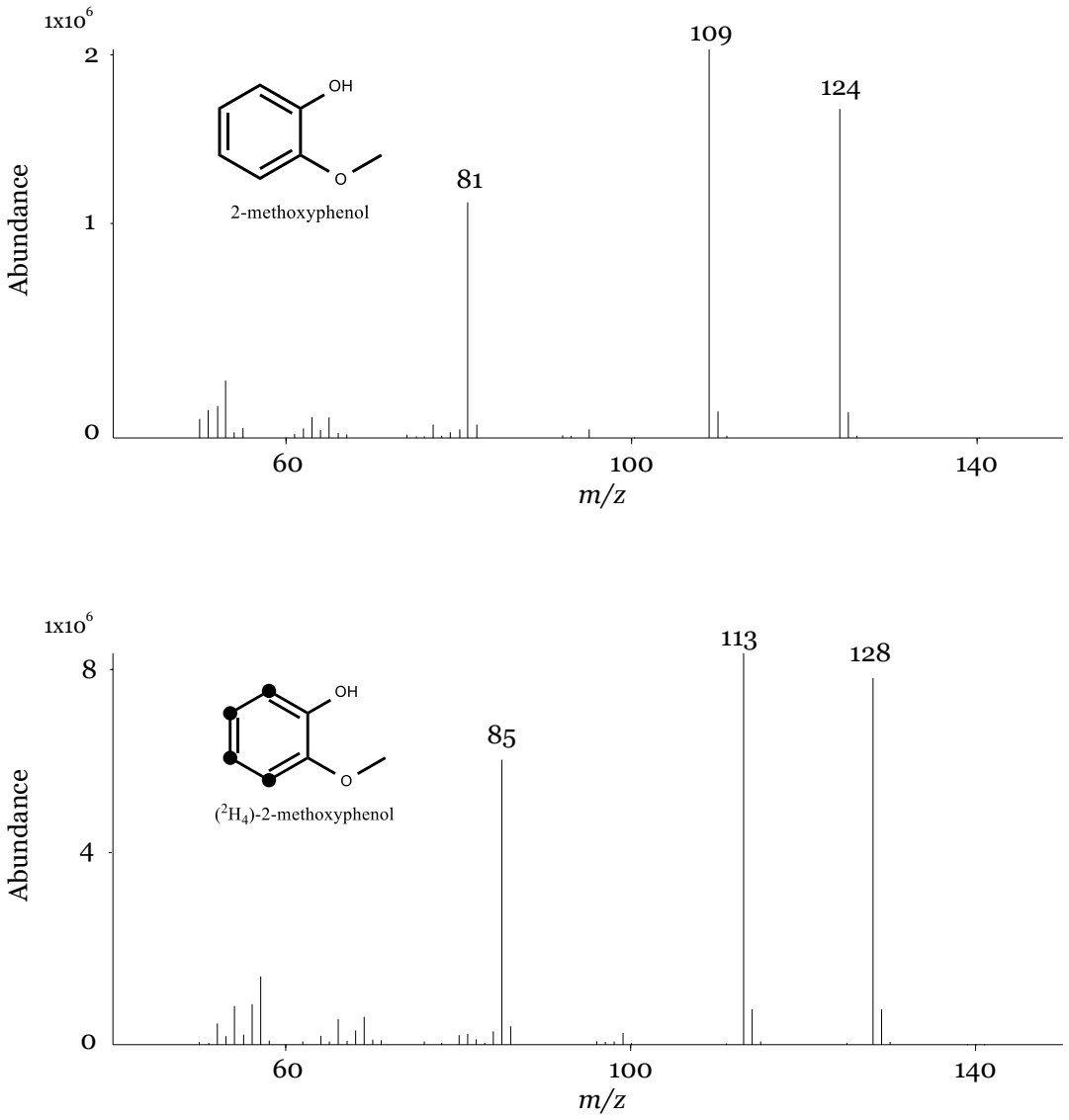


Figure 52 Mass spectra of 2-methoxyphenol and isotope analogue

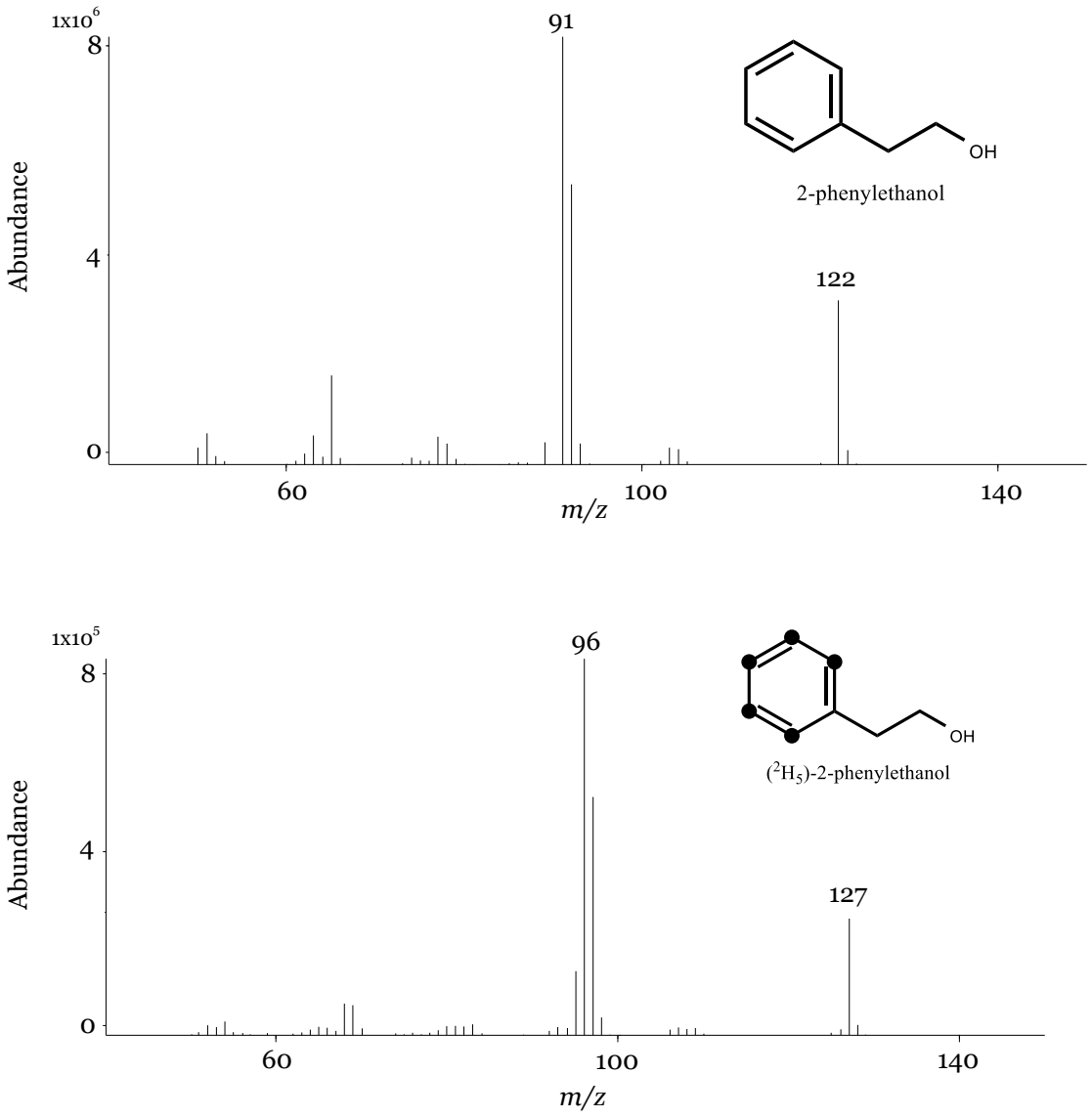


Figure 53 Mass spectra of 2-phenylethanol and isotope analogue

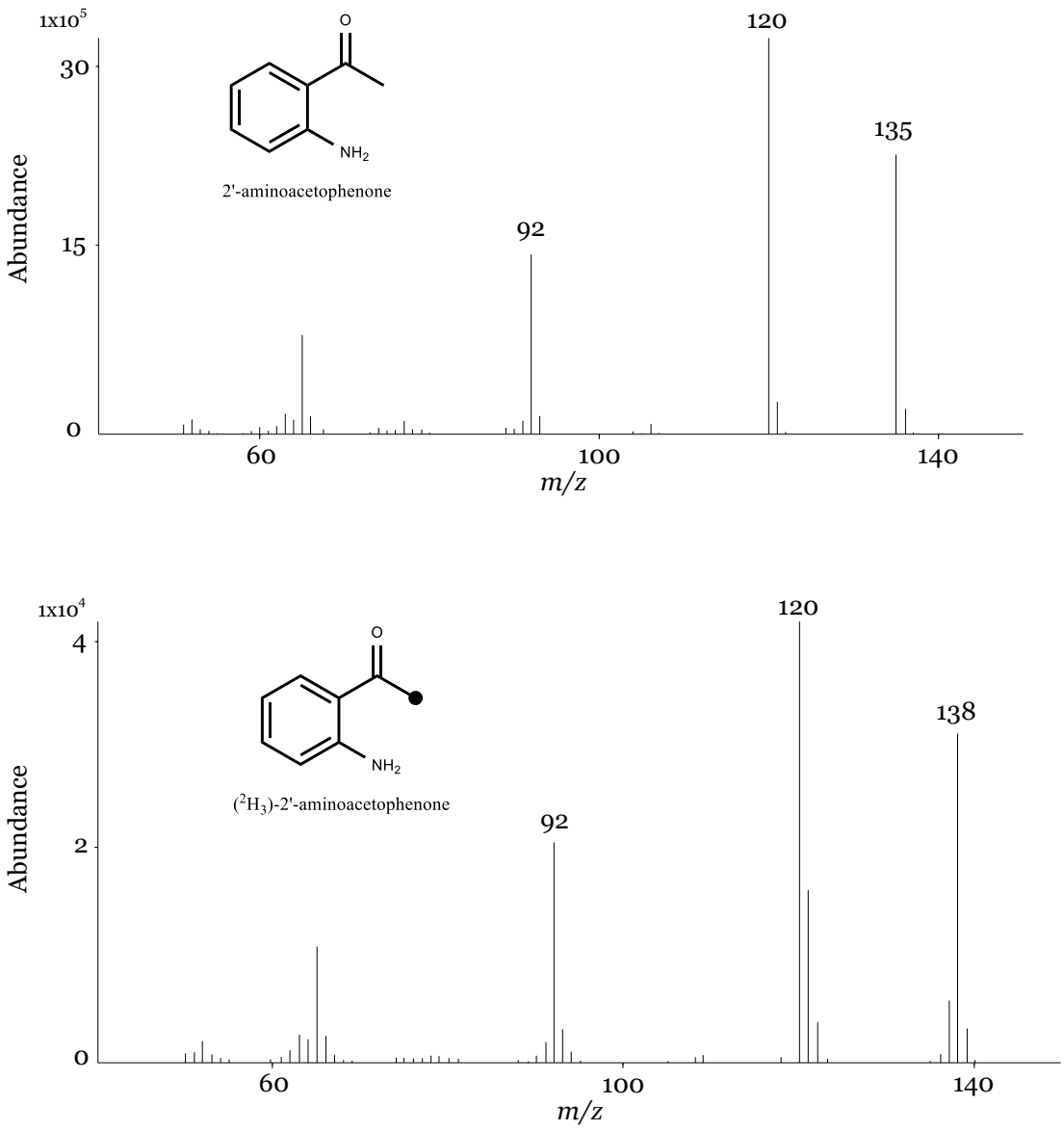
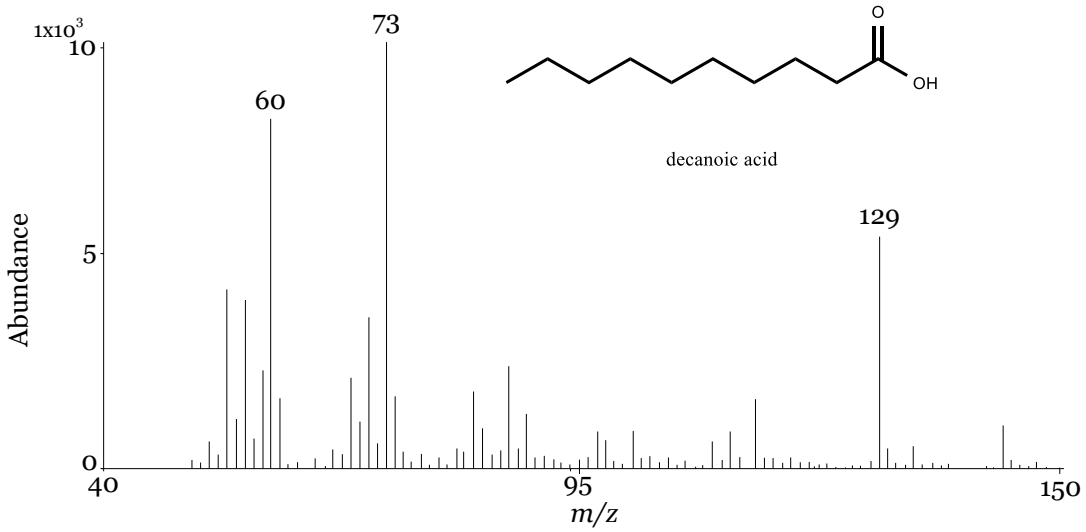


Figure 54 Mass spectra of 2′-aminoacetophenone and isotope analogue



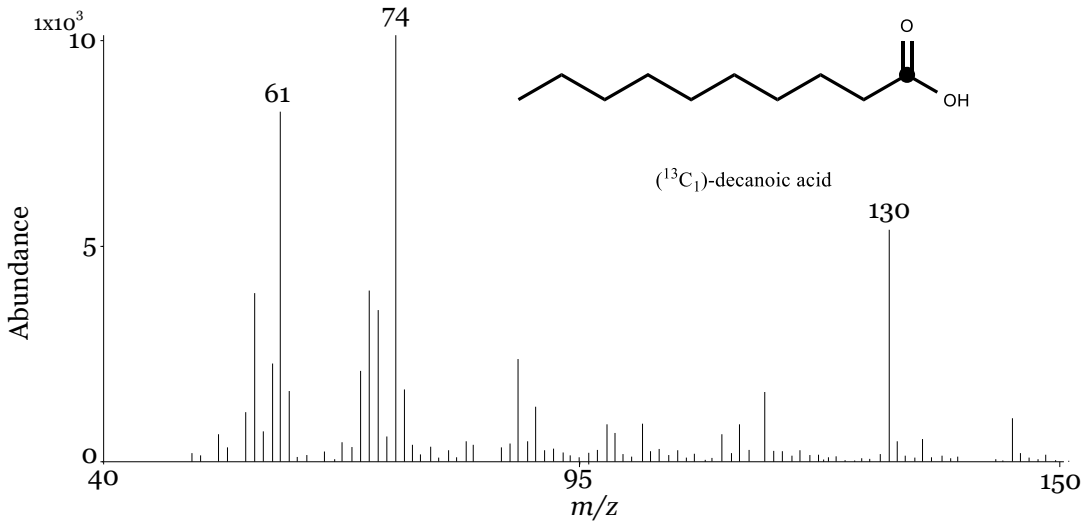


Figure 55 Mass spectra of decanoic acid and isotope analogue

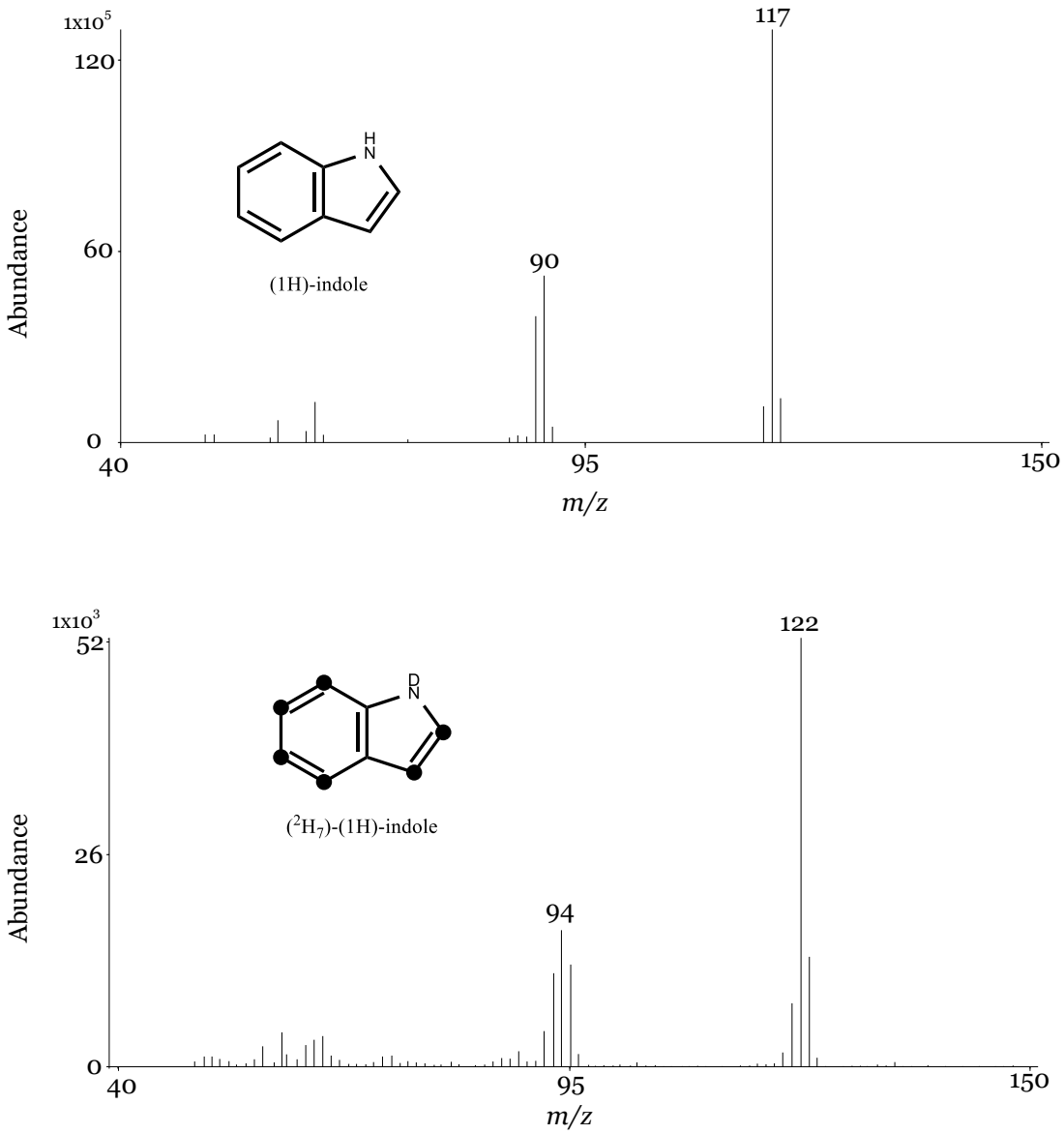


Figure 56 Mass spectra of 1*H*-indole and isotope analogue

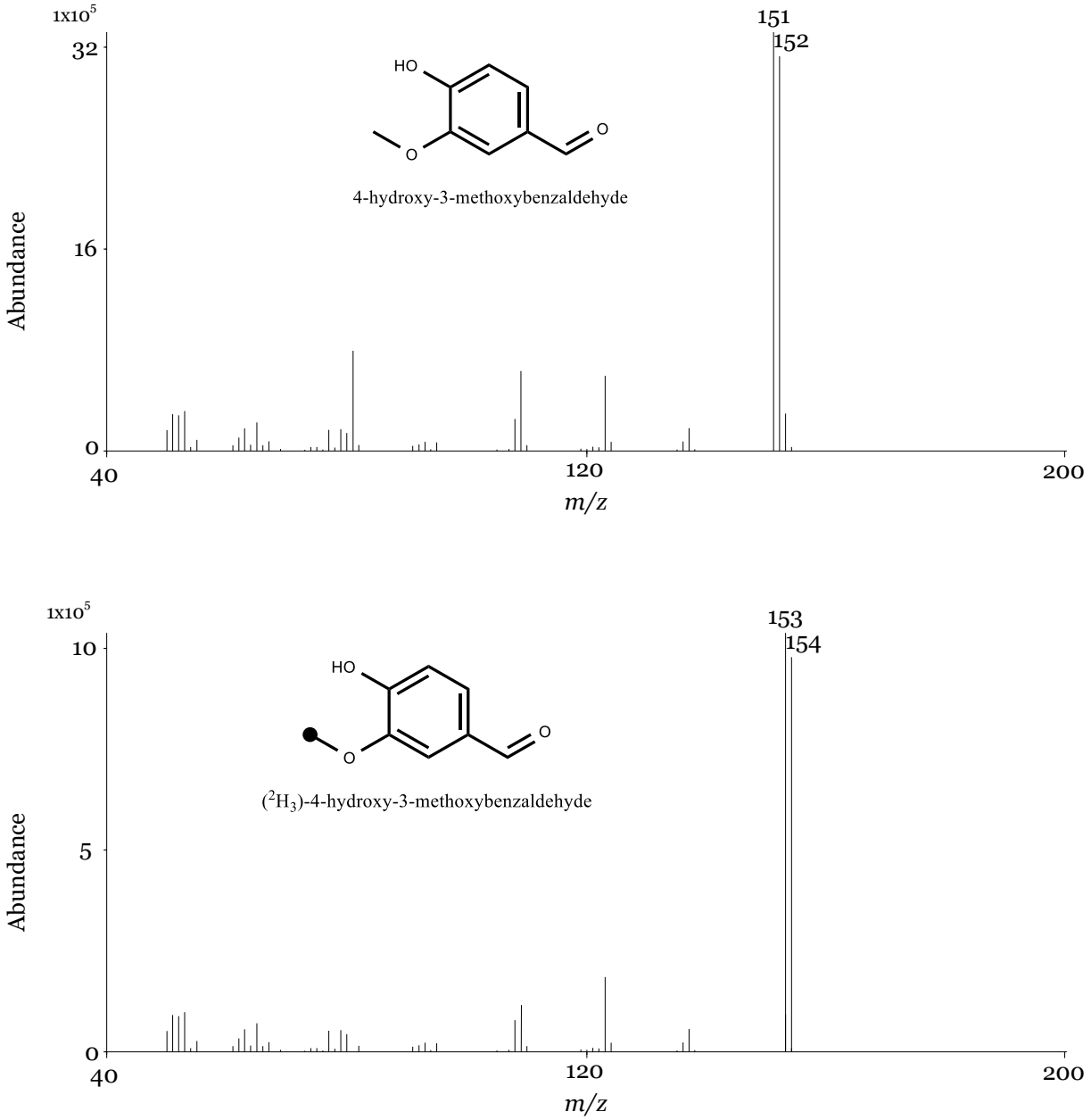


Figure 57 Mass spectra of 4-hydroxy-3-methoxybenzaldehyde and isotope analogue

Vita

Trent was born and raised in Knoxville, Tennessee. He attended Hardin Valley Academy for high school where he graduated in 2012. He attended the University of Tennessee Knoxville where he graduated with a Bachelor of Science in Food Science with minors in physics and chemistry. He then continued at the University of Tennessee to pursue a Master of Science in Food Science full time.