An overview of some natural products with two A-level science club natural products experiments

Michael Hal Sosabowski, George W. J. Olivier, Hala Jawad and Sieja Maatta

ABSTRACT Natural products are ubiquitous in nature but do not form a large proportion of the A-level syllabuses in the UK. In this article we briefly discuss a small selection of natural products, focusing on alcohols, aldehydes and ketones, and alkaloids. We then outline two natural product experiments that are suitable for A-level chemistry clubs or similar. Experiment 1 is the isolation and analysis of caffeine from tea, and Experiment 2 is the extraction and characterisation of a volatile oil, eugenol, from cloves. These experiments include a variety of laboratory techniques, including steam distillation, determination of melting point, thin-layer chromatography and column chromatography, infrared spectroscopy and ultraviolet spectrophotometry.

The A-level chemistry syllabuses outlined in the specifications of the UK exam boards commonly include only peripheral allusions to natural products; one might expect to find simple discussion of carbohydrates, fats and proteins, and these are examined in greater depth in the biology syllabus.

There are many learning outcomes that can be captured when examining natural products, in terms of reactivity but also in terms of their physical properties such as melting and boiling points and their relative partitioning between aqueous and non-aqueous media, especially at differing pH values.

One of the simplest natural products, ethanol, illustrates such a learning outcome when set into the context of its homologous series, the alcohols. We are all familiar with the fact that ethanol and water are completely miscible, i.e. any amount of water will mix with any amount of ethanol and vice versa. The polar nature of the hydroxyl group in ethanol and its subsequent attraction to the dipole in water more than compensate for the fact that the non-polar ethyl group is hydrophilic. However, as one ascends the homologous series, hydrophilicity drops at an almost exponential rate owing to the increasing influence of the ever-growing R- group: octanol (C_8) can be regarded as completely immiscible in water. Octanol is so hydrophobic (and therefore lipophilic) that it can be used as a simple model for fat solubility; the partition coefficient, K_{OW}, is a measure of how much of a compound X dissolves in the water fraction versus the octanol fraction when compound X is shaken with a water/octanol mixture.

The boiling points of alcohols are similarly affected by the electron distribution. Alcohols boil at a far higher temperature than the corresponding isomeric ethers. Ethanol boils at 78.5 °C whereas its isomeric ether, dimethyl ether, boils at −24 °C. This is due to the fact that ethanol has a protic hydrogen and can undergo intermolecular hydrogen bonding whereas dimethyl ether does not, although it has a small dipole.

Table 1 shows the boiling points and miscibility with water of a number of alcohols of increasing molecular mass.

The effect of the presence or absence of dipoles or charges on solubility is critical in drug design and delivery. Clearly, ionic compounds or, in the case of natural products, those containing ions will be more hydrophilic than lipophilic. Changing the amount of charge in a molecule will have a marked effect on its reactivity but also its bio-uptake. Many natural products are found as a quaternary ammonium salt, such as cocaine hydrochloride. When this drug is used as the hydrochloride, it cannot be smoked because of its high boiling point caused by

Table 1 Boiling points and water miscibility of some alcohols

Name	Formula	Boiling point, °C	Solubility in water, g(100 g)$^{-1}$ at 20 °C
Methanol	CH_3OH	65	Completely miscible
Ethanol	CH_3CH_2OH	78.5	Completely miscible
1-propanol	$CH_3CH_2CH_2OH$	97	Completely miscible
1-butanol	$CH_3CH_2CH_2CH_2OH$	117.7	7.9
1-pentanol	$CH_3CH_2CH_2CH_2CH_2OH$	137.9	2.7
1-octanol	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2OH$	155.8	0.3

Data from Hart, Craine and Hart (2003).

the ions; moreover, the rate at which it crosses the blood–brain barrier is relatively slow. By increasing the pH, the free base is liberated to make 'crack' cocaine. This has a far lower boiling point and can therefore be inhaled as a vapour, and the absence of ions makes the rate at which it crosses the blood–brain barrier higher than its hydrochloride analogue.

Carbonyl compounds also afford several natural products with which the reader may be familiar; Table 2 shows some common natural carbonyl compounds, their occurrence and any noteworthy features.

One of the most fascinating groups of natural products is the alkaloids (Latin: 'alkali-like'), so called because many of them contain a basic amine-nitrogen. The group contains many disparate compounds, united only by the presence of the basic nitrogen.

Table 2 A selection of naturally occurring aldehydes and ketones

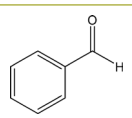
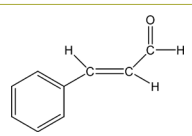
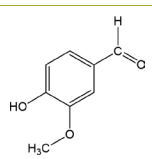
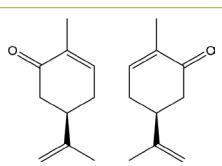
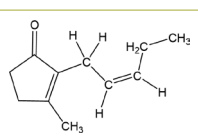
Name	Structure	Notes
Benzaldehyde		Benzaldehyde is responsible for the smell of almonds and therefore the smell of marzipan. The almondy smell of many fruit pips or stones is attributed to it; somewhat more sinisterly, that version of the almondy smell is caused by the breakdown of amygdalin into benzaldehyde and hydrogen cyanide (prussic acid), an assassins' tool.
Cinnamaldehyde		Cinnamaldehyde is what give cinnamon its characteristic smell. The double bond affords *E* and *Z* isomerism; natural cinnamon contains mostly the *E* (trans) isomer. It also has some commercial applications as a fungicide.
Vanillin		Vanillin is found in vanilla pods, which come from *Vanilla planifolia*, and is responsible for the characteristic ice-cream smell. Vanilla extract is vanillin along with several other compounds that highlight the vanilla flavour; it is also used in perfumery to mask any unwanted smells.
Carvone		*R*- and *S*-carvone are a pair of enantiomers. *R*-carvone (left) gives spearmint its characteristic smell; caraway, *S*-carvone, the other enantiomer, smells completely different and is used as a spice in various styles of cooking.
Jasmone		Jasmone is one of the active compound in jasmine flowers (*Jasminum officinale*). It is widely used in perfumes.

Table 3 A selection of alkaloids

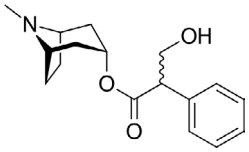
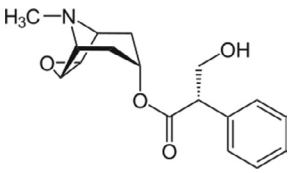
Name	Structure	Notes
Atropine		Named after Atropos, one of the Fates in Greek mythology as seen in Disney's *Hercules*. Found in deadly nightshade, *Atropa belladonna*, the 'deadly' referring to its exceptionally high toxicity and the *belladonna* referring to the fact that it dilates pupils, an apparently beauty-enhancing change. Atropine was once used by opticians to see a patient's retina.
Hyoscine (Scopolamine)		Along with atropine (above), hyoscine is one of the active ingredients in mandrake roots (of Harry Potter fame). These became prized by witches who were fascinated by the fact that prisoners who were executed by hanging often sported an erection and some even ejaculated. This has been attributed to an increased pressure in the cerebellum. The myth grew that, from the seed of a dying man, sprung the mandrake root, which can take a vaguely human appearance. Moreover, a bite of mandrake would give the biter a hallucinogenic trip, albeit sometimes fatally. Mandrake is toxic owing to the presence of atropine and scopolamine. In an effort to avoid mandrake-induced death, the witches experimented with various delivery methods. The best method they found was to rub it onto a stick upon which they would sit and the drug would cross the vaginal mucous membrane. As soon as the subject started to hallucinate but before they lost control of their faculties, they would drop the stick, having titrated an 'effective' dose but not overdosed. Hence the witches' broomstick (Figure 1).

Figure 1 An 18th century engraving of a witch being prepared for the Sabbat; the application of atropine can be seen in the background

Table 3 (continued) A selection of alkaloids

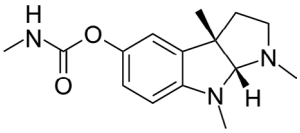
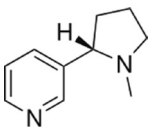
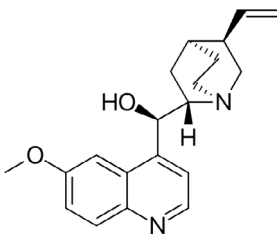
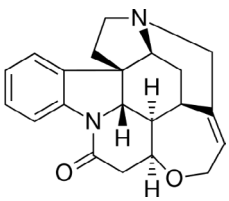
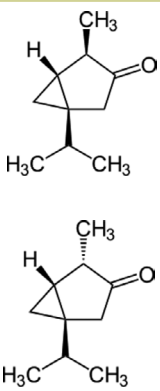
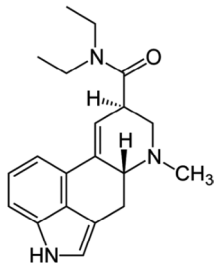
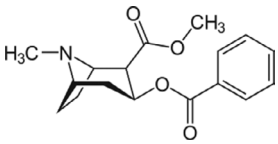
Name	Structure	Notes
Physostigmine		Physostigmine is another molecule that has associations with witchcraft. It is found in the Calabar bean, and is toxic. In the trial by ordeal of those accused of witchcraft, the suspect was made to eat several beans. If they vomited and survived, they were deemed innocent; if they did not, they were guilty.
Nicotine		Nicotine is found in the nightshade family of plants, and in trace amounts in tomatoes. Like many alkaloids it is a stimulant, which can partly explain its addictiveness. It is relatively toxic: if one were to take the entire nicotine content of a packet of twenty cigarettes in a single bolus, it would probably prove fatal.
Quinine		Quinine is the active component of tonic water. The term 'tonic' originally referred to something that had some medicinal quality and in the case of quinine it was a prophylactic against malaria. Its bitter taste was rendered more palatable by the addition of an alcoholic extract of juniper berries, which we now call gin. It is not the only example of a spirit having its origins in medicine: pastis (brands include Pernod and Ricard) had its origins in a treatment for worms.
Strychnine		Strychnine is another assassins' tool as it is toxic to most animals including humans. It acts as an inhibitor of acetylcholine esterase, which is the enzyme that breaks down acetylcholine after an action potential has crossed a nerve synapse. This means that all motor neurones are effectively telling their respective muscles to tense, leading to suffocation and the classical arched back (opisthotonus) of the victim.
Thujone		Thujone is found in the grand wormwood tree, *Artemisia absinthium*. Napoleon, the first modern general, who coined the phrase 'an army marches on its stomach', noticed that many troops together would unwittingly pass intestinal worms to each other, causing weight loss. He ordered them to be given the oily alcoholic extract liquid of the wormwood tree, the thujone content of which kills tapeworms. The bitter taste was mitigated by the addition of fennel, cinnamon and liquorice, and this mixture was later commercialised as Absinthe, and a later thujone-less version is still sold as Pernod Fils.

Table 3 (continued) A selection of alkaloids

Name	Structure	Notes
Lysergic acid diethylamide (LSD)		Lysergic acid diethylamide (LSD) was synthesised in 1938 by Albert Hoffman, a Sandoz employee. He extracted the parent acid from the ergot fungus and accidently ingested some: he immediately became aware of its effects, which include synaesthesia, the ability to taste colour and see sounds. Later, he deliberately took a dose on a day now called 'bicycle day' since he described the unusual cycle home. LSD is now sold as micro-dots on account of its high potency, and is outlawed in most countries.
Cocaine		Cocaine is one of a class of tropane alkaloids. It was originally isolated from the coca plant, *Erythroxylum coca*, by indigenous peoples of South America who found that it enabled them to work longer and have a curious imperviousness to cold when it was chewed. It was used as a dental anaesthetic and this property, quite separate from the narcotic effect, is caused by blockage of sodium channels in sensory nerves (Figure 2). Cocaine hydrochloride was the drug-of-abuse-of-choice in the 1980s. It was largely superseded by liberating the free base from the hydrochloride salt by the addition of a stronger base. The 'free base' is what we call crack cocaine and it has a much lower boiling point, enabling it to be smoked, and it is far more lipophilic, which enables faster crossing of the blood–brain barrier.

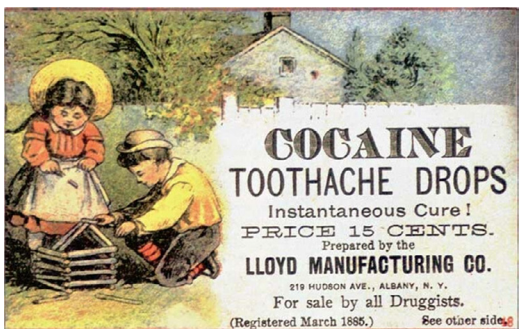
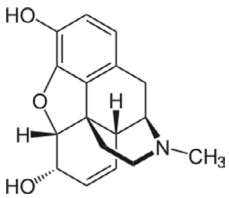
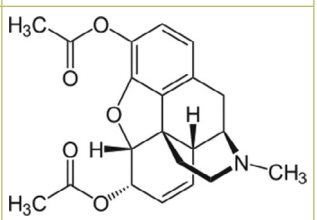
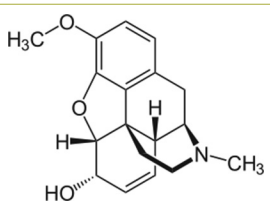
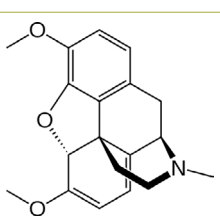
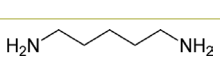


Figure 2 Lloyd Manufacturing advertisement for cocaine toothache drops

Table 3 (continued) A selection of alkaloids

Name	Structure	Notes
Morphine		Morphine, heroin, codeine and thebaine are treated in one entry in this table because they are so closely related and similar in structure. The opium poppy *Papaver somniferum*, when crushed, yields about 20 alkaloids, one of which is morphine (Greek: *Morpheus*, god of dreams). While the effects of morphine have been known for centuries, it was first isolated and analysed in 1804 by Friedrich Sertürner, who later made an analgesic elixir he called Laudanum (Latin: *laudere*, to praise). It was used as an anaesthetic in the American Civil War and the Crimean War before its addictive properties were fully realised. In 1898, Bayer was researching methods by which morphine's analgesic properties might be enhanced, and focused on its delivery. By replacing the two –OH groups with acetyl groups, the lipophilicity was increased, thus allowing the drug to cross the blood–brain barrier faster. Diacetylmorphine (diamorphine) was to be named heroin (German: *heroisch*, heroic). Hoffman (of LSD fame) played a part in this discovery, if by accident: he was trying to synthesise codeine by boiling morphine with a methylating agent, in an effort to find a medicine that acted as an antitussive agent (cured coughs) without the addictive nature associated with morphine (Figure 3). Heroin was sold as an over-the-counter cough medicine until about 1925. Thebaine has two methoxy groups in the place of the terminal –OH groups in morphine. This inhibits binding to the opioid receptors and so does not have the analgesic effects of morphine or heroin. It can be converted into naloxone, which is used as an opioid antagonist since it does bind to the receptors, thus blocking any opioid taken at the same time.
Heroin		
Codeine		
Thebaine		
Cadaverine		Cadaverine and putrescine are described together since they occur simultaneously as flesh putrefies, and they differ only by a CH_2– group. As the amino acids ornithine and lysine in proteins break down, they decarboxylate, leaving simple carbon chains with amine groups on them. The smell of fish is similarly an amine (trimethylamine); fresh fish doesn't smell of anything at all.
Putrescine		

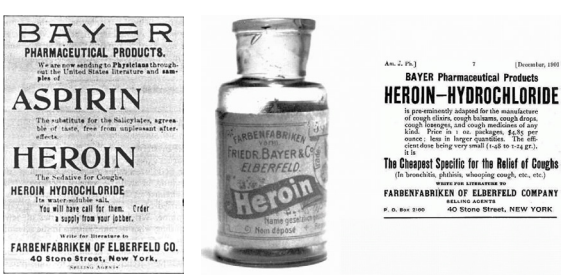


Figure 3 Farbenfabriken advertisements for heroin cough medicine

Table 3 shows some common alkaloids, their occurrence and any noteworthy features.

As an introduction to extraction and characterisation of natural products, there follow two experiments suitable for A-level chemistry clubs or similar that not only introduce the natural products but involve a wide variety of experimental procedures such as steam distillation, thin-layer chromatography (TLC), determination of melting point and recrystallisation.

Experiment 1: Isolation and analysis of caffeine from tea

Objectives

1 To isolate and recrystallise caffeine from tea.
2 To analyse the crude and purified extracts by melting point determination, UV and IR spectroscopy.

Introduction

The methyl derivatives of xanthine form an important group of pharmaceutically relevant natural products. There are three common methyl xanthines: caffeine (trimethylxanthine), theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine) (Figure 4). Of these, caffeine is the most significant, being the stimulant in drinks such as tea and coffee and the active ingredient in products such as Pro Plus®.

Plants containing methylxanthines

Tea consists of the prepared leaves of *Thea sinensis* L. (*Camellia thea*), a shrub cultivated in East Africa, India, Sri Lanka, China and Japan. It contains 1–5% caffeine and small quantities of theobromine and theophylline.

Coffee consists of the seeds of *Coffea arabica* and other *Coffea* species that are prepared by the removal of the seed coat followed by roasting. Prepared coffee contains 1–2% caffeine.

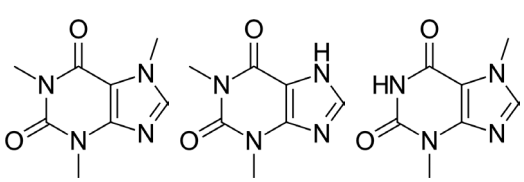


Figure 4 Three common methyl derivatives of xanthine: (left) caffeine; (middle) theophylline; (right) theobromine

Mate tea consists of the dried and cured leaves of *Ilex paraguariensis* and other *Ilex* species indigenous to South America. It contains 0.2–2% caffeine.

Guarana is the dried paste prepared from the seeds of *Paullinia cupana*, which is found in the upper Amazon. The paste is dried and powdered before mixing with water to make a drink. It contains 2.5–5% caffeine.

Kola nuts are the dried cotyledons of the seeds of various species of *Cola*, especially *Cola nitida*. The trees are grown in West Africa, the West Indies, Brazil and Java. Kola seeds contain 1–2.5% caffeine and also some theobromine.

Cocoa seed are obtained from the tree *Theobroma cacao*. Cocoa is produced in South and Central America, the West Indies, West Africa, Ceylon and Java. The kernels are removed after processing and are used in the production of chocolate. The kernels and husks both contain theobromine (0.9–3%) and a lesser amount of caffeine. Theobromine is obtained commercially from the husks. Oil of theobroma is used pharmaceutically as a suppository base.

Experimental procedure

This experiment involves the isolation of caffeine from tea leaves by extraction with methanol in a Soxhlet extractor, followed by partitioning between water and chloroform with recrystallisation of the product from ethanol.

Health and safety precautions

- Laboratory personal protective equipment (PPE) is to be worn at all times.
- Methanol is a toxic and highly flammable solvent and should not be handled near an open flame under any circumstances. Care *must* be taken to ensure that no liquids are spilt into the heating mantle used in this experiment.
- Chloroform is a toxic solvent. Containers holding this chemical and any solutions in chloroform should be stoppered wherever possible. Avoid inhaling the vapours. Report any spillage immediately to an appropriate member of staff.

Extraction and purification of caffeine

1 Weigh and then place ten tea bags in the Soxhlet extractor (Figure 5). (Cut open a tea bag and remove the tea. Weigh the bag. Take into account the weight of the bag multiplied by the number of bags used in your original

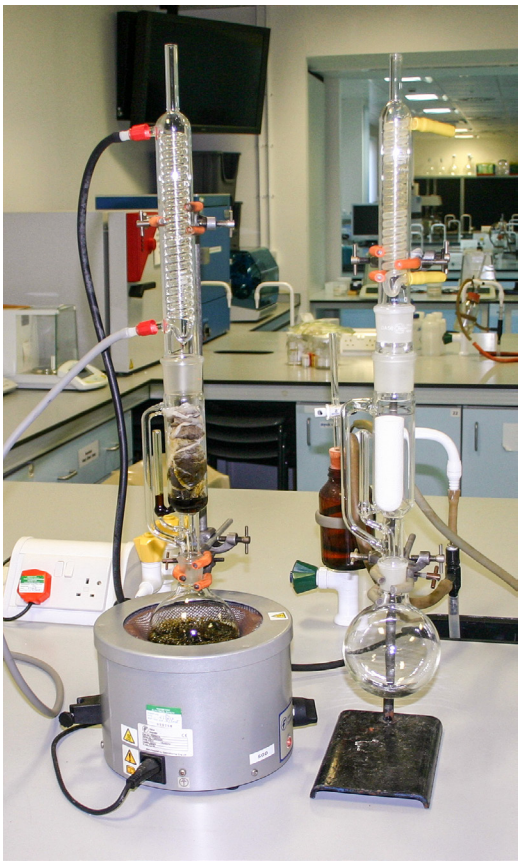


Figure 5 Soxhlet extraction apparatus: (left) extraction of caffeine from teabags; (right) the use of a porous thimble within the Soxhlet apparatus to extract caffeine from loose tea

sample and subtract it from the weight of the bagged tea.) Place 200 cm^3 of methanol in a round-bottomed flask with some anti-bumping granules and connect the Soxhlet extractor to the flask. Fill the extraction chamber of the Soxhlet with methanol and attach the reflux condenser. Bring the methanol in the system to the boil and allow the extraction to proceed for 45 minutes.

2 Allow the system to cool. Remove the methanol in a rotary evaporator.

3 Add approximately 10 cm^3 of the filter aid Celite® to the flask and suspend the dried residue in 100 cm^3 of boiling water. Add 20 cm^3 of 10% sulfuric acid. Allow the mixture to stand for 5 minutes and filter under vacuum. Repeat with a further 100 cm^3 of boiling water.

4 Add approximately 10 cm^3 of Celite® to the filtrate and filter again under vacuum. Wash the filter with 50 cm^3 of boiling water.

5 Allow the aqueous extract to cool and then extract with five portions of chloroform (10 cm^3). Combine the chloroform extracts and wash them with 1% sodium hydroxide (10 cm^3) and then with water (10 cm^3).

6 Place the chloroform extract in a tared round-bottomed flask and remove the solvent on a rotary evaporator.

7 Recrystallise the residue using the minimum volume of boiling ethanol on a hot water bath, and then filter into a dry 50 cm^3 beaker to crystallise.

8 Recover the product by vacuum filtration and dry it in an oven (100 °C) for 1 hour.

9 Weigh the product and then bottle and label it.

Characterisation of caffeine

Melting point

Carry out a melting point determination on your product.

Infrared spectroscopy

Collect an infrared (IR) spectrum for your product. The spectrum you collect should be annotated with assignments for the absorbances observed.

Ultraviolet spectrophotometry

Collect an ultraviolet (UV) spectrum for your product using an appropriate solvent and appropriate cuvettes. The spectrum you collect should be annotated to identify the λ_{max} and indicate the functional group(s) likely to absorb light at this wavelength.

Results

Extraction and purification of caffeine

Mass of tea taken (g):	
Mass of crude extract (g):	
Mass of purified caffeine (g):	
Determined % of caffeine in starting material (%w/w):	

Characterisation of caffeine

Melting range for product (°C):	From:	
	To:	
IR spectroscopy	The spectrum of your compound should be annotated and attached to this report.	
UV spectrophotometry	The spectrum of your compound should be annotated and attached to this report.	

Questions

1 Indicate what you might expect to see in the 1H-NMR spectra of the three methylxanthines, and how you might differentiate them from each other.
2 In step 3 of the method, sulfuric acid is added to the aqueous solution/suspension. Explain why this is done, given that many plant pigments are weak acids.
3 Given that the absorbance value (1%, 1 cm) for caffeine in water is 504 at 273 nm, show your calculations for producing a solution that would give an absorbance value of between 0.75 and 1.0 at 273 nm. Report your answer as mg per 100 cm^3.

Experiment 2: Extraction and characterisation of a volatile oil, eugenol, from cloves

Objectives

1 To isolate the volatile oils from a sample of cloves.
2 To analyse a sample of the volatile oil using TLC and IR spectroscopy.

Introduction

Volatile oils, also called essential oils, are volatile in steam. They differ entirely in both chemical and physical properties from fixed oils. Volatile oils are used pharmaceutically as flavouring agents, carminatives and counter-irritants. A number of them, including eugenol (Figure 6), have been reported to possess antibacterial properties. Many plants containing volatile oils are used in folk medicines and alternative therapies.

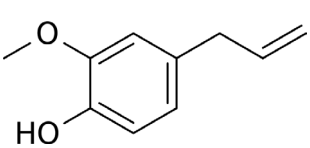


Figure 6 Eugenol

Eugenol is the primary constituent of clove oil. Cloves are the dried flower buds of *Syzygium aromaticum* (*Eugenia caryophyllus*), a tree indigenous to the Moluccas (also known as the Spice Islands or Clove Islands). It was traditionally cultivated in Zanzibar and on neighbouring islands but is now produced principally in Madagascar, Brazil and Penang.

Oil of cloves is a colourless or pale yellow oil that is slightly heavier than water (relative density 1.047–1.060).

Health and safety precautions

- Laboratory PPE is to be worn at all times.
- When viewing plates under UV light, UV safety glasses must be worn.
- Ethyl acetate and petroleum spirit are toxic and highly flammable solvents and should not be handled near an open flame under any circumstances. Bunsen burners or other sources of ignition must under no account be used anywhere near the laboratory bench during this practical.
- Dichloromethane is a toxic solvent. Containers holding this chemical and any solutions in dichloromethane should be stoppered wherever possible. Avoid inhaling the vapours. Report any spillage immediately to an appropriate member of staff.
- Care *must* be taken to ensure that no liquids are spilt into the heating mantle used in this experiment.
- Under no circumstances must the silica gel plate used in the TLC analysis be removed from the laboratory – dust from the plate is easily dispersed and is harmful if inhaled.

Extraction of eugenol

1 Weigh 25 g of cloves, grind them finely in the apparatus provided and place in a 1000 cm^3 round-bottomed flask. Half fill the flask with hot deionised water and attach to the volatile oil distillation apparatus.
2 Add sufficient deionised water via the side arm to fill the separatory section of the apparatus.
3 Using the heating mantle, allow the contents of the round-bottomed flask to boil and the oil/water mixture to distil over into the side arm.
4 After 30 minutes of distillation, run out the oil/water mixture via the tap at the bottom of the side arm into the specialised measuring cylinder.

5 Allow the oil obtained to settle and measure the volume recovered.
6 Refill the side arm with deionised water and continue the distillation until the volume of oil in the side arm is constant. This may take as long as 3 or 4 hours.
7 Allow the apparatus to cool, run off the oil/water mixture as before and measure the volume of oil recovered.
8 Pour the total contents of the measuring cylinder into a 100 cm^3 separating funnel. Add 25 cm^3 of petroleum spirit (boiling point 60–80 °C) and shake carefully. Allow the two phases to separate and remove the aqueous layer (keep the aqueous layer for chemical tests). Place 1 cm^3 of the petroleum spirit aside for TLC analysis. Label as sample A.
9 Add 50 cm^3 of a 1% w/v solution of sodium hydroxide in 50% aqueous methanol (50 cm^3) to the separating funnel. Shake carefully and allow the phases to separate.
10 Remove the aqueous layer (lower) and place in a second 100 cm^3 separating funnel.
11 Extract the petroleum spirit layer with a further 20 cm^3 portion of the sodium hydroxide solution, allow to separate and add to the first hydroxide fraction.
12 Place the petroleum spirit fraction into a round-bottomed flask, evaporate to dryness using the rotary evaporator and use for TLC analysis. Label as sample B.
13 Neutralise the combined hydroxide extracts in the second separating funnel using 10% hydrochloric acid solution until the pH is below 8 (test against litmus paper).
14 Extract the neutralised aqueous solution with three 20 cm^3 portions of dichloromethane. Combine the organic layers (bottom) in a third separating funnel.
15 Wash the combined organic phase with 20 cm^3 of saturated brine solution, separate the dichloromethane layer and dry using a drying agent as detailed below. Filter the dried dichloromethane solution into a 100 cm^3 round-bottomed flask.
16 Remove the dichloromethane on a rotary evaporator and place the remaining sample into a suitable container for assessment, TLC and IR spectroscopy. Label as sample C and include the name of the product, weight of the product and date.

Drying organic solvents

Many anhydrous inorganic salts react with water to form hydrated salts and are useful as drying agents for the removal of water from organic solvents. For example:

$$Na_2SO_4 + 7H_2O \rightarrow Na_2SO_4 \cdot 7H_2O$$

$$MgSO_4 + 7H_2O \rightarrow MgSO_4 \cdot 7H_2O$$

The various drying agents differ in their rate of removal and capacity to remove water from organic solutions. The two most commonly used agents are anhydrous sodium sulfate and anhydrous magnesium sulfate.

17 Add a volume of drying agent (approximately 5% of the volume of solvent to be dried) to the organic solvent in a conical flask.
18 Gently swirl the mixture for 10–15 minutes. The drying agent will tend to become sticky as it reacts with the water. If all the drying agents appears gummy, add an additional 5% by volume.
19 When drying is complete, remove the drying agent by filtration. It may be necessary to wash the drying agent with small portions of the organic solvent to remove any solutes retained in the wet mass.
20 Remove the solvent with a rotary evaporator (also called a rotary film evaporator).

Characterisation of eugenol

Thin-layer chromatography

You should have at this point three samples (A, B and C) plus a reference standard to apply to a silica gel G TLC plate. Ensure that a suitable dilution in ethyl acetate or petroleum spirit of each sample is used for the TLC analysis. The developing solvent is hexane : ethyl acetate 85 : 15.

Visualisation is by viewing the plates first under UV light and then by spraying with 50% sulfuric acid followed by heating at 110 °C for 5 minutes. (NB. Make sure that you spray into the spraying cabinet in the fume cupboard.)

Sketch/copy your developed plate on a separate sheet of paper. Indicate the spots developed (and any colours). Also indicate which spots, if any, showed up under UV light. Attach the sketch to your practical report.

Ensure that the solvent from the TLC tank is placed in the appropriate waste disposal bottle at the end of the practical.

IR spectroscopy

Collect an IR spectrum for your product. The spectrum you collect should be annotated with assignments for the absorbances observed.

Chemical tests

Apply a chemical test suitable for the detection of one or more functional groups present in eugenol to samples taken at each stage in the extraction and purification process. Restrict the sample amount to a minimum so as not to reduce your final yield unnecessarily.

Results

Extraction and isolation of eugenol

Yields at various stages in the purification	
Yield of crude oil after 30 minutes of distillation (cm^3):	
Yield of crude oil after completion of distillation (cm^3)	
Total yield of crude oil (cm^3):	
Assuming a density of 1.05, calculate the weight of oil obtained (g):	
Yield of crude oil as a % of starting material (% w/w):	

Characterisation of eugenol

TLC of fractions of clove oil	Sketch/copy your developed plate in the space provided. Indicate the spots developed with colours. Also indicate which spots, if any, showed up under UV light.
IR spectroscopy	The spectrum of your compound should be annotated and attached to this report.

Question

Using chemical structures and with reference to your TLC and chemical tests results, outline the relevant stages in the purification of eugenol, indicating points at which impurities are removed. You should consider issues of pH, pK_a and polarity of molecules that will affect their partitioning and solubility in the various solvent systems.

Reference

Hart, H., Craine, L. E. and Hart, D. (2003) *Organic Chemistry*. 11th edn. New York: Houghton Mifflin.

Michael Hal Sosabowski is Professor of Public Understanding of Science in the School of Pharmacy and Biomolecular Sciences at the University of Brighton. Email: M.H.Sosabowski@brighton.ac.uk
George W. J. Olivier is a principal lecturer in the School of Pharmacy and Biomolecular Sciences at the University of Brighton.
Hala Jawad is a qualified pharmacist who runs her own company that deals with continuing professional development for pharmacists.
Seija Maata is a technician specialising in chemistry and pharmaceutical chemistry in the School of Pharmacy and Biomolecular Sciences at the University of Brighton.