Synthesis, characterisation and application of novel quinones for the detection of latent fingermarks

Joyce Chan

A thesis submitted for the Degree of Doctor of Philosophy (Science)

University of Technology Sydney (UTS)

February 2019

Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This research is supported by the Australian Government Research Training Program.

Signature of Student:

Joyce CHAN

Date: 01/02/2019

Acknowledgments

First and foremost, I would like to acknowledge my supervisors, Dr Barbara Stuart and Dr Ronald Shimmon. Barbara, thank you so much for your advice, and feedback. Ronald, thank you so much for all your help in the lab, your enthusiasm for chemistry is an inspiration. Barbara and Ron, you are truly the best supervisors anyone could ask for and I will be forever indebted to you.

Professor Claude Roux and Professor Chris Lennard your input and encouragement as co-supervisors have been greatly appreciated. Dr Xanthe Spindler, I'm in awe by the number of journal article references you keep catalogued in your head! Dr Linda Xiao, I am grateful your assistance and training with the instruments that were used in this project. Dr Alison Ung, your help in the lab has been so invaluable and thank you for listening to me and providing me with positive comments.

I must acknowledge my parents who have supported me throughout my life and especially during all the years at uni. Thank you for all the sacrifices that you have made so that I could be where I am today. Sheng, your work ethic is incredible and your determination in achieving your goals is inspirational.

All the friends that I have made throughout this PhD have made it more enjoyable than it should be. Amanda, you are the greatest friend anyone could ask for, I am so grateful that uni has brought us together. Now that you are living overseas, we will just have to travel further in search of delicious food! Adrian, the sibling I wish I had; your encouragement and the way you tackled your own PhD was especially motivating as I became a part-time student.

Natasha and Susan, you have both been incredible friends throughout the years. Thank you both for helping me with the LC work, it was really invaluable. Scotty and Mike, you two were a great team especially for Forensics at UTS. Anna, you were way too calm throughout your PhD and you have definitely been a good influence on Dave. Brian, thank you so much for reading my synthesis chapter - it definitely taught me a lot and I am so grateful for your feedback! Emily, I am so glad I met you. You're too funny and the most avid reader!

Thank you again to everyone who has helped me throughout the project from my family, supervisors, friends from before uni to those I made throughout.

Conference presentations

- 'Potential for the use of anthraquinones for the detection of fingermarks on porous surfaces.' NSW Police Fingerprint Conference, Expert Conference, 2009
- 'The use of anthraquinones for the detection of fingermarks on porous surfaces' International Fingerprint Research Group, Switzerland, 2009.
- 'Synthesis of anthraquinones and their use for the detection of fingermarks on porous surfaces.' 5th European Academy of Forensic Science, Glasgow, 2009. (poster)
- 'Preliminary results: Synthesis of anthraquinones and their use for the detection of fingermarks on porous surfaces.' 20th International Symposium on the Forensic Sciences, Sydney, 2010. (poster)
- 'Synthesis of anthraquinones and their application as fingermark detection reagents on porous surfaces.' 6th European Academy of Forensic Science, Hague, 2012. (poster)
- 'Synthesis of anthraquinones and their application as fingermark detection reagents on porous surfaces.' 21th International Symposium on the Forensic Sciences, Hobart, 2012. (poster)

Table of Contents

List of figures

List of Tables

Abbreviations

AFP	Australian Federal P01/0olice
Ag_2CO_3	Silver carbonate
AQ	1,4-Anthraquinone
AQS	1,2-Anthraquinone-4-sulfonic acid, ammonium salt
AR	Analytical reagent
$CDCl_3$	Deuterated chloroform
CID	Collision induced dissociation
COSY	Correlation spectroscopy
CTAB	Cetrimonium bromide
DFO	1,8-Diazafluoren-9-one
EFP-WG	European Fingerprint Working Group
ESI	Electrospray ionisation
FTIR	Fourier transform infrared
GC-MS	Gas chromatography-mass spectrometry
HAQ	2-Hydroxy-1,4-anthraquinone
HCl	Hydrochloric acid
HOSDB	Home Office Scientific Development Branch
HPLC	High-Performance liquid chromatography
Hr	Hour(s)
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IAI	International Association for Identification
IFRG	International Fingerprint Research Group
JP	Joullié's pink
KBr	Potassium bromide
LC-MS	Liquid chromatography-mass spectrometry
m/z	Mass-to-charge
MAQ	2-Methoxy-1,4-anthraquinone
MMD	Multi-metal deposition

mmol	Millimole
mol	mole
mp	Melting point
MS	Mass spectrometry
Na_2SO_4	Sodium sulfate
NMR	Nuclear magnetic resonance
NQS	1,2-Naphthoquinone-4-sulfonic acid, sodium salt
PD	Physical developer
LC-QTOF-MS	Liquid chromatography-quadrupole time-of-flight-mass-spectrometer/spectrometry
RP	Ruhemann's purple
s	Second(s)
SDS	Sodium dodecyl sulfate
SPR	Small particle reagent
TIC	Total ion chromatogram
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UK	United Kingdom
UTS	University of Technology Sydney
v/v	Volume/volume
VMD	Vacuum metal deposition
VSC	Video spectral comparator
w/v	Weight/volume

Abstract

Identification of an individual through fingermarkss is one of the oldest types of evidence in forensic science. A number of techniques are available for the detection of latent fingermarks on porous surfaces; for example 1,2-indanedione zinc (IND-Zn), 1,8-diazafluoren-9-one (DFO), and ninhydrin. While these techniques produce excellent results, each has their drawbacks. For example, ninhydrin requires secondary post-treatment and cooling with liquid nitrogen to produce fluorescent fingermarks. DFO developed fingermarks are difficult to detect on coloured or highly patterned surfaces. IND-Zn produces a highly fluorescent fingermark, however, under white light little contrast exists between the fingermark and the substrate. Therefore, there is need for research into the development of new fingermark reagents.

Quinones have been used for the development of amino acids in chromatography and biochemistry. Recent research into the use of 2-hydroxy-1,4-naphthoquinone (lawsone) has shown promising results for the development of latent fingermarks on porous surfaces. One of the aims of this thesis was to determine the reaction products between lawsone and three amino acids. These products were elucidated using Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and quadrupole time-of-flight liquid chromatography-mass spectrometry (LC-QTOF-MS). The proposed product is hypothesised to be similar between benzoquinones, naphthoquinones and anthraquinones as they differ only by π conjugation.

Another aim of this thesis was to synthesise a variety of quinones with differences in conjugation and substitution in order to compare and determine differences in quantum yield and whether these effects would influence their ability to develop latent fingermarks on porous surfaces. A number of quinones were successfully synthesised and characterised using FTIR and NMR spectroscopy and LC-MS.

In this preliminary study, the synthesised quinones and lawsone were then evaluated as potential reagents for the development of latent fingermarks on porous surfaces. The development conditions, reagent concentration, solvent system, pH, and metal

salts enhancements of each quinone were optimised using amino acids and fingermarks on different porous surfaces. All the quinones that were tested in this thesis did not produce coloured fingermarks and only developed faintly coloured amino acid test strips.

Slight improvements in luminescence were observed when comparing the results of the amino acids and fingermarks developed by naphthoquinones and anthraquinones. This is this is likely due to steric hindrance preventing anthraquinones from forming the desired products. Comparisons were also made between the fingermarks developed by the synthesised compounds and IND-Zn, with IND-Zn developed fingermarks being far superior in luminescence.

Chapter 1: Introduction

1.1 Fingerprints

1.1.1 Introduction

Fingerprint as a means of identification holds major significance in forensic science. It has been used to identify individuals since the 1800s and continuously in the judiciary system as a reliable source of evidence. The uniqueness of fingerprints stems from the immutability of the friction ridges on human skin. These friction ridges consist of ridges of skin with valleys in between each ridge. Each ridge unit, ranging from 100 to 300 µm in size, contains a sweat gland and a pore opening (1; 2). Ridges form patterns that are dependent on an individuals' skin morphology, such as the height and asymmetry and the buckling process in the layers of skin (3).The main purpose of these ridges is to increase the friction between the skin and another surface. These ridges are formed *in utero* appearing on the fingertips during the sixth or seventh week gestation (1; 3). The resulting patterns, devised by Sir Francis Galton (1892) and later refined by Sir Edward Henry (1900), can be classified as an arch, tent, loop or whorl (Figure 1-1) (2).

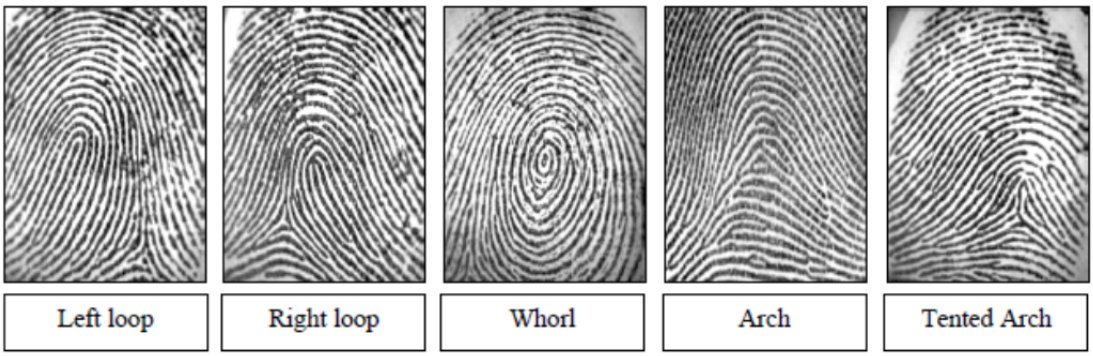


Figure 1-1 Fingerprint patterns in the Galton-Henry classification scheme (2)

The ridges are discontinuous, non-uniform and can end, branch, turn or combine depending on the underlying skin surface (1). These features are known as *minutiae* and are unique. There are seven basic types of minutiae (see Figure 1-2); however, the number of basic minutiae may vary depending on the fingerprint expert. For example, the American National Standards Institute recognise four classes of minutiae – ridge ending, bifurcations, compound and undetermined (2; 4).

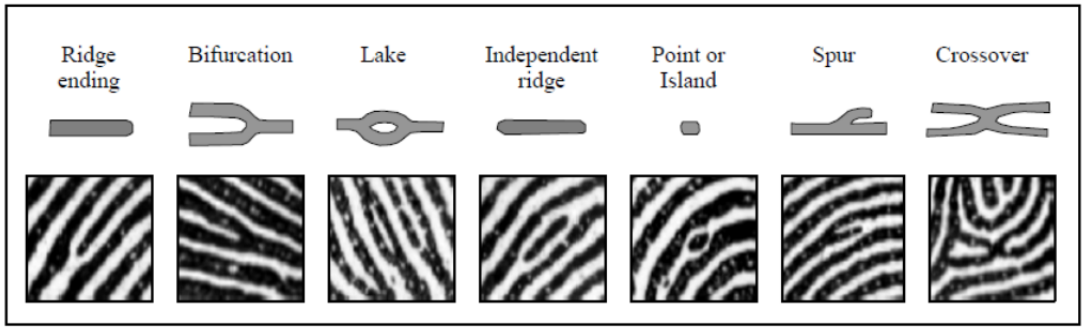


Figure 1-2 Common minutiae types (2)

The combination of these characteristics as well as random pore locations on the finger enhances the uniqueness of the friction ridge skin (1; 5). Furthermore, its immutability allows for identification. The friction ridge pattern from the finger deposited on a surface is called a *fingerprint*. The impression made on the surface is a negative image, that is, although the minutiae has the same coordinates on the finger, ridge endings would appear as bifurcations (2).

The terms *fingerprint* and *fingermark* are frequently used interchangeably. It should be noted that these are in fact different. The term *fingerprint* is used when a comparison print is taken for identification purposes (the known print), whereas, a *fingermark* describes the print that is left on a surface (the unknown print) (6).

1.1.2 History of fingermark identification

The use of friction ridge skin has been utilised for the identification of individuals. For example, archaeologists have speculated on the impressions that have been found in pottery and clay slabs as means of protecting against forgeries or a means to recognise ones work. In Babylon (around 3000 B.C.), finger impressions were used in clay tablets to create legally binding contracts (7), bricks had impressions of the moulder's finger (8) and references allude to the collection of fingerprints from criminals (7; 9). In ancient China, from as early as first century B.C., the fingerprint impression was commonly used to seal official documents (8). These examples indicate the possibility and/or use of fingerprints for the purpose of identification. Despite the early uses of

fingerprints, a scientific methodology regarding its classification and use in the justice system was not evident until 1892 with the publication of *Finger Prints* by Sir Francis Galton (9). Galton referenced the work of Johannes Purkinje who observed the four basic patterns of a fingerprint. Galton also attempted to address the issue of uniqueness; he calculated the probability of two people having an identical fingermark as 1 in 64 billion. No mention was made, however, to the work that was published in the journal *Nature* entitled *On the Skin Furrows of the Hand* by Dr Henry Faulds (1880) (1) or Sir William Herschel's response to the article (9). The significant findings of these men are briefly discussed in this section.

Dr Henry Faulds, a Scottish physician, was working as a missionary when he noted that fingerprint ridges are immutable and the pattern of formed vary between individuals. Faulds remarked on the use of fingerprints to include / exclude suspects in a crime in his publication in *Nature*. This publication is the first of its kind in documenting the use of fingerprints in forensic science (1; 7).

Sir William Herschel, a senior administrative officer in India, responded to Faulds letter in *Nature*. In the letter he noted that he had been documenting fingerprints over a period of 20 years and these observations suggested their permanence (10). In contrast to Faulds, Herschel did not observe ethnic and hereditary correlation in fingerprints. He claimed to be the pioneer in the use of fingerprints as a means of identification stemming from his use of fingerprints in keeping track of wages and to identify prisoners (7; 10). As Berry and Stoney mentioned, Herschel did not associate the use of fingerprint identification for crime scene analysis (7), but rather the importance of friction ridges as a means of identification.

The implementation of fingermarks as a tool in forensic science was only made possible through the system of classification that was influenced by Galton, Alphonse Bertillon, Juan Vucetich and Edward Henry. This system of classification eventually led to the use of fingermark identification worldwide and a cornerstone for forensic science. The use of fingermark evidence in court as a means of identification is based on three main principles, these are (11):

First principle: Fingerprints are unique.

Second principle: Fingerprints are immutable.

Third principle: Classification of fingerprint ridge patterns is possible due to their minutiae.

1.1.3 Identification using fingermarks

The permanence of the fingerprint over an individual's lifetime, as well as its uniqueness, is the fundamental principle for their use in identification. Due to various reasons as discussed in Section 1.1.1, the friction ridge skin is unique. The term 'uniqueness' has been subject to debate and it is often defended with probabilistic estimations to quantify the term. For example, the analogy of the variability in snowflakes where it was hypothesised that due to the large number of snowflakes, finding one that is identical to another would be "virtually impossible" (12). This analogy has been compared with the probability of finding two identical fingerprints (13). A more systematic study to validate fingermark evidence involved the examination of 50 000 left loops. This was conducted by the United States government in preparation for *United States vs. Mitchell*, however, as commented by Kaye, the study is not without its flaws (12). This study consisted of two experiments, the first experiment involved the comparison of 50 000 fingerprint images against itself using the Automated Fingerprint Identification System (AFIS) algorithms and then to the remaining 49 999 images in the database. It was found that the probability of finding two identical fingerprints is less than $1x10^{-97}$. The second experiment was conducted using the same method, except the latent prints used for the comparison were cropped images of the original. From the second experiment, it was concluded that the probability of a false match ranged from $1x10^{-27}$ (for 4 minutiae) to $1x10^{-97}$ (for 18 minutiae) (14). Despite the weaknesses in experimental design, the *Mitchell* case has set precedence to the uniqueness of fingerprints.

Legal challenges, for instance, *Daubert vs. Merrell Dow Pharmaceuticals (1993)* and *United States vs. Llera Plaza (2002)*, have emphasised the need to ascertain the reliability and validity of scientific evidence and techniques used. These cases have also questioned the admissibility of the expert testimony, in particular, fingerprint evidence (13; 15; 16).

The current methodology used by fingerprint examiners when comparing a fingerprint and a fingermark visually is through Analysis, Comparison, Evaluation and Verification (ACE-V) (6). During the analysis stage of the ACE-V protocol, fingerprint examiners assess the quality and quantity of minutiae within the fingermark. Therefore, it is of utmost importance that there is clarity of the fingermark which may be the result of the detection and enhancement process. Clarity can be improved by maximising contrast and increasing sensitivity of a fingerprint reagent towards the targeted components present in fingerprint secretions. The ACE-V methodology has come under scrutiny after the *Mayfield* case with regards to contextual and confirmation bias of the fingerprint examiners. An understanding of these issues would minimise inconsistencies between experts and improve the reliability of the comparison process as well as strengthen forensic evidence as in judicial proceedings (17; 18).

The comparison stage can be divided into three levels of detail, they are as follows:

> **First Level Detail:** describes the general pattern on fingerprints. It is considered to be a class characteristic; hence, identification cannot be drawn (6).
>
> **Second Level Detail:** describes the minutiae or other points of identification. Second level details are individual characteristics (6).
>
> **Third Level Detail:** describes the ridge shapes and location of pores. Third level details are individual characteristics (6).

A quantitative approach known as the 'numerical standard' or the 'empirical' approach has been used by fingerprint examiners when establishing identification. It relies on a minimum number of minutiae that must be present for a positive identification. Most countries use the numerical standard, however, the threshold differs between

countries (19). There is another approach that is based on a non-numerical standard. This approach takes into consideration of factors such as the number of concurring points, rarity of the general pattern, type and frequency of the minutiae and the presence of ridge edges (19).

In 2009 the National Academy of Sciences published a paper that acknowledged the techniques used in forensic science lacks scientific basis. As a result, forensic institutions such as the International Association for Identification (IAI) and the European Fingerprint Working Group (EFP-WG) have supported the development of statistical models based on research to describe the value of evidence (20). In 2011 the IAI stated that "*there currently exists no scientific basis for requiring a minimum amount of corresponding friction ridge detail information between two impressions to arrive at an opinion of single source attribution"* (19).

Although fingermark evidence has passed the '*Daubert* test', various experts have aimed to provide a statistical model (e.g. likelihood ratio) to describe minutiae variability as further affirmation of uniqueness. These models aim to strengthen the fingermark identification process. The statistical analysis / model is beyond the scope of this thesis, however, reference material can be found in Stoney and Thornton (21), Neumann *et al.* (22-24), Egli *et al.* (25) and Abraham *et al.* (26). It should be noted that the use of the likelihood ratio is currently advocated as an objective way to evaluate the weight of fingermark evidence. In order to assist in the likelihood ratio calculation, the frequency of different types of minutiae between populations has been brought to light. Some population studies include South American (Argentinean and Spanish samples), North American (European and African samples), and Eastern European (20; 27; 28).

1.1.4 Composition of fingermarks

An understanding of the composition of fingermark secretion as well as their interactions with the substrate on which they are deposited is crucial in the detection and enhancement process. A fingermark is composed of a mixture of natural

secretions and environmental contaminants (6). The human fingerprint consists of various ridges interspersed with pores that act as outlets for the secretions on the skin. The secretions are made by three types of glands (6):

- apocrine glands,
- sebaceous glands, and
- eccrine glands.

A summary of the constituents and the location of these three glands are provided in Table 1-1.

Table 1-1 Fingermark constituents, adapted from Champod *et al*. and Ramotowski (6; 29)

	Location	Constituents	Solubility
Apocrine	Axillary and anogential areas	Iron Water Proteins Carbohydrates Sterols Vitamins	Water soluble
Sebaceous	All over the body with the exception of palms and soles	Fatty acids Alcohols Glycerides Sterols and esters	Mainly non-water soluble
Eccrine	All over the body, most abundant on the forehead, palms, and soles of the feet	Chlorides Metal ions (Na^+, K^+, Ca^{2+}) Ammonia Water Amino acids Sugars Urea Creatinine Uric acid	Water-soluble

Although sebaceous glands are not located on the fingerprint ridges their secretions are a major component of fingermarks. Their presence is likely the result of transfer from with the sebum producing parts of the body (6; 30). Eccrine secretions offer the greatest potential for chemical enhancement due to the abundance of eccrine glands

throughout the friction ridge as well as its interactions with the substrate. The concentration of amino acids in eccrine secretions is listed in Table 1-2.

Table 1-2 Concentration of amino acids as percentage of serine in eccrine secretions, adapted from Ramotowski (29)

Amino acid	Mass (µmol)	Serine Ratio (Hamilton) (31; 32)	Serine Ratio (Hardon *et al.*) (33)	Serine Ratio (Oro and Skewes) (34)
Serine	0.106	100	100	100
Glycine	0.071	64	54	59
Ornithine	0.034	32	45	59
Alanine	0.029	27	35	28
Aspartic acid	0.023	22	11	22
Threonine	0.018	17	9	18
Histidine	0.018	17	13	14
Valine	0.013	12	10	9
Proline	0.011	10	-	-
Leucine	0.011	10	7	10
Lysine	0.011	10	5	-
Tyrosine	-	6	3	5
Phenylalanine	-	7	5	5

1.1.5 Types of fingermark evidence

Fingermark evidence can be classified as either *visible* or *latent*. Visible fingermarks can be visualised without any treatment if there is enough contrast, however, it may be necessary to use optical illumination techniques (such as oblique lighting) to enhance them (6). Visible fingermarks may be:

- **Positive:** these are formed on a surface after a fingerprint has been in contact with a coloured material such as blood (6);
- **Negative:** this occurs following the removal of the surface such as dust by fingerprint ridges (6);
- **Indented (plastic):** these are formed as a three-dimensional mould of the fingerprint on a malleable surface such as clay (6).

In the law enforcement environment, the most common type of fingermark evidence encountered is latent fingermarks. Latent fingermarks are deposited on a surface; however, they are invisible to the naked eye. Therefore, they need to be treated prior to visualisation by optical, physical or chemical techniques. The application of these techniques is dependent on the composition of the fingermark and the surface on which they are deposited (6). Fingermarks can be deposited on three types of surfaces:

- porous,
- semi-porous, and
- non-porous.

Porous surfaces such as paper absorb water-soluble constituents of latent fingermarks within seconds of deposition. Upon absorption, the water evaporates and amino acids, urea and chlorides remain. Amino acids remain stable whilst urea and chloride diffuse across the surface. The non-water soluble deposits remain on the surface longer than water-soluble surfaces (6). The depth at which the fingermarks secretions penetrate the surface is highly dependent on environmental conditions such as temperature and humidity, as well as the porosity of the paper (35). Water-soluble constituents migrate faster with high relative humidity while the non-water soluble deposits are more

affected by temperature (6). When fingermarks are deposited on porous surfaces, adsorption is likely the result of hydrogen-bonding between the hydroxy groups of the cellulose chain and the adsorbed molecule (36). Therefore, if the reaction between the fluorogenic fingermark reagent and the residue occurs within the cellulose matrix, the product that is formed would be incorporated into the matrix leading to stronger luminescence (35). The interaction between the components in a fingermark and a substrate has been described by Champod *et al.* (6) and illustrated in Figure 1-3.

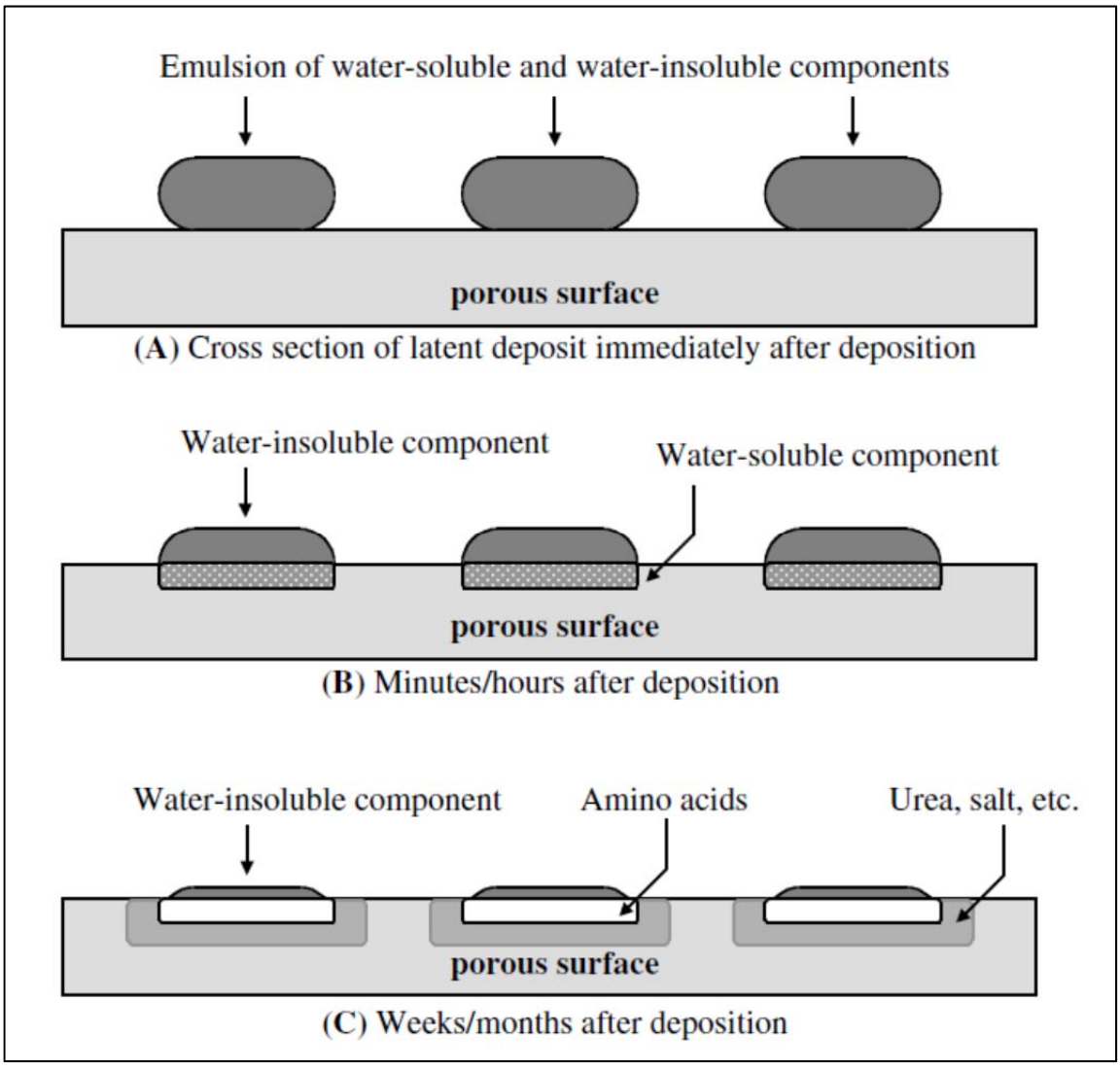


Figure 1-3 Fingermark deposit on porous surfaces over a period of time, adapted from Champod *et al.* (6)

Non-porous surfaces such as plastics, glass and metal, do not absorb fingermark deposits. Therefore, these fingermarks are fragile and can be rubbed off or removed

by organic solvents. Water will remove the water-soluble materials while the water-insoluble constituents remain. However, semi-porous materials have characteristics of porous and non-porous surfaces (e.g. polymer banknotes, waxed wrapping paper). They absorb water soluble components slowly and non-water soluble components tend to stay on the surface for shorter amounts of time than on non-porous surfaces (37; 38). The aging of a fingermark on a non-porous substrate is illustrated in Figure 1-4.

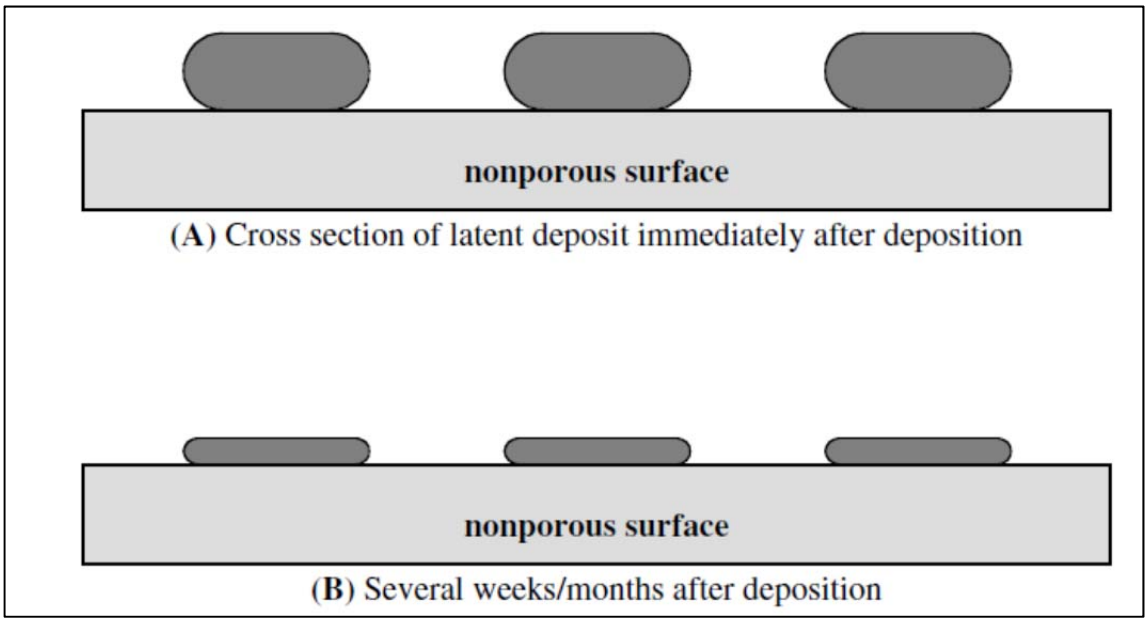


Figure 1-4 Fingermark deposit on non-porous surfaces over a period of time, adapted from Champod *et al.* (6)

1.1.6 Current fingermark detection techniques

Environmental factors and characteristics of the aforementioned surfaces determine the type of chemical and/or physiochemical enhancement techniques that are utilised to visualise the latent prints (39). A brief discussion of the most commonly used fingermark detection techniques for each surface type is provided in this section.

1.1.6.1 Detection techniques for semi-porous surfaces

The enhancement of fingermarks on semi-porous surfaces can be conducted using vacuum metal deposition (VMD) (40). This technique involves the deposition of a layer of gold film that penetrates into the fingermark deposit, followed by zinc onto the gold film. At the end of the VMD process the resulting print is transparent with the background coated with the zinc (37; 40). For the development of prints on polymer banknotes, pre-treatment with cyanoacrylate fuming is also needed before VMD, with subsequent staining also required. It should be noted that VMD can also be used for non-porous surfaces such as low density polyethylene (38; 41). However, the main disadvantage of this technique is the need for specialised equipment (42).

1.1.6.2 Detection techniques for non-porous surfaces

There are a number of methods that are suitable for the development of latent prints on non-porous surfaces. These methods involve adherence or reaction with the sebaceous components of the fingermark deposit. Most of these methods with the exception of powdering require specialised laboratory equipment due to their hazardous nature. Powder dusting is a widely used physical detection method for non-porous surfaces. There are different types of powders available, for example, aluminium flake, fluorescent and magnetic powders (38). A small particle reagent (SPR) or wet suspensions can be used to detect fingermarks on a surface that has been wet. The molybdenum disulfide particles adhere to the water non-soluble components of a fingermark (43). The sensitivity of SPR is an issue and it is mainly used at the crime scene on objects which are difficult to transport to a laboratory (38). Research conducted by Jasuja *et al*., has shown the use of aqueous electrolytic solutions for the development of latent fingermarks on non-porous surfaces (44).

Chemical enhancement techniques include iodine, gentian violet and cyanoacrylate fuming. Iodine fuming can be used for non-porous and porous surfaces and is based on the process of sublimation. It is adsorbed by the sebaceous component of the

fingermark deposit to produce yellow-brown ridge patterns. Although this technique is versatile, the fumes are toxic and the developed prints are not permanent and quickly fade. Its sensitivity is also limited. Therefore, photography is needed immediately after the development (38). The longevity of the print can be enhanced with the use of a fixative, 7,8-benzoflavone, which then leads to dark blue ridge details (45) alternatively, the use of a brucine solution has also been suggested by Jasuja and Kumar (46). Iodine fuming has been shown to recover text from thermal paper after its initial treatment with conventional reagents for porous surfaces such as ninhydrin (47).

Gentian violet can be used on many different types of surfaces including adhesive tapes. It is a dye that produces a purple colour when reacted with the sebaceous deposit within fingermark ridges. Gentian violet is toxic as it contains phenol, however, a non-phenol formula has been formulated (38). More recently with the application of nanoparticles for fingermark detection, research has shown that a cadmium selenide (CdSe) suspension may be an alternative to gentian violet (48). Sticky side powder is another wet suspension method; however, it is only used for the detection of fingermarks on the adhesive side of sticky tape. This means that the non-adhesive side of the tape will need to be subjected to standard non-porous development/enhancement techniques prior to its use (49). Sudan black can also be used to detect latent fingermarks on non-porous surfaces (43; 50; 51).

The traditional methods used for the enhancement of bloodied fingermarks on dark non-porous surfaces involve the use of acid dyes (e.g. acid yellow, violet and black). The United Kingdom (UK) Home Office Scientific Development Branch (HOSDB) has suggested the use of a white wet powder suspension, which reacts with the non-bloodied part of the fingermark, as a complementary step in the sequence. Au *et al*. showed that the suspension interacts with the non-bloodied part of the fingermark thus improving contrast between the acid dye enhancement (52). Bossers *et al.* (53) provides a more in-depth review on the enhancement of fingermarks in blood.

Cyanoacrylate

The use of cyanoacrylate fuming was first reported by the Criminal Identification Division of the Japanese National Police agency and the Northampton Police (UK) (54). Cyanoacrylate fuming is based on the polymerisation of cyanoacrylate esters, usually methyl or ethyl esters, in the presence of water or bases (e.g. sodium hydroxide). These nucleophiles help to initiate the "superglue" polymerisation process. Components of the fingermark such as amines and alcohols can also act as nucleophilic initiators (54; 55). The fuming is performed in an enclosed chamber at ambient temperatures. When the fingermark comes into contact with the cyanoacrylate monomer, it polymerises as a white deposit on the ridges while little polymerisation occurs on the background. The polymer provides sufficient contrast for visualisation of the latent fingermark if the surface is dark (55; 56), but may lack contrast on light coloured surfaces. Stains such as gentian violet, rhodamine 6G and basic yellow can also be used in conjunction with cyanoacrylate fuming to aid in the visualisation procedure (37; 38; 57). The sublimation of anthraquinone dyes has also been used to develop latent fingermarks. These adhere to the cyanoacrylate ester polymer to produce red or blue prints, which can then be easily transferred for recording (58). The use of these dyes in an operational setting may be hindered by the high temperatures required and health and safety concerns.

Research has indicated that storage temperatures and the age of fingermarks can have adverse effects on the polymerisation stage of cyanoacrylate fuming. This may be the result of degradation and/or loss of the chemical components within the fingermark. These effects may be moderated by lowering the temperature during the polymerisation phase of the fuming (59). The structure of the cyanoacrylate polymers, and consequently the quality of the developed fingermarks, are also affected by the relative humidity during the fuming process. It was found that a relative humidity of approximately 70 – 90 % provides optimal contrast between the background and the fingermark (60). These issues, as well as the potential for hydrogen cyanide generation from the heating of cyanoacrylate at above 200 °C, should be taken into consideration when using this technique (61).

1.1.6.3 Detection techniques for porous surfaces

The main techniques for the detection of latent fingermarks on porous surfaces are discussed in this section; however, some techniques that are used on non-porous surfaces are also applicable to porous surfaces. For example, multi-metal deposition (MMD) coupled with a silver nitrate physical developer (62) and the sublimation of copper phthalocyanine (42). The main chemical reagents used for the detection of latent fingermarks on porous surfaces are 1,2-indanedione, 1,8-diazafluoren-9-one and ninhydrin. These reagents react with amino acids (eccrine components) that are present in the fingermark deposit.

Physical developer (PD)

Physical developer (PD) is a technique that is sensitive to the sebaceous components of the fingermark and, as such, can be used on porous surfaces that have been wet. PD is a system that is based on the redox (reduction/oxidation) reaction of iron (II) and silver (I) ions. The silver formed in the reaction is deposited preferentially on the fingermark residues (63), resulting in fingermarks that have a dark grey to black colouration. The overall equation, adapted from Wilson *et al.* (64), is hypothesised as follows:

$$Fe^{2+}_{(aq)} + Ag^{+}_{(aq)} \rightleftharpoons Fe^{3+}_{(aq)} + Ag_{(s)}$$

Australian law enforcement agencies in their fingerprinting sequence may use PD after treating the substrate with amino acid targeting reagents (49). It is the last step in the sequence because it was hypothesised that PD was targeting different components of the fingermark. However, current research indicates that PD is reactive towards eccrine components only in the presence of other non-water soluble components of a fingermark. The non-water soluble components of a fingermark protects the eccrine components from being dissolved during the various wetting processes in the PD method (65; 66).

There are multiple steps in the PD method, these include alternating between rinsing of the substrate in water, maleic acid and the physical developer solution. The use of the maleic acid rinse helps to neutralise the basic components such as calcium carbonate in paper (67), and prevents the formation of silver oxide which causes a black discolouration in paper (64). The Australian Federal Police (AFP) recommends the use of maleic acid, however, malic acid and dilute nitric acid are also common acid pre-wash solutions (49).

PD has a number of drawbacks such as its instability which may result in large variations in the quality of developed fingermarks. The best results are only obtained with fresh solutions and clean glassware (64). Other issues with this technique include background development and low contrast with darker substrates as the silver particles are not fluorescent. Other disadvantages to PD include the cost, the difficulties in the preparative method and the destructive nature of the technique. Although PD suffers from a number of drawbacks, it is still regarded as the primary technique for wetted porous surfaces. Recent development of other reagents such as Oil Red O (67) and nile red (68) have shown promising results in terms of their ease of use and effectiveness in enhancing fingermarks on porous wetted surfaces (69).

Multi metal deposition (MMD)

The use of multi metal deposition (MMD) was proposed as a fingermark development technique in 1989 by Saunders (62). MMD is a technique that is based on the deposition of gold nanoparticles onto fingermark secretions followed by a metal-based treatment to visualise the latent fingermark in a modified PD solution (62).

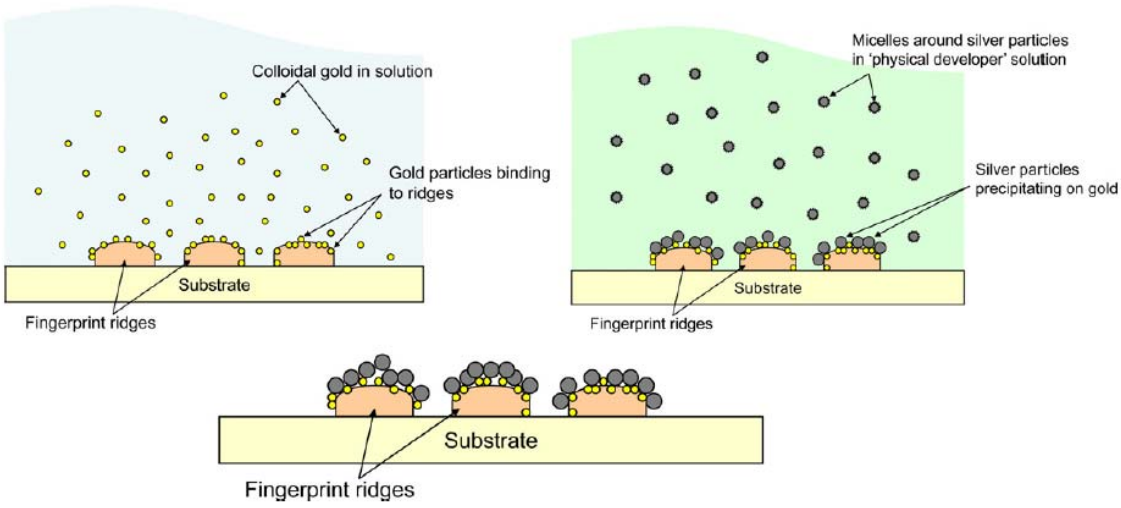


Figure 1-5 Schematic diagram of the deposition of particles onto fingermark impressions in the MMD technique, adapted from *Fairley et al.* (62).

The deposition of gold nanoparticles is possible due to electrostatic forces of attraction between the nanoparticles and fingermark secretions (70). The resulting fingermarks, depending on the colour of the substrate, are dark grey to black in colour. Although this technique may be used on a wide number of surfaces, including both porous and non-porous surfaces, its use is restricted operationally due to the number of steps required to enhance the latent impression (62). There have been numerous research projects conducted to improve MMD (62; 70; 71) including the exploration of a single-metal deposition as an alternative to MMD (72; 73). A schematic diagram showing the deposition of particles onto fingermark ridges is shown in Figure 1-5.

Ninhydrin

Ninhydrin was discovered by Siegfried Ruhemann in 1910 during the isolation of 1,2-indanedione from the synthesis of dicarbonyl compounds (74). Ruhemann found that ninhydrin reacts with ammonia and amines to produce coloured compounds. The coloured compound is known as Ruhemann's Purple (RP). RP has been used for the detection and quantification of amino acids (74; 75). Although the structure of RP was elucidated by Ruhemann in 1911, the first accepted mechanism for its formation was

established by Friedman and Williams (74; 76). An extensive mechanistic review of the formation of RP was later published by Friedman (77).

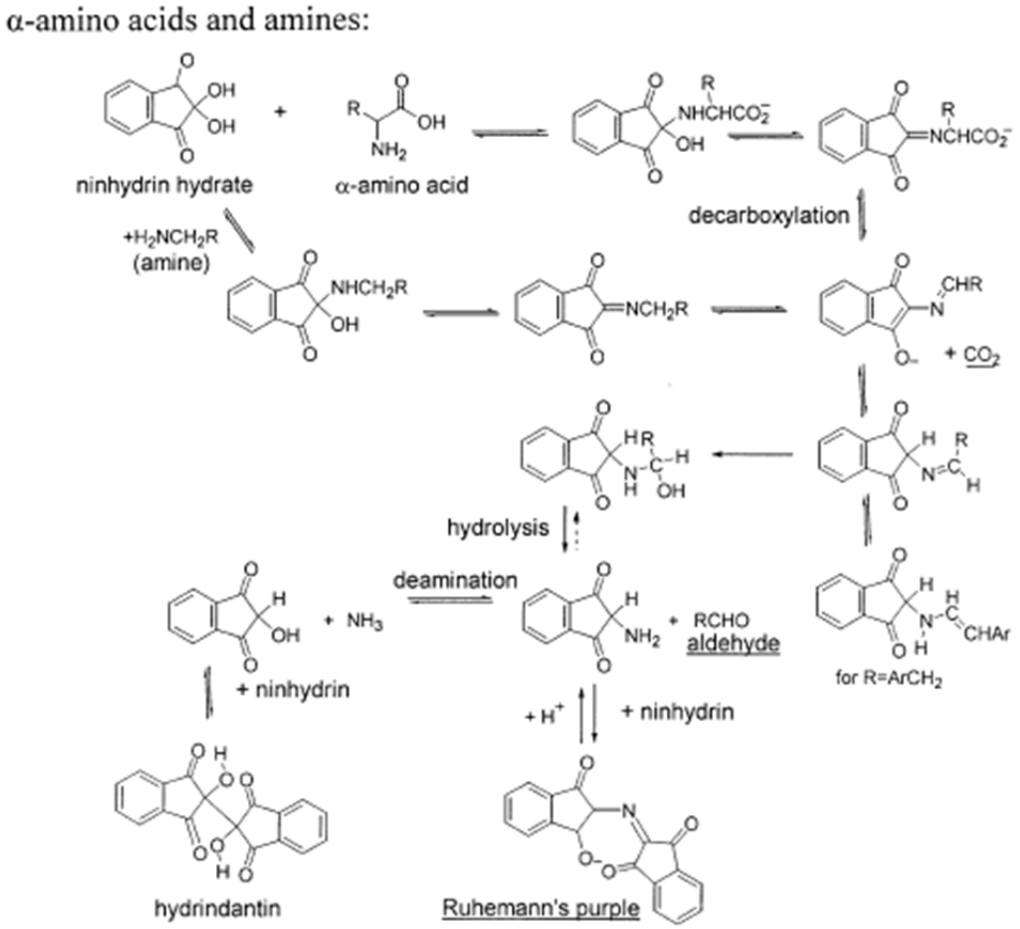


Figure 1-6 Mechanism of reactions of amino acids and amines with ninhydrin, adapted from Friedman (77).

The proposed mechanism describes the formation of an azomethine ylide intermediate from the Schiff base condensation reaction between ninhydrin and an amine/amino acid. The intermediate then undergoes hydrolysis and subsequently forms RP. The reaction mechanism between amino acids and ninhydrin is depicted in Figure 1-6. Friedman noted a number of side reactions that can occur depending on the conditions of the reaction, especially the pH and to a lesser extent the reaction time.

In 1954, Oden and von Hofsten suggested that ninhydrin could be used to detect latent fingermarks (78). The early formulations of ninhydrin were problematic as a result of

the flammability of the carrier solvents which contained a mixture of diethyl and petroleum ether, acetone and ethanol (6). These organic solvents coupled with the use of acetic acid caused inks on paper to run, compromising the sensitivity as well as causing high background staining (74). The problems resulting from the carrier solvents were solved with the development of 1,1,2-trichlorotrifluoroethane (CFC113 or Freon113) (74; 78; 79). The formulation required the addition of ethanol as ninhydrin has low solubility in the non-polar CFC113 (79-81). Concentration of ethanol, greater than 10 % v/v, can lead to diffusion of fingermark ridges as water is produced from its reaction with acetic acid (77; 82).

Under the Montreal Protocol 1987, the use of CFC113 was banned as it caused the depletion of the ozone layer. Therefore, 3M™ Novec™ HFE7100 was developed as a non-toxic, non-ozone depleting replacement (74; 83). The polarity of the formulation, contrast and visualisation issues remained challenging despite the changes. Contrast and visualisation issues were alleviated using a post-treatment solution of zinc chloride. Herod and Menzel conducted further research into this reaction product and found that a fluorescent complex was formed (84). Strong ninhydrin prints (i.e. a higher amino acid concentration) resulted in a red complex with zinc chloride (1:2 metal : ligand ratio), while weak prints produced an orange complex (1:1 metal : ligand ratio) (85). The maximum absorption occurs at 490 nm and fluorescence at 550 nm. Cadmium salts can also be used with absorption and fluorescence at 505 and 590 nm, respectively.

The photoluminescence of the developed print is only observed under cooling with liquid nitrogen, that is, without cooling no fluorescence is observed (86; 87). The reaction scheme of the formation of RP and the subsequent formation of the metal complex is shown in Figure 1-7.

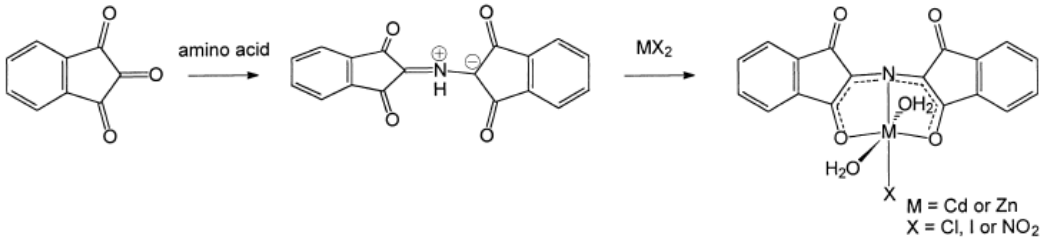


Figure 1-7 Formation of RP from ninhydrin and amino acids and its subsequent metal complexes, adapted from Conn *et al.* (88).

Ninhydrin can be applied to the substrate using a brush or spray (74). Fingermark development occurs over a period of 24 – 48 hours at room temperature, preferably in the dark with humidity around 50 to 80 % (37). Due to the time that is needed for development (up to seven days), the need for secondary metal salt treatment and cooling in order to produce fluorescence, the use of ninhydrin is a time-consuming and cumbersome process (89). Ninhydrin is considered a better reagent for the development of coloured fingermarks because RP is easily detected with the naked eye (39). While heat and humidity can be used to increase the rate of development, it decreases the contrast between the developed fingermarks and the substrate because of the heavy background staining that may occur (74). As a result, it is generally not recommended.

The development of new reagents was necessary in an attempt to improve the optical and fluorescent properties of ninhydrin. Numerous researchers have explored the syntheses and use of ninhydrin analogues. Some of these analogues include benzo[*f*]ninhydrin, 5-methoxyninhydrin, thieno[*f*]ninhydrin, naphtha[*f*]ninhydrin and isatin (74; 90-92). Other compounds of interest are 1,8-diazafluorene-9-one (DFO) and 1,2-indanedione.

1,8-Diazafluoren-9-one (DFO)

in 1990, Grigg and Pounds prepared 1,8-Diazafluoren-9-one (DFO) during the synthesis of ninhydrin analogues (75; 93). Wilkinson (81) proposed that DFO forms an unstable

hemiketal in the presence of a solvent that is then attacked by the nitrogen from the α-amino acids. Following the loss of water, an imine intermediate is formed that then undergoes decarboxylation and hydrolysis to yield an aromatic amine and an aldehyde. The aromatic amine then reacts with another DFO molecule to produce the final product. The proposed reaction scheme for the reaction of DFO and l-alanine is shown in Figure 1-8. Characterisation of the final product determined it to be analogous to RP (81). Unlike ninhydrin, which produces non-fluorescent dark purple prints, DFO produces pink-purple fingermarks that are highly fluorescent at room temperature without secondary treatment with metal salts and cooling (74; 88; 93; 94). The product formed has a maximum absorption and emission occurring at 530 nm and 570 – 580 nm, respectively.

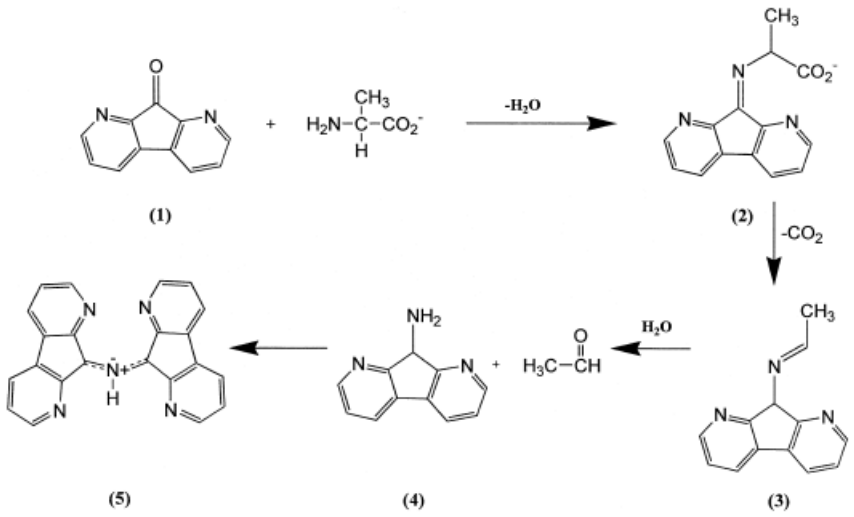


Figure 1-8 Possible reaction scheme of DFO and L-alanine, adapted from Wilkinson (81).

A mixture of CFC 113, methanol and acetic acid was used by Pounds *et al*. to develop early formulations of DFO that had similar disadvantages associated with ninhydrin (7; 95). High concentrations of methanol in the formulation caused inks to diffuse while the CFC 113 carrier solvent was banned due to environmental concerns. Reformulations with heptane, xylene and petroleum ether led to issues with flammability as well as inferior developed fingermarks when compared with the original formulation with CFC 113 (83).

It was later proposed that a new formulation using HFE7100 would contain a small percentage of methanol as it is a necessary component to dissolve DFO in the non-polar carrier solvent. The formation of a reactive hemiketal between DFO and methanol is necessary for its reaction with amino acids in fingermarks (81). NMR spectroscopy indicated that approximately 20 % of DFO exists as the hemiketal in methanol; however, this percentage decreases as the aliphatic chain of the alcohol increases. Therefore, the solvent has a large impact on the number of fingermarks detected with the greatest number detected when DFO was dissolved in methanol and followed by ethanol (81).

DFO is more sensitive than ninhydrin, developing 2-3 times more fingermarks (6; 96). Operationally, the use of DFO is followed by ninhydrin and physical developer in order to maximise the detection of latent fingermarks (49). Researchers have found that this is possible due to the slow reaction rate and/or incomplete reaction between DFO and amino acids, thus allowing for further enhancement with ninhydrin. Due to the slow reaction rate of DFO, many operational procedures call for “double dipping” of the treated fingermarks to improve their fluorescence (74; 94; 95; 97; 98).

Initially the development of fingermarks treated with DFO required the application of heat with a dry oven (20 min at 100 °C), however, the use of a dry heat press (10 s at 180 ˚C) was found to produce equal results (17). It was noted that the absence of humidity when developing fingermarks with DFO was reported to produce the best results. The high temperature used could burn the fingermark and substrate, although placing the sample in between sheets of paper towel alleviates the problem (39; 99). The fluorescence produced is at the maximum after heating and decreases with time. DFO can be considered a ‘dual’ fingermark reagent, producing a coloured and fluorescent product at room temperature, however, the light pink chromophore it produces is difficult to detect on coloured or highly patterned surfaces (13; 37; 75; 93; 98; 100). Although the use of DFO has been widely accepted operationally, it is not without its drawbacks of high costs and poor solubility in non-polar solvents (94; 95).

1,2-Indanedione

The disadvantages of both ninhydrin and DFO have contributed to the pursuit of a reagent that can improve both the colour and fluorescence of the developed latent fingermark. In general, many approaches have focused on ninhydrin analogs with the utilisation of 1,3-indanedione as the precursor. Hauze *et al.* (93) used 1,2-indanedione as a precursor and synthesized 6-methylthio-1,2-indanedione which developed fluorescent fingermarks and faint orange ridges. The fluorescence of the fingermarks could be further enhanced by the application of a zinc nitrate solution. As a result, 1,2-indanedione and its analogues were examined (94; 101).

1,2-Indanedione was synthesised as an intermediate during the preparation of substituted ninhydrin by Ramotowski *et al.* (102) . Similar to DFO, the application of 1,2-indanedione to amino acids and fingermarks produces a pale pink coloured product that is fluorescent in a single-step process. The coloured product is referred to as Joullié's Pink (JP), named after Professor Joullié's who developed the indanedione reagent (103; 104).

The reaction mechanism of 1,2-indanedione and its analogs have been studied by Petrovskaia *et al.* (105). The reaction mechanism between 1,2-indanedione and an amino acid is illustrated in Figure 1-9. It was postulated that an imine could be formed between 1,2-indanedione (I) and an α-amino acid which then undergoes decarboxylation to form an azomethine ylide (C-N-C 1,3 dipole) (III). The resulting dipole, in a Strecker degradation, generates 2-amino-1-indanedione (VI), thus forming a RP-like product in excess 1,2-indanedione. It was further hypothesised by Spindler *et al* (106) that the addition of zinc (II) ions leads to the rapid conversion to 2-amino-1-indanedione. 2-Amino-1-indanedione then reacts with another mole of 1,2-indanedione to form JP (VII).

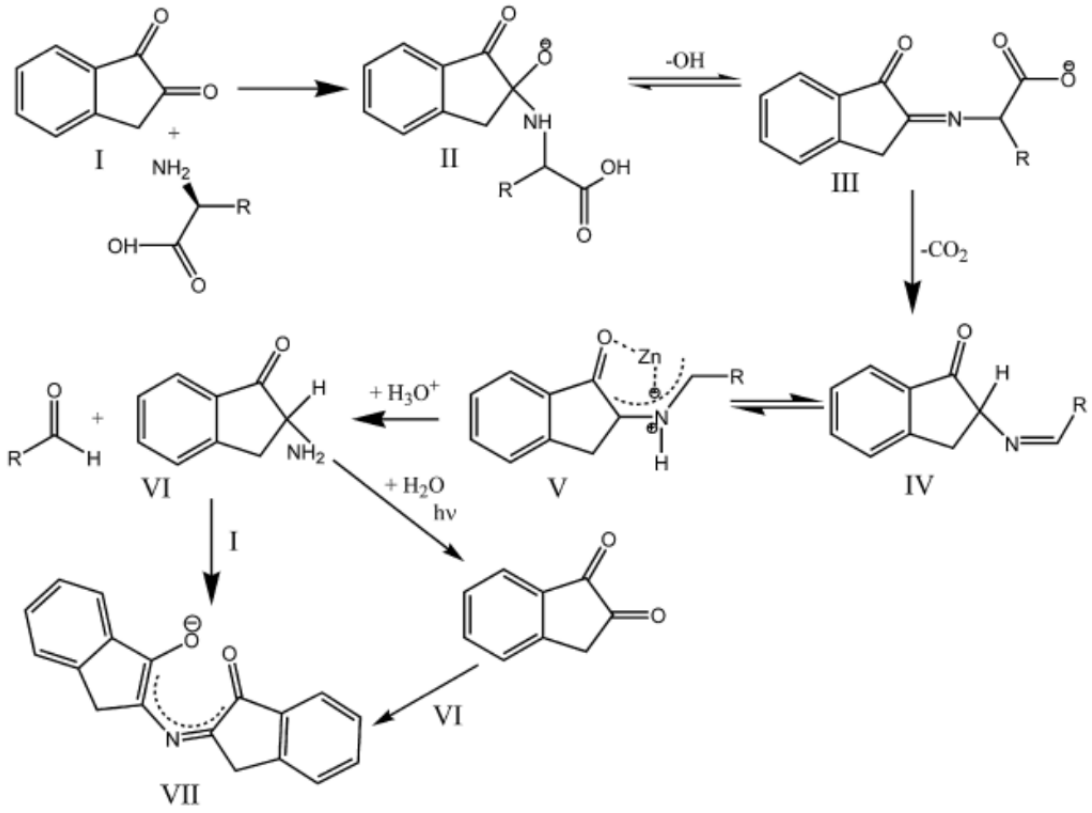


Figure 1-9 Reaction mechanism between 1,2-indanedione and amino acid, adapted from Spindler *et al.* (106).

There have been other attempts at elucidating the structure of the reaction product between 1,2-indanedione and amino acids. Alaoui *et al.* used glycine as the representative amino acid in their attempts (107). The reaction of glycine and 1,2-indanedione in methanol at room temperature was found to produce two fluorescent reaction products. The fluorescent products were identified as 2-carboxymethyliminoindanone (I) and 1,2-di(carboxymethylimino)indane (III) (Figure 1-10) (107).

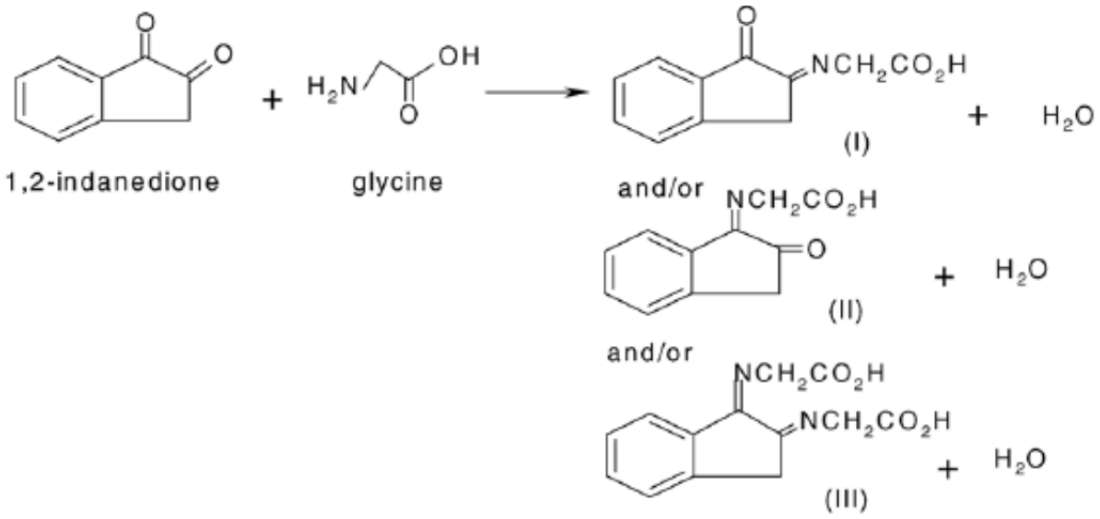


Figure 1-10 The proposed reaction products of 1,2-indanedione and glycine in methanol, from Alaoui *et al*. (107).

It has been hypothesised that based on the structural similarities between 1,2-indanedione and ninhydrin, their reaction with amino acids would yield a similar product. However, the products that Alaoui *et al.* found were not analogous to RP but rather to its intermediates. The imines formed could be the result of an incomplete reaction between 1,2-indanedione and glycine as the research by Alaoui *et al*. was conducted at room temperature with no heat application.

The reaction between 1,2-indanedione and amino acids is dependent on the substrate, humidity, the solvent system and the application of heat. Almog *et al.* has found in methanolic solutions, the formation of a stable 1,2-indanedione hemiketal, thus preventing it from reacting with amino acids standards and latent fingermarks (103). The concentration of acid in the formulation has a significant effect on the reaction. Similarly to the reactions between DFO and ninhydrin, the increase in acid concentration improves the fluorescence of the developed fingermarks. Concentrations of acetic acid above 10 mL/L has adverse effects on the contrast between the background and the fingermark (95). The addition of small amounts of acetic acid enables the interaction with the paper substrate through a neutralisation process, thus improving the reaction. However, an excess of acetic acid can lead to diffusion of fingermarks (106; 108). The addition of acetic acid in the formulation is a matter of contention as Wiesner *et al*. has found that 1,2-indanedione produced

superior results on both acidic and neutral papers in the absence of acetic acid (98). The effects of different solvents on the formulation were highlighted by Roux *et al*. with the detection limit for glycine in luminescence mode increasing from 3.1 x 10^{-4} mg/mL (petroleum ether) to 7.6 x 10^{-5} mg/mL (Arklone) (94).

The reaction between 1,2-indanedione and amino acid is sensitive to the ambient humidity at the time of development. This is problematic when comparing results/formulations in different countries and is a likely cause for the contentious results in different countries (99). In a parallel study conducted at the University of Technology Sydney (UTS) and the Canberra Institute of Technology, the effect of ambient humidity was emphasised (109). Conditions common in Canberra such as low relative humidity, resulted in reduced sensitivity of the reagent. While in Sydney, with its warmer and more humid climate, 1,2-indanedione produced better results (106; 109). In both cases, 1,2-indanedione consistently delivered superior results when compared with DFO. Sears *et al*. obtained the converse results in the UK (110). In all studies, the addition of zinc chloride appeared to stabilise and improve the fluorescence of 1,2-indanedione (106).

The trend in performance was observed by Bicknell *et al.* where humidity and the moisture content of the paper have been reported to play a crucial role in maximising fluorescence intensity (101). In the study three different types of paper (two different types of white copy paper and a yellow copy paper) and an optimised solution of 1,2-indanedione with petroleum ether as the carrier solvent were used. The two white copy papers and the yellow copy paper had a critical moisture content level of 3.0 % and 1.9 %, respectively, below which both the colour and fluorescence decreased. The values corresponded to a minimum ambient relative humidity of 69 % for the white copy papers and 72 % for the yellow copy paper. Below these values, it was recommended that the use of a humidity chamber and oven development is used (101).

The use of a metal salt in the enhancement of fluorescence has been well documented with ninhydrin and DFO. As a result, researchers employed similar techniques with 1,2-

indanedione. Research conducted involved post-treatment of fingermarks with zinc chloride. Hauze *et al*. noted that following zinc salts post-treatment, the colour of the amino acid spots as well as fingermarks changed from light pink to dark pink. Subsequent research on the post-treatment with zinc salts used liquid nitrogen during luminescence visualisation led to improved luminescence of the developed fingermarks (94). Research by Wallace-Kunkel *et al.* obtained incongruent results where liquid nitrogen did not deliver superior luminescence (99). This is likely due to the different formulations that were used.

The addition of zinc salts in the 1,2-indanedione formulation was also assessed as it would greatly decrease the time needed for processing. The results of these tests mirrored those of the zinc post-treatment. The incorporation of zinc salts in the 1,2-indanedione formulation was also tested resulting in improvements in the longevity of the fingermark luminescence and negating the effects of humidity (94). Stoilovic *et al*. also reported the addition of zinc chloride made the 1,2-indanedione solution less susceptible to environmental changes (62). However, it has been noted the addition of zinc chloride decreased the stability of the solution, it was proposed that this was the result of self-condensation where zinc is acting as a catalyst (101). This was later confirmed by Spindler *et al*. (106) It was found that zinc chloride acts as a Lewis acid catalyst by stabilising the azomethine ylide in low humidity environments of below 50 % relative humidity. In humidity levels above 70 %, 1:25 to 1:5 molar ratio of zinc chloride to working solution appeared to increase the rate of product formation (106).

1,2-indanedione treated fingermarks may develop at room temperature over 24 – 48 hours (94), however, the formation of JP can be accelerated with the application of heat. Gardner and Hewlett reported that fingermarks developed at 100 °C showed an increase in fluorescent intensity after a week of storage in darkness, indicating that the reaction is incomplete (95). In support of previous findings, data showed that development using a conventional oven at 100 °C for 10 min did not drive the reaction to completion (99). Other research showed the formation of undesirable non-fluorescent oligomeric by-products after heating for prolonged periods at 70 – 100 °C in low humidity conditions (106). The use of the heat press at 165 °C for 10 s produced

the greatest colour and fluorescent fingermarks (99). Other research has also shown that the 1,2-indanedione solution reacts with all the amino acids present in the fingermark and no new fingermarks could be detected after redevelopment (111). The photodegradation of the fingermark has also been reported with samples in a light restricted environment producing increased luminescence longevity (111) .

JP has an absorption maximum at 550 nm and a luminescence emission at 555 nm when excited with a blue-green light source (15; 112). Currently the Australian Federal Police (AFP) recommends the use of a dry heat press at 160 °C for 10 s in between clean sheets of absorbent paper to prevent burning of the substrate (49).

1.1.6.4 Miscellaneous detection techniques for porous surfaces

Genipin

Toxicity issues associated with the use of the conventional fingermark developing reagents led to studies utilising natural compounds (113). Genipin is the product from the hydrolysis of geniposide by *β*-glucosidase. The fruit of *Gardenia jasmonoides Ellis* and *Genipia americana* are the natural sources of geniposides (114). Genipin reacts specifically with primary amino acids, and also with peptides and proteins forming a dark blue coloured adduct (114). Due to its non-toxic and colour characteristics (a natural source of blue pigment), genipin has been used as a food colourant and dye in some Asian countries (115). The cross-linking properties with various molecules, such as chitosan or proteins, have also seen the application of genipin in the synthesis of biomedical products and pharmaceuticals preparations and in analytical chemistry as an amino assay reagent (116; 117).

In 2004, Almog *et al.* (113), recognised the potential of genipin as a 'dual' fingermark detection reagent. When genipin reacts with amino acids, it is transformed from a colourless reactant into a blue luminescent product, thus highlighting its specificity to amino acids (118). Currently, genipin is not routinely used in forensic casework as it is considered a novel reagent. However, it could be used on substrates with highly

luminescent backgrounds as its emission wavelengths are longer than those of the conventional reagents, thus improving the signal-to-noise ratio (97). With optimisation and development of its working formulation, it may become an alternative technique for fingermark detection on porous surfaces.

The reaction mechanism between genipin and amino acids has been studied with conflicting outcomes. For example, Touyama *et al.* (97; 118), found that more than one compound could be the precursor of the blue product, whilst Fujikawa (97; 119; 120) has proposed the formation of a monomeric adduct. The results may not be accurate as they were carried out in solution phase as opposed to on porous substrates (97). Although the exact mechanism of action is unclear, it has been found that the blue product is more stable in alkaline (pH 9) than in neutral (pH 7) and acidic solutions (pH 5) (115). The determination of the mechanism of reaction and the structure of the product may lead to different approaches in the optimisation of the working solution for genipin.

Lawsone

Lawsone (2-hydroxy-1,4-naphthoquinone) is a natural colourant and is responsible for the dyeing property of henna. Lawsone is present as a glycoside in the leaves of *Lawsonia inermis* L. (Lythraceae) at a concentration of 1.0 – 1.4 % (121; 122). Plants containing lawsone are native to a number of countries spanning across Africa, the Middle East and South Asia such as Afghanistan, Bangladesh, Egypt, Pakistan, Saudi Arabia, Sudan, Tunisia, UAE and Yemen (123).

Lawsonia inermis L. has been used in traditional medicine as a treatment for a number of ailments such as jaundice, various skin diseases, burns, inflammation and bacterial infections (124). Due to its staining properties, henna is also used as a hair, skin and beard dye (123). The ancient Egyptians were believed to have used henna for its staining properties (125). As a result of its natural origins and relative safety in comparison to synthetic dyes, there is growing interest in the use of lawsone in

consumer products. Due to its increasing popularity, genotoxicity studies were conducted by Kirkland and Marzin (121). It was concluded that the use of henna posed no genotoxic risk to the user; despite results indicating lawsone is haematotoxic due to the concentration and frequency that it is used. Other research has found that lawsone could be absorbed through the skin following the application of henna-based cosmetic products (126).

Lawsone was first introduced as a latent fingermark detection reagent in 2008 by Jelly *et al.* due to the dyeing properties of henna (127). Furthermore, naphthoquinones such as 1,2-naphthoquinones-4-sulfonate acid, sodium salt have been used in analytical chemistry for the detection of amino acids in chromatography (128-132). In the studies conducted by Jelly *et al*. a range of naphthoquinones were purchased (Table 1-3) and used to detect latent fingermarks on porous surfaces. Utilising the existing formulations for ninhydrin, DFO and IND-Zn, four different formulations were tested (Table 1-4) (133).

Table 1-3 Names and structures of naphthoquinones used by Jelly *et al.* (133).

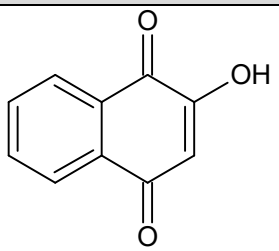
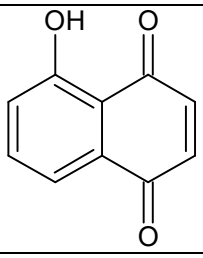
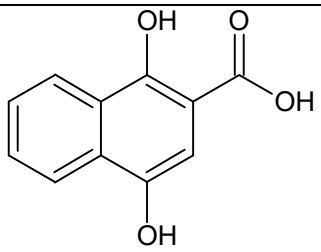
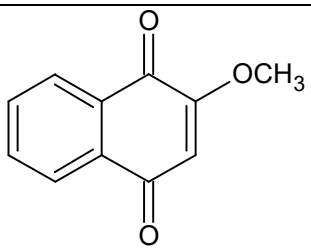
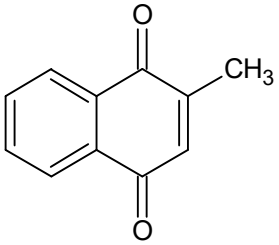
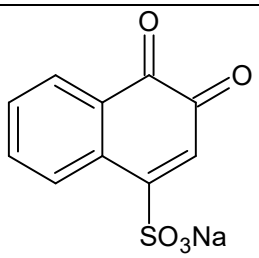
Compound	Structure
Lawsone (2-hydroxy-1,4-naphthoquinone)	
5-Hydroxy-1,4-naphthoquinone	
1,4-Dihydroxy-2-naphthoic acid	
2-Methoxy-1,4-naphthoquinone	
2-Methyl-1,4-naphthoquinone	
1,2-Naphthoquinone-4-sulfonic acid, sodium salt	

Table 1-4 Formulations of working solutions used for naphthoquinones, adapted from Jelly *et al*. (133).

Formulation A	Formulation B	Formulation C	Formulation D
0.1 g naphthoquinone 3 mL dichloromethane 6.4 mL ethanol 0.6 mL acetic acid 90 mL HFE7100	0.1 g naphthoquinone 3 mL dichloromethane 6 mL ethyl acetate 1 mL acetic acid 0.1 mL zinc chloride solution 90 mL HFE7100	0.1 g naphthoquinone 8.5 mL ethanol 0.7 mL ethyl acetate 0.8 mL acetic acid 90 mL HFE7100	0.1 g naphthoquinone 20 ml ethyl acetate 80 mL HFE7100

The results indicated formulation D (1 mg/mL) provided optimum colour and luminescence. The other formulations were unstable, causing precipitation of lawsone from the solvent system, and diffusion of fingermarks. A variety of development conditions were also investigated:

- dry steam iron at 170 °C for 30 – 60 s
- oven at 140 – 170 °C for 1 hr

Optimum luminescence of amino acid standards and fingermarks was achieved through oven development at 150 °C for 1 hr. Jelly noted optimum luminescence for lawsone and the other substituted naphthoquinones (Table 1-3) occurred at an excitation wavelength at 590 nm and 530 nm, respectively, with a red long-pass filter (133).

These preliminary results demonstrated the potential use of naphthoquinones for the detection of latent fingermarks on porous surfaces. Jelly proposed a reaction mechanism (Figure 1-11) for the reaction of lawsone and amino acids. This is based on the ninhydrin reaction mechanism where an imine intermediate is formed via a nucleophilic attack on the carbonyl group of lawsone by the non-protonated amino acid followed by a loss of carbon dioxide and water. The imine intermediate then loses an aldehyde with one less carbon than the amino acid following hydrolysis. However,

Jelly was not able to determine the reaction product(s) between lawsone and amino acids through synthetic studies or gather additional information with regards to the reaction products of the naphthoquinones and fingermarks using synchrotron infrared microscopy. Furthermore, a limited range of solvent systems were used, indicating additional analyses are necessary to optimise the formulation.

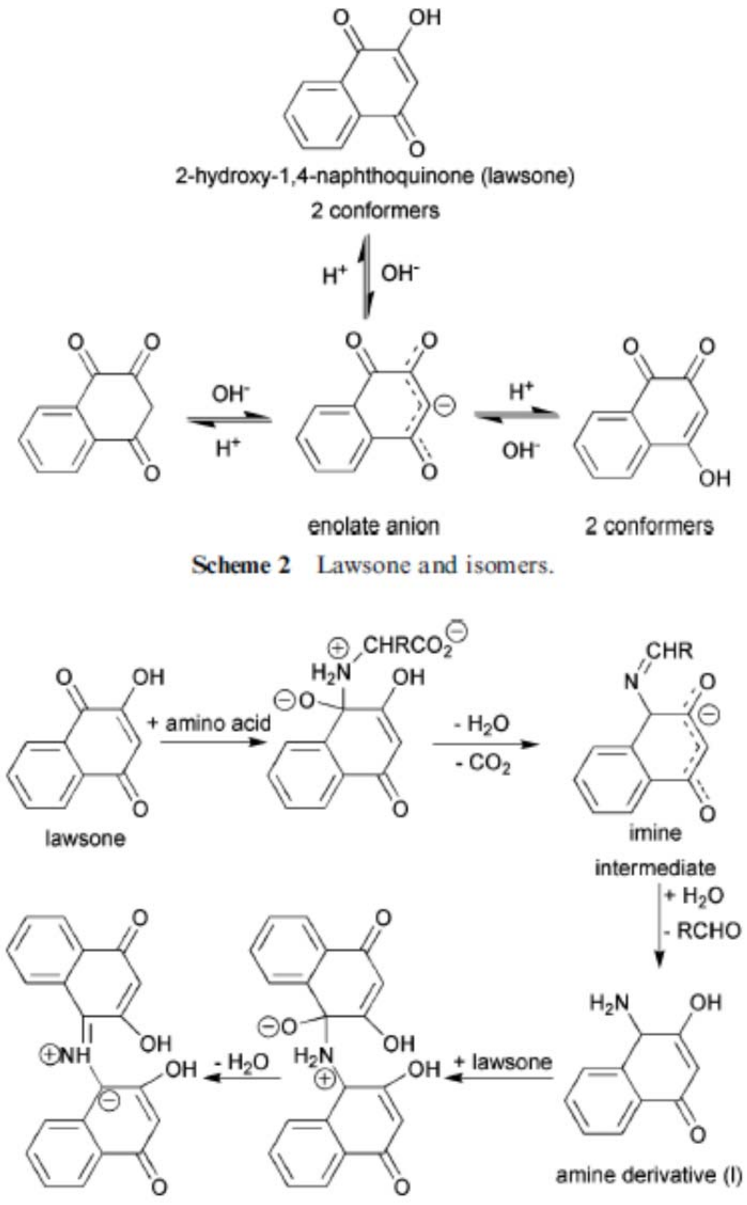


Figure 1-11 Proposed mechanism of the reaction between lawsone and primary amino acids, adapted from Jelly *et al.* (127).

More recently, Thomas and Farrugia (134) compared the use of genipin and lawsone with ninhydrin and DFO in the enhancement of fingermarks in blood on paper. Both genipin and lawsone (1 mg/mL) were found to degrade the fingermarks. It was hypothesised that fluorescence quenching occurred as a result of dyes or chemicals within the substrate, which may have contributed to the lack of positive enhancement on different paper types that were studied. Furthermore, fluorescence enhancement was limited coupled with noticeable diffusion of fingermark ridges when developed with genipin and lawsone. It was found that while genipin showed potential in the enhancement of latent fingermarks, it did not improve the fluorescence of fingermarks in blood across all paper samples in the study. Lawsone did not provide enhancement of fingermarks under white-light or in luminescence mode with.

1.2 Project aims

Research into fingermark detection reagents on porous surfaces has come a long way since the discovery of ninhydrin by Ruhemann in 1910. The disadvantages of each fingermark reagent have led to the synthesis and application of novel compounds. The aim of this project was to compare the effects and reactions of different functional groups on a fingermark reagent. Of particular interest were compounds with the quinone moiety, and to use these compounds to enhance latent fingermarks on a variety of porous surfaces and backgrounds.

The research conducted using lawsone demonstrated its potential for the detection and enhancement of latent fingermarks. The current project aims to further optimise these parameters as well as to synthesise novel quinones with differences in conjugation. A number of key areas were identified and these are:

- The determination of the reaction product(s) between lawsone and amino acids. These results will act as a model for the products formed with more conjugated quinone systems.
- The synthesis and characterisation of quinones which may be used as reagents for the detection of latent fingermarks on porous surfaces.

- The optimisation of parameters, including formulation, development and enhancement conditions, for the synthesised quinones for the detection of latent fingermarks on porous surfaces.
- A comparison of the relative fluorescence of fingermarks of varying ages and amino acid standards developed by the quinones.
- A comparison of the fingermarks developed with the novel reagents and the IND-Zn developed fingermarks is to be conducted. Noting that IND-Zn is the routine reagent used by law enforcement agencies in Australia.

Chapter 2: Structural Elucidation of the Reaction Products of Lawsone and Amino Acids

2.1 Introduction

Structural elucidation of the reaction products between lawsone and amino acid(s) is beneficial so that a reaction mechanism may be proposed. It would allow us to determine the solvent systems that could stabilise any intermediates that may be formed, thereby, improving the yield of the final product(s). The use of lawsone for the detection and enhancement of latent fingermarks was discussed in detail in Section 1.1.6.4.

It was briefly noted in Section 1.1.6.4 that Jelly *et al.* (127) proposed a reaction mechanism between lawsone and amino acids (Figure 1-11) reacting in a 2 : 1 ratio that is analogous to that of ninhydrin, DFO and 1,2-indanedione. According to Jelly (135), the reaction of glycine and lawsone in ethanol, over 48 hours, yielded two products of two distinct colours, yellow and red. The two products were separated by silica gel chromatography using petroleum spirits and ethyl acetate in a ratio of 4: 1.

The yellow product, identified through a library search using gas-chromatography mass spectrometry (GC-MS) data, appeared to have a mass to charge ratio, accurate to one decimal place, consistent with 2-amino-1,4-naphthoquinone. Jelly hypothesised that the structure of the red product is the product of lawsone and glycine, however, this was undetermined (135). The ^{1}H and ^{13}C NMR spectra of the red product were documented (Figure 2-1 and 2-2). However, neither heteronuclear single quantum coherence (HSQC) nor correlation-spectroscopy (COSY) spectra were provided and/or obtained.

The long reaction time over which the study was conducted could have led to side reactions and/or degradation products. Jelly (135) noted the lack of distinct peaks from GC-MS and NMR analysis and attributed these issues to the limited amount of product obtained from the reaction. The lawsone-glycine product may not have been volatilised or could have been thermally unstable and, therefore, may not have been suitable for GC-MS analysis. It is possible that another analytical technique should be employed in the characterisation of the product.

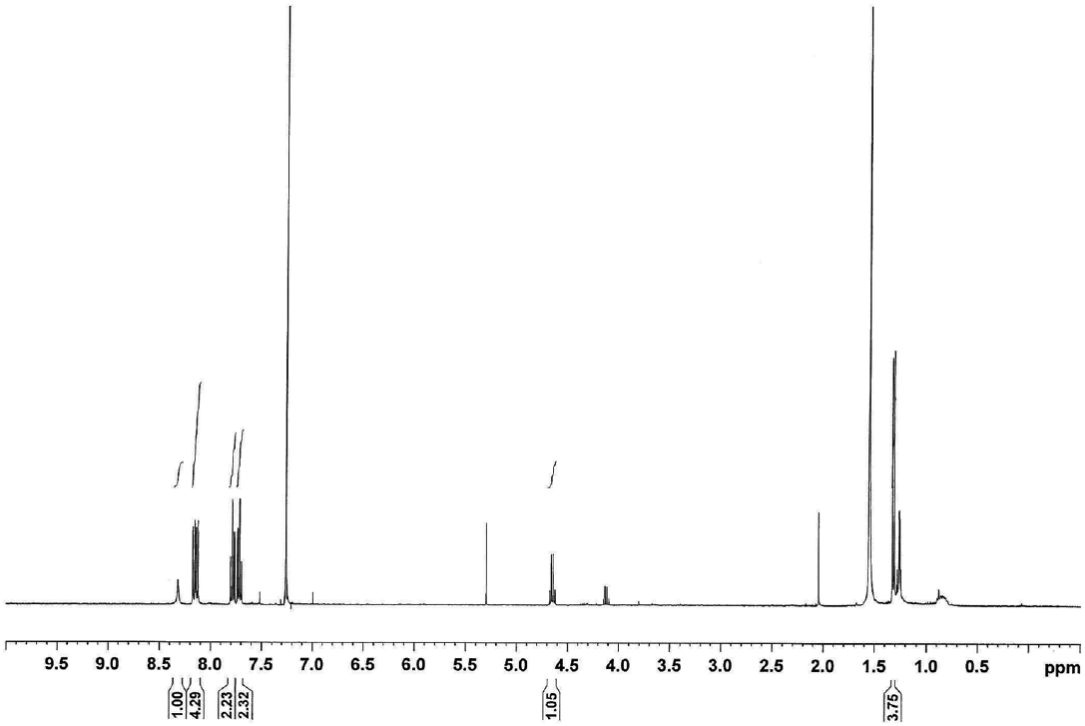


Figure 2-1 ^{1}H NMR spectrum of the 'red product' from the reaction of lawsone and glycine, from Jelly (135).

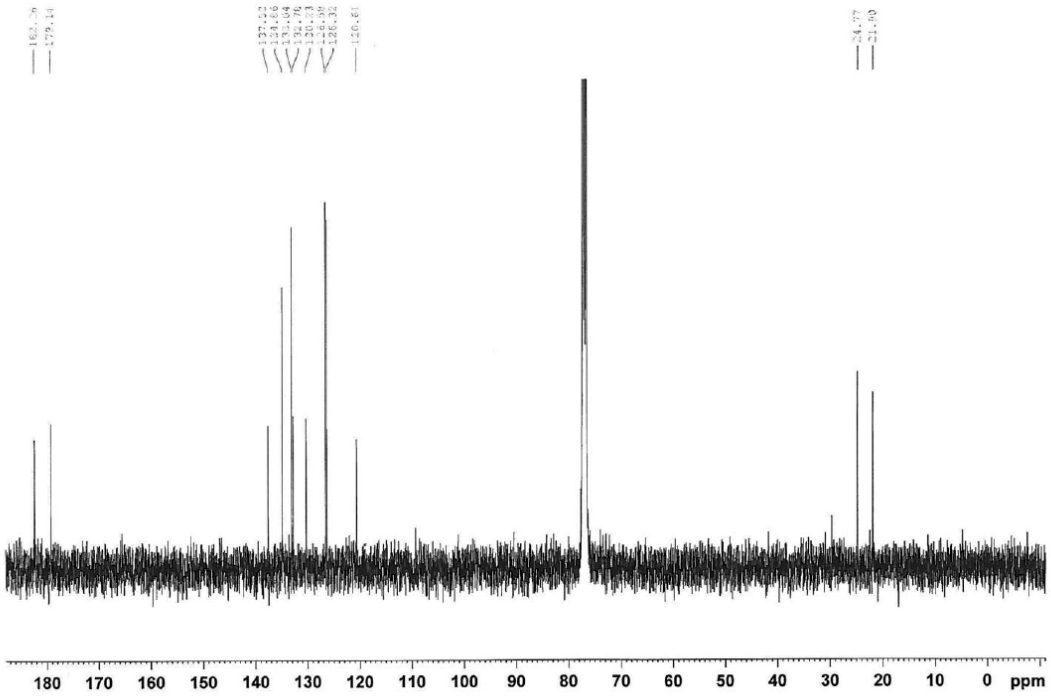
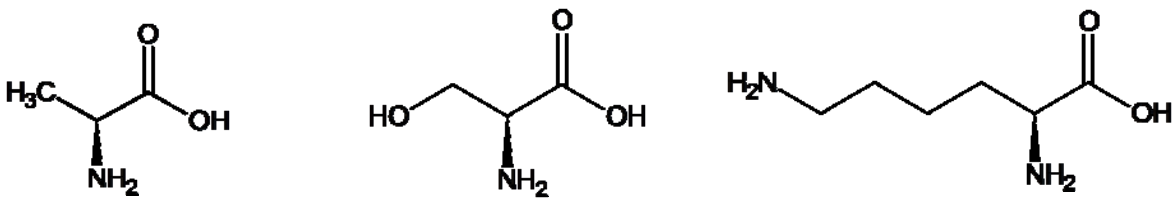


Figure 2-2 ^{13}C NMR spectrum of the 'red product' from the reaction of lawsone and glycine, from Jelly (135).

The synthesis of the reaction products between lawsone and three different amino acids, alanine, lysine and serine, are documented in this chapter. The structures of the three amino acids are shown in Figure 2-3. Alanine, a non-polar amino acid, and serine, a polar amino acid resulting from the presence of the terminal hydroxy group, were chosen due to their abundance in eccrine secretions. Lysine contains two terminal amines both of which may act as potential nucleophilic sites during the formation of the azomethine ylide. Due to the multiple reactive sites on lysine, it is possible that various side reactions may occur that can prevent the formation of the desired fluorogenic product. This phenomenon was documented by Friedman's research conducted on the mechanism of ninhydrin with various amino acids (77). It was noted that amino acids with multiple nucleophilic sites other than the α-amino group may form cyclic intermediates that only form RP slowly or prevents the formation of RP.



(a) (b) (c)

Figure 2-3 Amino acids used in the reaction (a) L-alanine; (b) L-serine; (c) L-lysine.

The reactions of amino acids with lawsone in a solution of methanol were conducted with a 2 : 1 stoichiometric ratio of lawsone to amino acid. Methanol was employed in the reactions due to its polarity aiding in the dissolution of amino acids and lawsone, thus reducing the volume of solvent required for the reaction. The reactions were performed using two different methods; the first involved the reaction between lawsone and the amino acid in solution, while the second involved the impregnation of amino acids onto cellulose followed by its reaction with lawsone. The first method aimed to provide a more simplistic view of the interaction between the two reagents, while the second aimed to mimic the interactions between porous substrates / amino acids / lawsone.

Liquid chromatography (LC) analysis was coupled with an accurate mass quadrupole time-of-flight mass spectrometer (QTOF-MS). LC -MS was used as an alternate analytical technique to GC-MS for the analysis of the products formed in the reaction. Analysis with LC-MS negates the use of derivatising agents. Derivatisation is generally required for polar and thermally labile compounds such as amino acids or the product that may be formed. Forgoing the derivatisation step is beneficial as it may lead to a mixture of derivatives that can complicate the resulting chromatogram (136; 137), hence adding ambiguity to the structural elucidation process. LC-QTOF-MS also provides an accurate mass for the compounds that are found, thus allowing for greater accuracy when proposing possible structures for the unknowns and their fragmentation pathways, as well as distinguishing between two compounds of similar mass.

The aim of this chapter was to analyse the reaction mixtures of lawsone and the three aforementioned amino acids and to determine whether the proposed product(s) by Jelly *et al*. (127) and Jelly (135) was formed. Attempts were also made to tentatively elucidate structures and fragmentation pathways of the compound(s) formed.

2.2 Materials and Methods

All reagents and solvents were obtained from Sigma-Aldrich unless otherwise stated: Reagent grade L-alanine ≥ 98 % [CAS 56-41-7], L-lysine ≥ 98 % [CAS 56-87-1], L-serine *ReagentPlus*® ≥ 99 % [CAS 56-45-1] were ground into a fine powder immediately prior to use. 2-Hydroxy-1,4-naphthoquinone (lawsone) 97 % [CAS 83-72-7], chloroform-d 99.8 atom %D containing 0.05 % (v/v) tetramethylsilane (TMS) (Aldrich) [CAS 865-49-6] and chromatography-grade cellulose (SigmaCell, Type 101) [CAS9004-34-6] were used as supplied. Analytical reagent (AR) grade methanol [CAS 67-56-1] was obtained from Chem-Supply Pty Ltd and was used as supplied.

^{1}H and ^{13}C NMR spectra were recorded using an Agilent Technology 500/54 (500MHz/54 mm bore) premium shielded NMR spectrometer coupled to a 7510-AS autosampler operating at 500 MHz and 125 MHz, respectively. Spectra were calibrated using residual protic solvent (i.e. chloroform-d). Chemical shifts are reported in parts per million (ppm) relative to Me_4Si (TMS) as an internal reference. Spectral processing included proton integration, multiplicity assignment (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of a doublet), determination of coupling constants (Hz) and proton assignment.

A Foster and Freeman Video Spectral Comparator (VSC) 6000 was used to visualise the thin layer chromatography (TLC) plates.

A Varian Cary Eclipse Fluorescence Spectrometer was used to measure the fluorescence of the amino acid solutions.

UV-Visible spectroscopy was performed on an Agilent Technologies Cary 100 UV-Vis Spectrophotometer with the Cary WinUV software.

A Thermo Scientific Nicolet 6700 FT-IR spectrophotometer was used to collect infrared spectra of the amino acid products in transmission mode as potassium bromide (KBr) disks. The corresponding software used was OMNIC 8.3 Thermo Fisher Scientific Inc.

Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) was performed on an Agilent 1200 series liquid chromatograph coupled to an Agilent 6500 series QTOF mass spectrometer. The column used was a Phenomenex C18 column (150 x 3 mm x 5 µm). MassHunter software (Agilent Technologies) was used for instrument control, data acquisition and data analysis. All experiments were run in full scan mode and MS/MS mode. The fragmentation peaks for the mass spectra were reported if they had greater than 10 % abundance relative to the base peak.

2.2.1 Reaction of lawsone with amino acids

A 50-mL two-neck round bottom flask, fitted with a magnetic stirrer and a water cooled condenser, was charged with the amino acid (Table 2-1) and methanol (5 mL). After the complete dissolution of amino acid in methanol, lawsone (0.05 g, 0.29 mmol) was added into the reaction flask. The mixture was brought to reflux and was monitored in 30 min intervals by TLC using a mixture of dichloromethane, ethyl acetate and acetic acid in a ratio of 30 : 70 : 2. The TLC plate was visualised in luminescence mode using the VSC6000 system under an excitation wavelength of 485 - 530 nm with a 610 nm long-pass camera filter. The reaction mixture was allowed to reflux until the reagents were consumed to form the crude product, refer to Table 2-1.

The crude product from each of the reaction mixtures was filtered. The products were analysed without further purification. Luminescence and UV-Vis examinations of the solutions were conducted using 0.1 mL aliquots of each sample that was placed in a quartz fluorescence cuvette and diluted to 3 mL with methanol. FTIR analysis was conducted in transmission mode, where 2 mL aliquot of each sample was vacuum dried prior its to preparation as KBr disks. Prior to NMR analysis, the products (approximately 5 – 7 mg) were dried under vacuum and performed in $CDCl_3$.

Table 2-1 Amino acids and their respective masses used in the reaction

Amino acid	Mass of amino acid (g)	Reaction time (hr)
Alanine	0.0128	5.5
Lysine	0.0210	2
Serine	0.0151	5.5

2.2.2 Reaction of lawsone and amino acids impregnated into cellulose

Preparation of the amino acid impregnated cellulose substrate was conducted following the method described by Spindler *et al.* (106). A 50-mL beaker fitted with a magnetic stirrer was charged with the amino acid (refer to Table 2-1), MilliQ water

(18.2 mΩ, 5 mL) and chromatography-grade cellulose (SigmaCell type 101, 2.5 g). The slurry was allowed to air-dry overnight and was ground into a fine powder prior to use.

A 50-mL two-neck round bottom flask fitted with a magnetic stirrer and a water cooled condenser, was charged with the amino acid impregnated cellulose, lawsone (0.05 g, 0.29 mmol) and the minimum volume of methanol. The mixture was brought to reflux and was monitored in 30 minute intervals by TLC using a mixture of dichloromethane, ethyl acetate and acetic acid in a ratio of 30 : 70 : 2. The reaction product was separated from the cellulose substrate following filtration and washed with methanol. The product was then dried under vacuum prior to analysis.

2.3 Results and discussion

The experiments conducted with amino acids embedded in cellulose following the outlined methods detailed in Spindler *et al.* (106) proved unsatisfactory. In the current study, the reaction proved unsuccessful as it was difficult to determine whether the amino acids were completely embedded within the cellulose structure and therefore a 2:1 stoichiometric ratio could not be ensured. Further, the isolation of the products from cellulose was problematic. The reaction products were analysed by NMR spectroscopy and revealed large amounts of impurities. Separation of the products was attempted using column chromatography and preparative TLC. The resulting products were re-analysed by NMR spectroscopy which still showed significant amounts of impurities. Thus, an alternative method for synthesis was sought, where lawsone was refluxed with amino acids in methanol. The results of this alternative method are described in the sections below.

It was observed in the *in situ* reaction of the lawsone reaction with the three amino acids in methanol under reflux that the colour of the solution was indicative of product formation, that is, the colour of the solution changed from yellow to red. It was also observed that the reaction of lysine with lawsone is faster than the other two amino acids that were tested. This was likely due to the differences between the structures of the three amino acids. The presence of two amino groups on lysine (Figure 2-3) may

have led to products other than the proposed product and/or other intermediates. The structures of these proposed products/intermediates are elucidated by the analyses conducted detailed in the following sections.

2.3.1 FTIR spectroscopy

Evidence of lawsone reacting with amino acids was provided by FTIR analysis. Figure 2-4 shows three overlaid spectra of the product following the reaction with three different amino acids. It also shows the FTIR spectrum of the reaction of lawsone with amino acids, which is used as a reference to highlight the conversion of lawsone to the reaction product. Figure 2-5 shows (a) the reaction product of lawsone and amino acids proposed by Jelly (127) and (b) the reaction product of lawsone and amino acids proposed in this thesis.

The spectra from the three reactions, show a peak at 3400 cm^{-1} for the amine (N-H) stretch, which is in the expected region indicating the presence of a secondary amine. A comparison of the absorption bands of lawsone and the lawsone-amino acid product indicate the lack of carbonyl (C=O) stretch arising from C1 of lawsone (literature value 1644 cm^{-1} (C1=O)) in the product spectra. It is likely that increasing the conjugation of the lawsone-amino acid product has moved the absorption of the carbonyl (C=O) to a lower wavenumber (~1600 cm^{-1}). The carbonyl (C=O) stretch (~1680 cm^{-1}) at C4 is present in all four spectra. The formation of the product can be supported by the appearance of the peak at 1550 cm^{-1}, which corresponds to the formation of the imine functional group. The quinone absorption band at ~1300 cm^{-1} is present in the product, albeit at a lower wavenumbers than lawsone but this may be attributed to differences in conjugation. The presence of these peaks is indicative of a reaction occurring and is further supported by the spectral differences between the reaction products and lawsone.

Figure 2-4 FTIR spectra of lawsone, and the products from the reaction of lawsone and three different amino acids.

2.3.2 NMR spectroscopy

NMR spectroscopy was used in an attempt to analyse and characterise the reaction product and/or products formed between lawsone and the three amino acids. A combination of one dimensional and two dimensional NMR experiments were conducted, proton (^{1}H) and carbon (^{13}C), heteronuclear singular quantum coherence spectroscopy (^{1}H – ^{13}C HSQC) and correlation spectroscopy (^{1}H – ^{1}H COSY). As mentioned in the previous sections, Jelly *et al.* proposed the formation of a product, as shown in Figure 2-5(a) (127).

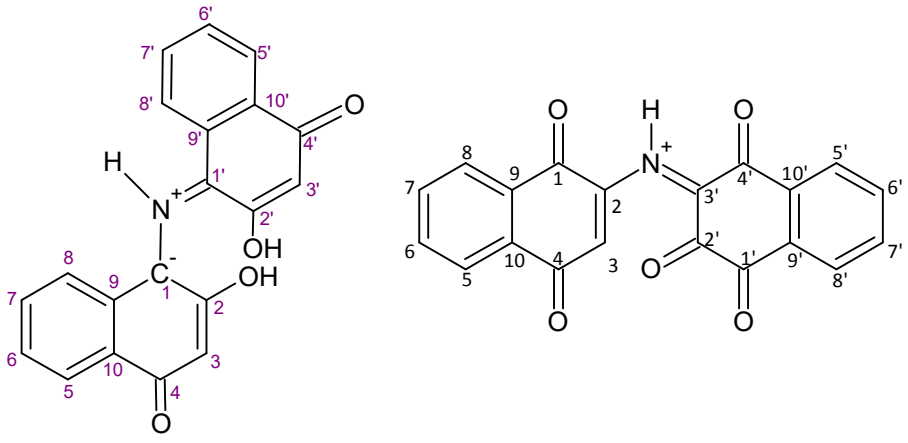


Figure 2-5 (a) Structure of proposed lawsone-amino acid product formed by Jelly (127) (b) structure of proposed lawsone-amino acid product based on the results of this thesis.

The following NMR results aim to support the structure depicted in Figure 2-5 (b). In the experiments conducted, it was determined that the ^{1}H NMR spectra of the compounds from the three different reactions showed the peaks that contained the same splitting patterns. These peaks include a doublet owing to C5,5'-H and C8,8'-H integrating to four protons, a doublet of a triplet owning to C6,6'-H and C7,7'-H integrating to four protons, a singlet owing to C3-H integrating to one proton and a singlet owing to the amine (NH) integrating to one proton. Figure 2-6 shows the ^{1}H NMR spectrum of a selected region, δ2.0 ppm to δ9.0 ppm, of the lysine and lawsone

reaction product, while Figures 2-7 to 2-9 show the expanded region of the lawsone reaction product with the three different amino acids.

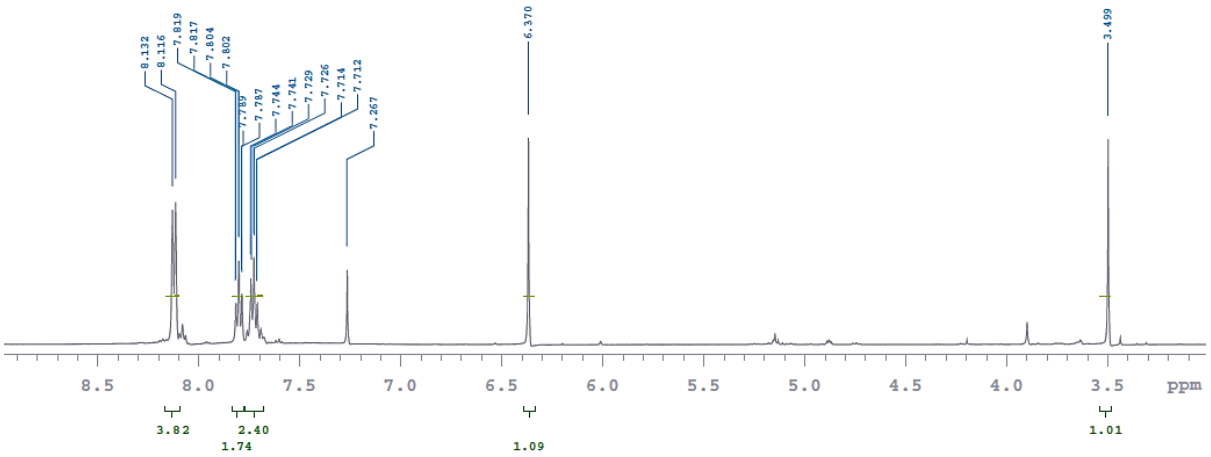


Figure 2-6 ^{1}H NMR spectrum of lysine and lawsone reaction product in $CDCl_3$.

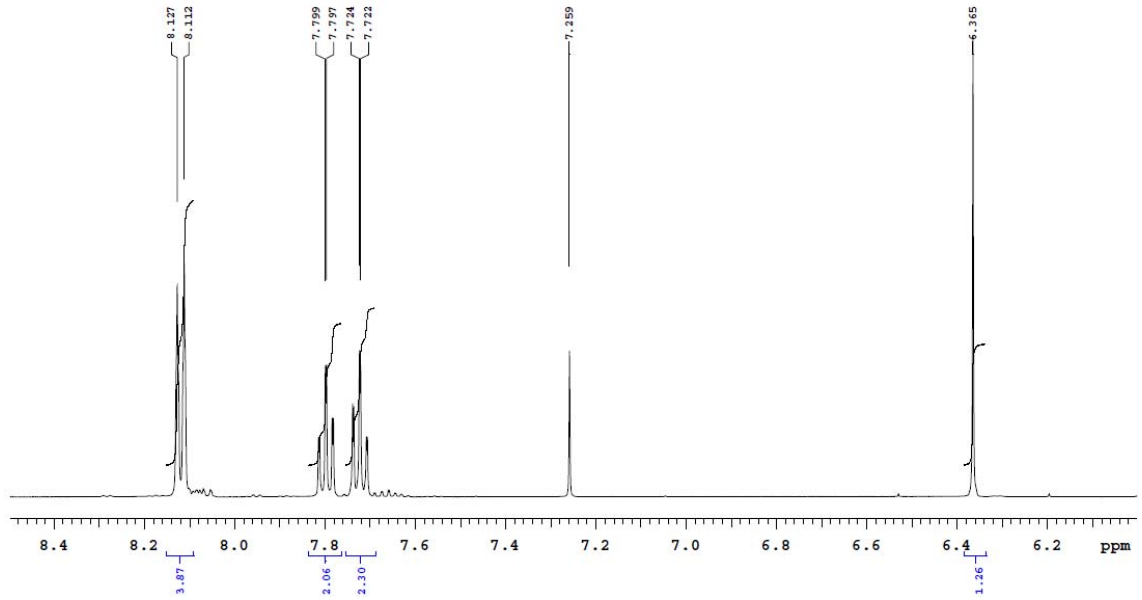


Figure 2-7 ^{1}H NMR spectrum of alanine and lawsone reaction product (6.0 – 8.4 ppm region) in $CDCl_3$.

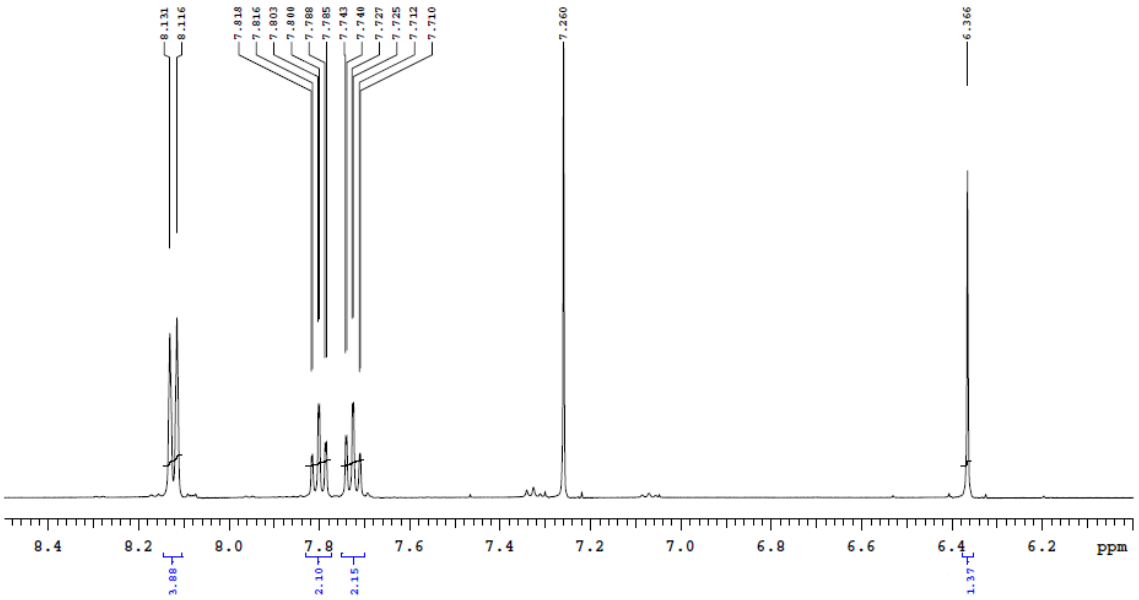


Figure 2-8 ^{1}H NMR spectrum of lysine and lawsone reaction product (6.0 – 8.4 ppm region) in $CDCl_3$.

.

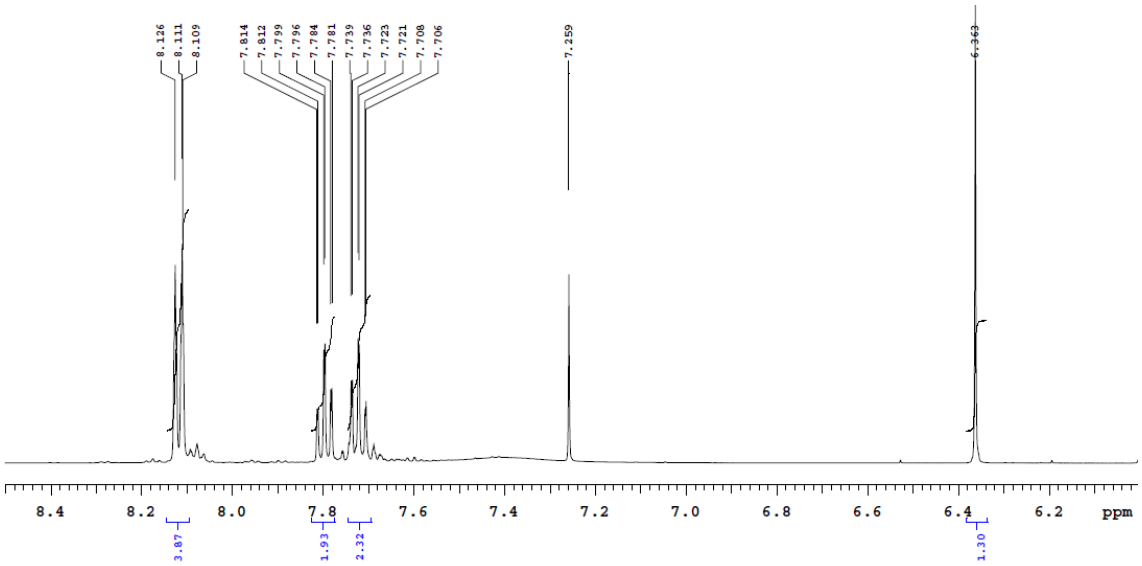


Figure 2-9 ^{1}H NMR spectrum of serine and lawsone reaction product (6.0 – 8.4 ppm region) in $CDCl_3$.

Based on the structure of the lawsone-amino acid product as depicted in Figure 2-5(b), it is predicted to show five different proton environments in a ^{1}H NMR spectrum. Four of the five were described above and were in the expected regions. A comparison of the experimental data and the spectrum of lawsone standard (Figure 2-10) indicated

that the proton owing to the hydroxy group is absent within the ^{1}H NMR spectra of the three reaction products.

A comparison of the ^{1}H NMR spectrum of lawsone (Figure 2-10) and the reaction products (Figures 2-7, 2-8, and 2-9) show that the chemical shifts of the aromatic protons as well as the vinyl proton (C3-**H**) have not experienced a dramatic change in their chemical shift. Also of note are the similarities of the chemical shifts of the corresponding protons and splitting patterns in the products from the three different reactions. The data indicates that the reaction product retained the core structure of lawsone.

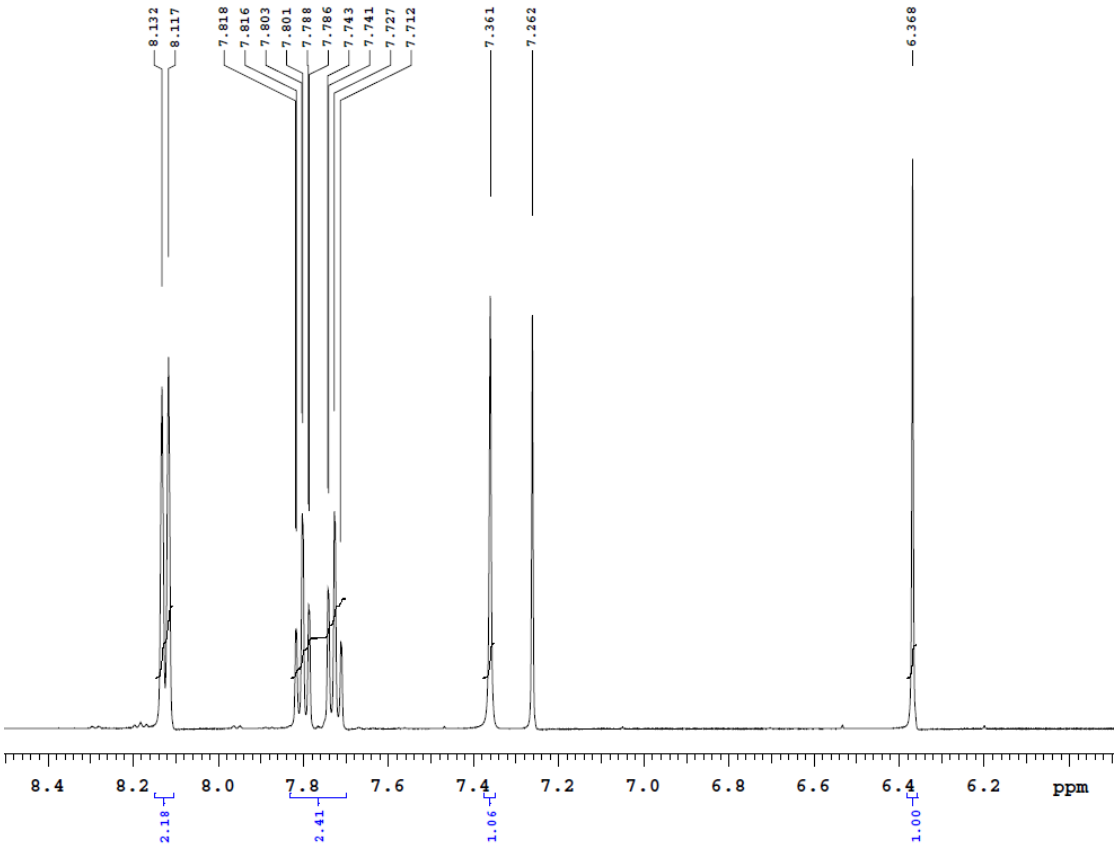


Figure 2-10 ^{1}H NMR spectrum of lawsone (6.0 – 8.4 ppm region) in $CDCl_3$.

The chemical shifts, number of proton environments due to molecular symmetry and integration values indicate the formation of the product in a 2:1 ratio of lawsone to amino acid. The NMR spectroscopy results of the three different reactions produced

the same number of chemical environments with correspondingly similar chemical shifts, suggesting the three different reactions produced the same product.

The yellow product, 2-amino-1,4-naphthoquinone that Jelly (135) noted in their synthetic studies was not present in the three different reactions that were conducted as part of this research. The ^{1}H and ^{13}C NMR spectra did not have the characteristic peaks as indicated in literature for 2-amino-1,4-naphthoquinone (124).

The ^{13}C NMR spectrum of the reaction product of lawsone and serine is shown in Figure 2-11. Confirmation of the presence of the amino group was possible from the ^{13}C NMR spectra. The chemical shift, δ50.9 ppm, for the CN in the proposed structure is present as a singlet in ^{13}C NMR spectrum and is in the expected region. The resonances of other functional groups, as well as the number of different chemical environments, are also observed in the expected region. The C3 singlet is present at δ110.7 ppm, the carbonyl singlets are present at δ179.5, δ184.9 and δ181.9 ppm, while the carbons within the aromatic system are present with chemical shifts ranging from δ135.2 – δ126.4 ppm. The chemical shifts of these carbon environments also closely correspond to the reported literature values for lawsone. This is further confirmation of the formation of the proposed structure. The assigned values of the product are shown in Figure 2-11.

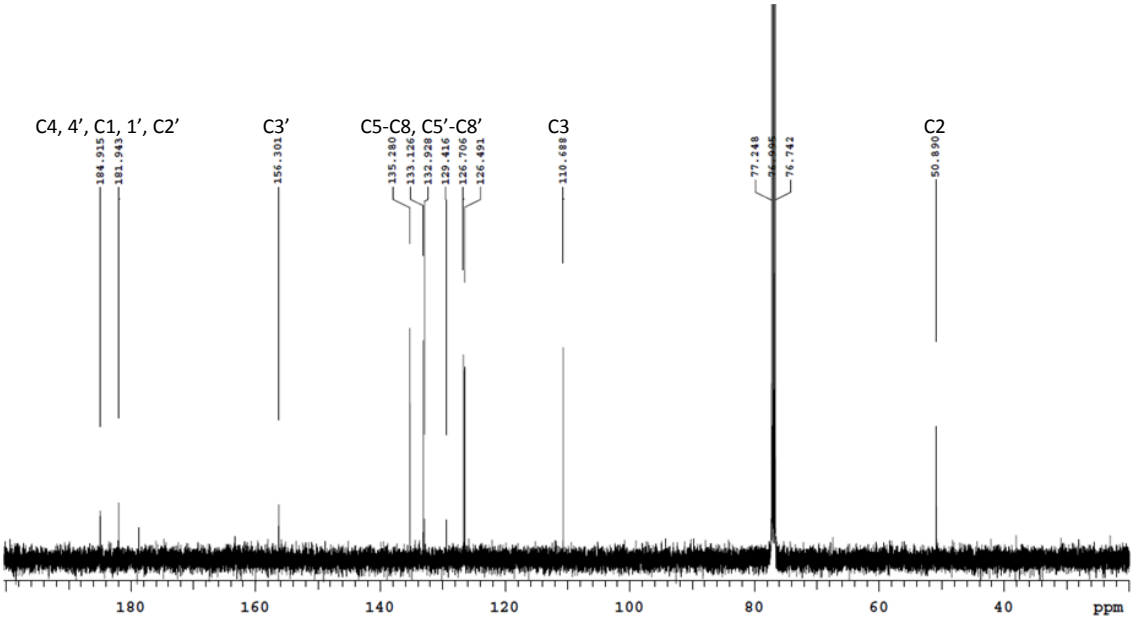


Figure 2-11 ^{13}C NMR spectrum of the proposed serine and lawsone reaction product in $CDCl_3$.

Two-dimensional (2D) NMR spectroscopy, including ^{1}H – ^{13}C HSQC and ^{1}H – ^{1}H COSY experiments, were also conducted on the reaction products of the three reactions. Similarly to the ^{13}C and ^{1}H NMR spectra, the ^{1}H – ^{13}C HSQC and ^{1}H – ^{1}H COSY spectra for the three products were comparable and indicated the correct assignment of the relative position of the carbons and protons that are present in the molecule. The ^{1}H – ^{13}C HSQC spectra are shown in Figure 2-12 and Figure 2-13.

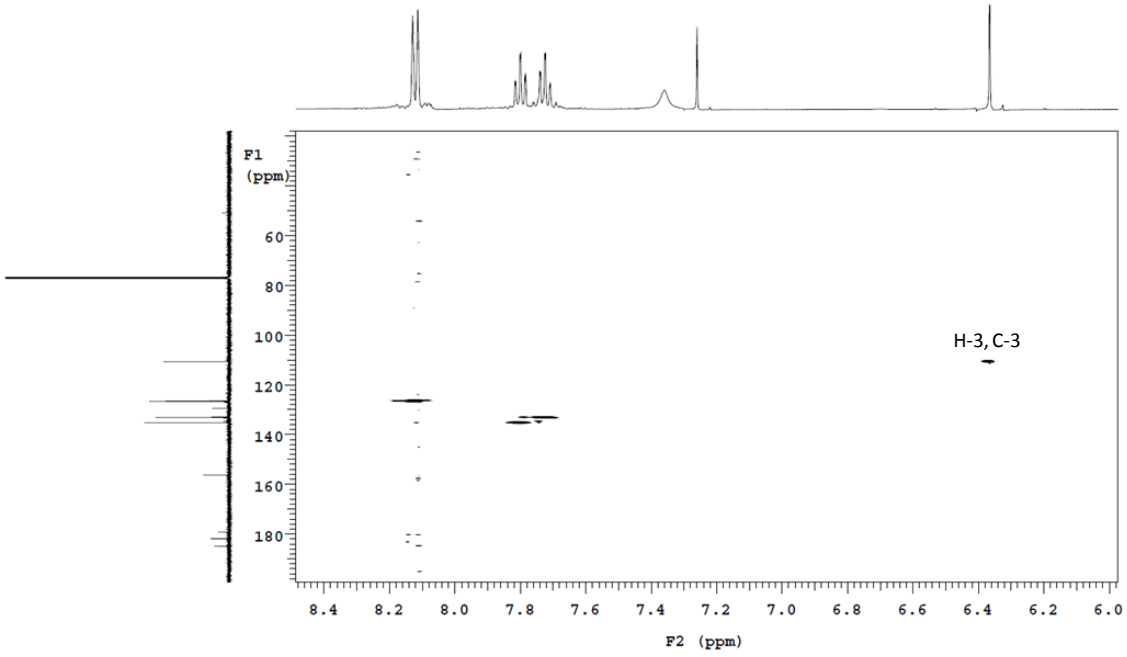


Figure 2-12 Selected region (6.3 – 8.4 ppm) of ^{1} H- ^{13}C HSQC spectrum of serine-lawsone reaction product in $CDCl_3$.

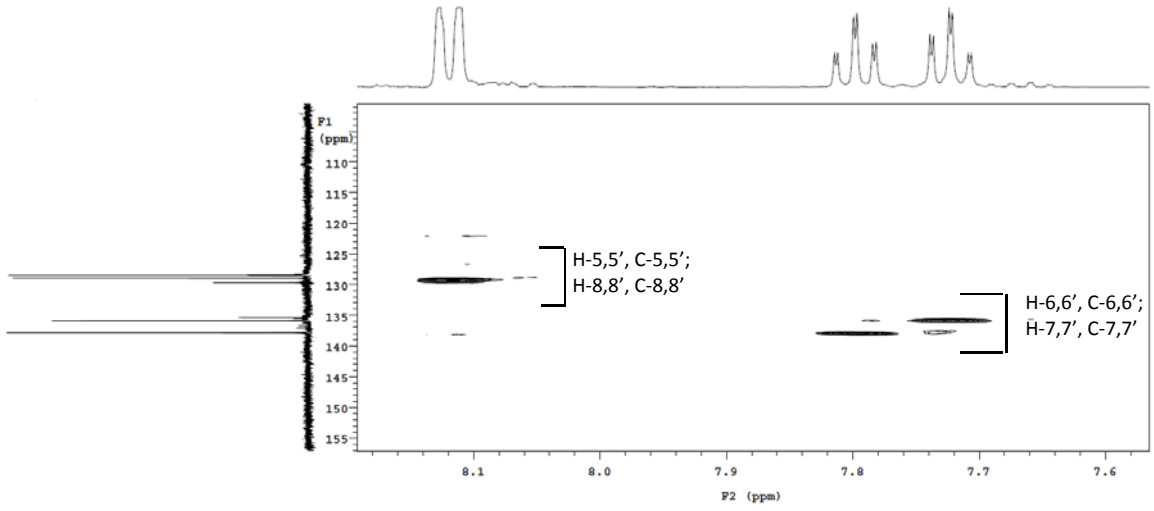


Figure 2-13 Expanded region (7.6 – 8.2 ppm) of the ^{1}H – ^{13}C HSQC spectrum from Figure 2-12.

The ^{1}H – ^{1}H COSY experiments were conducted to show coupling between protons usually up to three bonds and occasionally four to five bonds apart. Figure 2-14 demonstrates the ^{1}H – ^{1}H COSY NMR spectrum of the lysine and lawsone reaction product and showed further evidence of the correct chemical shift assignments of the

protons. As Figure 2-14 illustrates, there are correlation spots between the protons in the multiplex resonating at δ7.8 ppm and the protons resonating at δ8.1 ppm.

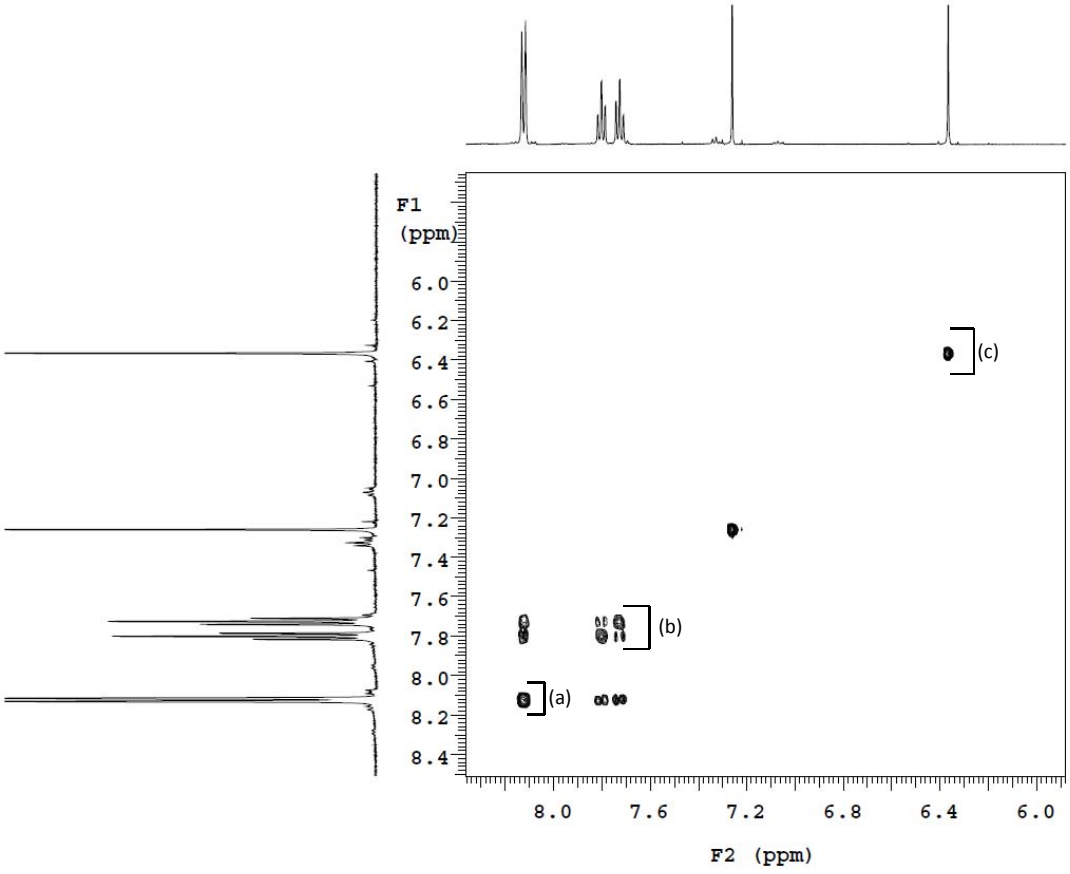


Figure 2-14 Selected region of ^{1}H-^{1}H COSY spectrum of the lysine and lawsone reaction product depicting (a) H-5, 8, 5' and 8' (b) H-6, 7, 6' and 7' and (c) H-3.

2.3.3 LC-MS

LC-MS experiments were also conducted on the three different reaction mixtures to determine whether any by-products were formed that were below the limit of detection for NMR spectroscopy. These experiments produced an accurate mass for the compounds detected, making it possible to propose structures and their MS fragmentation pathways.

A number of different compounds that may be of interest were found in the three different reactions. Figure 2-15(a) indicates that there are four peaks present

indicating that there may be four compounds of interest in the lawsone and alanine reaction.

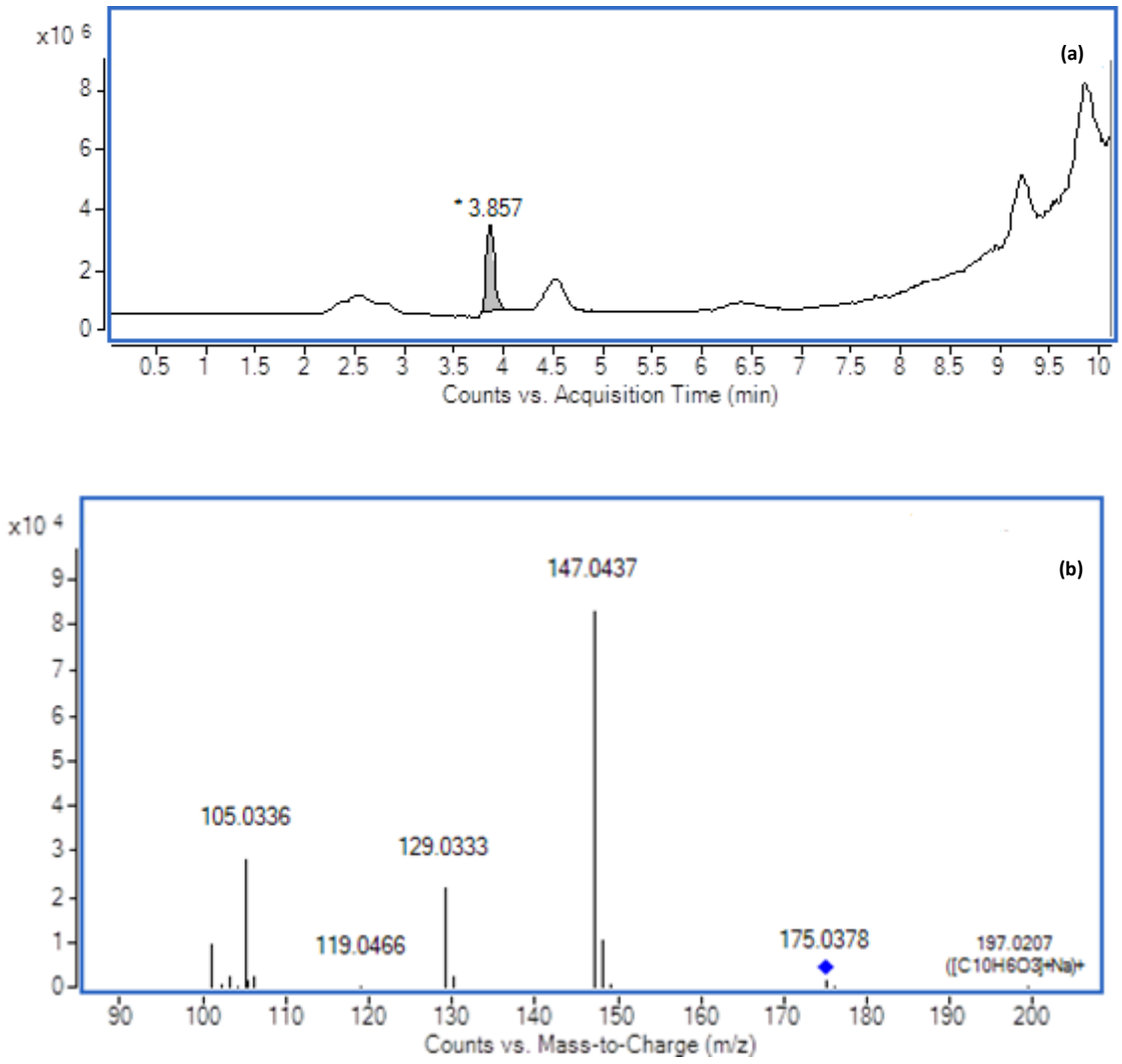


Figure 2-15 (a) TIC chromatogram of the alanine and lawsone reaction mixture (FE = 170 V), and (b) product ion scan spectrum of the product with $[M+H]^+$ of 175.0378 and $[M+Na]^+$ of 197.0207 (FE = 170 V, CE = 25eV).

The HRMS product ion scan spectrum of the product (Figure 2-15 (b)), at R_t = 3.8 min, shows the $[M+H]^+$ at *m/z* 175.0378, correlates with the molecular formula for protonated lawsone ($C_{10}H_6O_3$, 0.68 ppm mass accuracy error). In further support of this finding, the MS peak corresponding to the sodium adduct of lawsone ($[M+Na]^+$) is also present at *m/z* 197.0207. The fragmentation pattern also correlates with the fragmentation of lawsone reported in literature (138).

The product ion scan spectra of the products from at R_t = 4.5 min and 9.3 min are shown in Figure 2-16 (a) and (b), respectively.

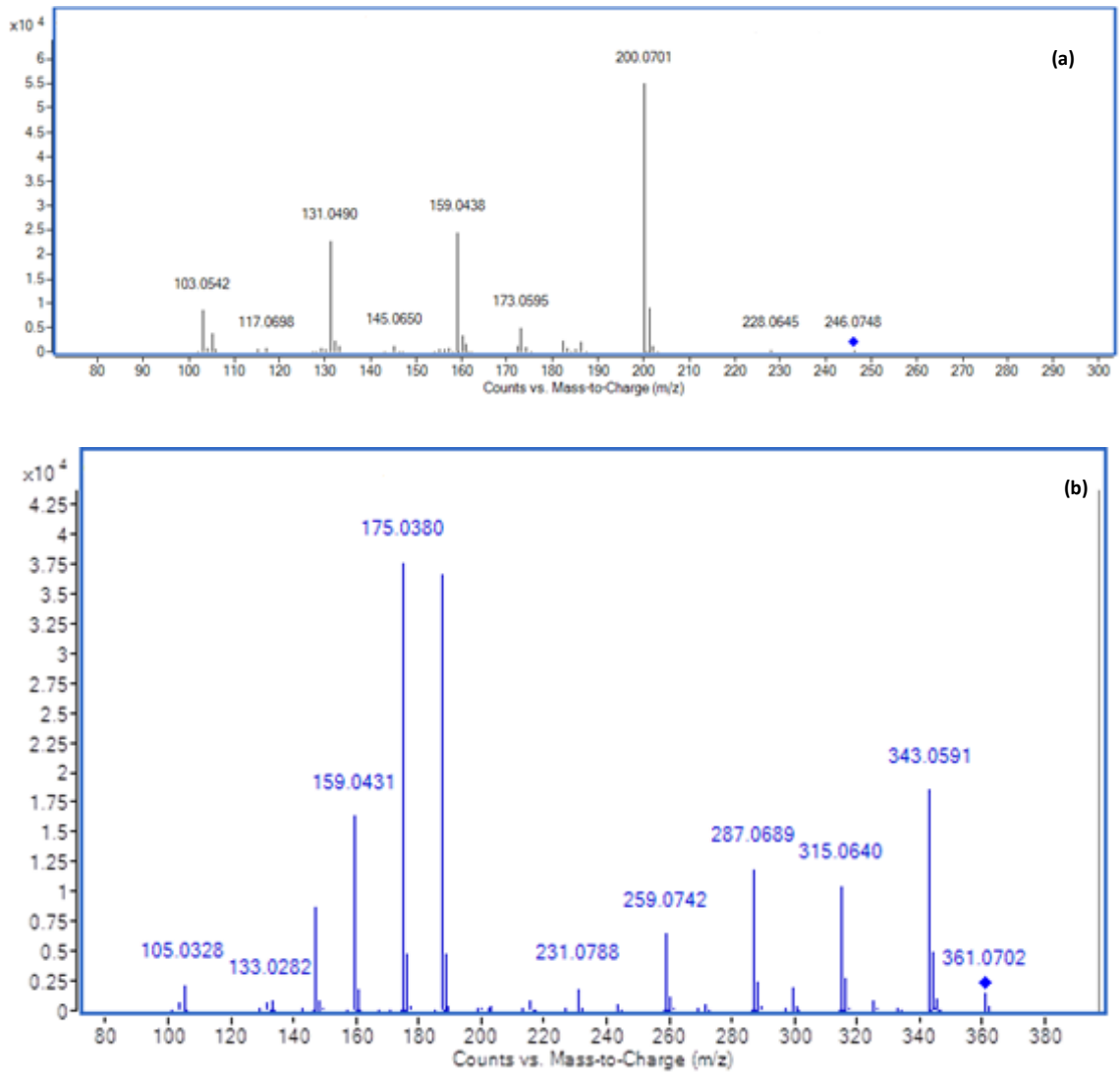


Figure 2-16 (a) Product ion scan spectrum of the product with $[M+H]^+$ = 246.0748, and (b) product ion scan spectrum of the product with $[M+NH_4]^+$ of 361.0702 (FE = 170 V, CE = 25 eV).

Figure 2-16 (a) shows the $[M+H]^+$ at *m/z* 246.0748, which correlates with the molecular formula for $C_{13}H_{11}NO_4$, mass accuracy error -0.43 ppm, with R_t = 4.5 min. Figure 2-16 (b) shows the $[M+NH_4]^+$ at *m/z* 361.0702, which corresponds to the molecular formula for $C_{20}H_{13}N_2O_5$, mass accuracy error 35.3 ppm, with R_t = 9.3 min. The proposed

structures for the two products at *m/z* 246.0748 and *m/z* 361.0702 and their fragmentation pathways are depicted in Figure 2-17and Figure 2-18, respectively.

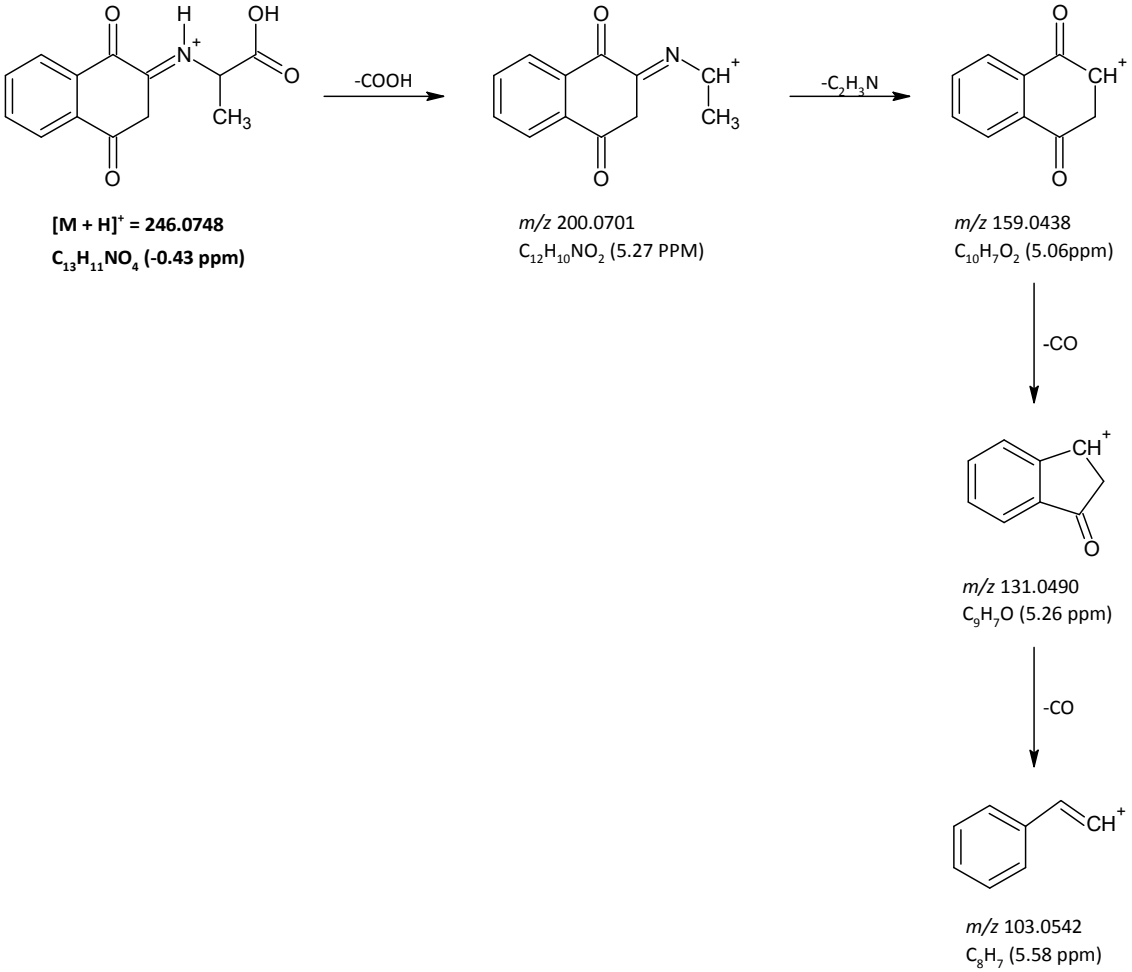


Figure 2-17 Proposed structure and MS fragmentation pathway for product with $[M+H]^+$ of 246.0748.

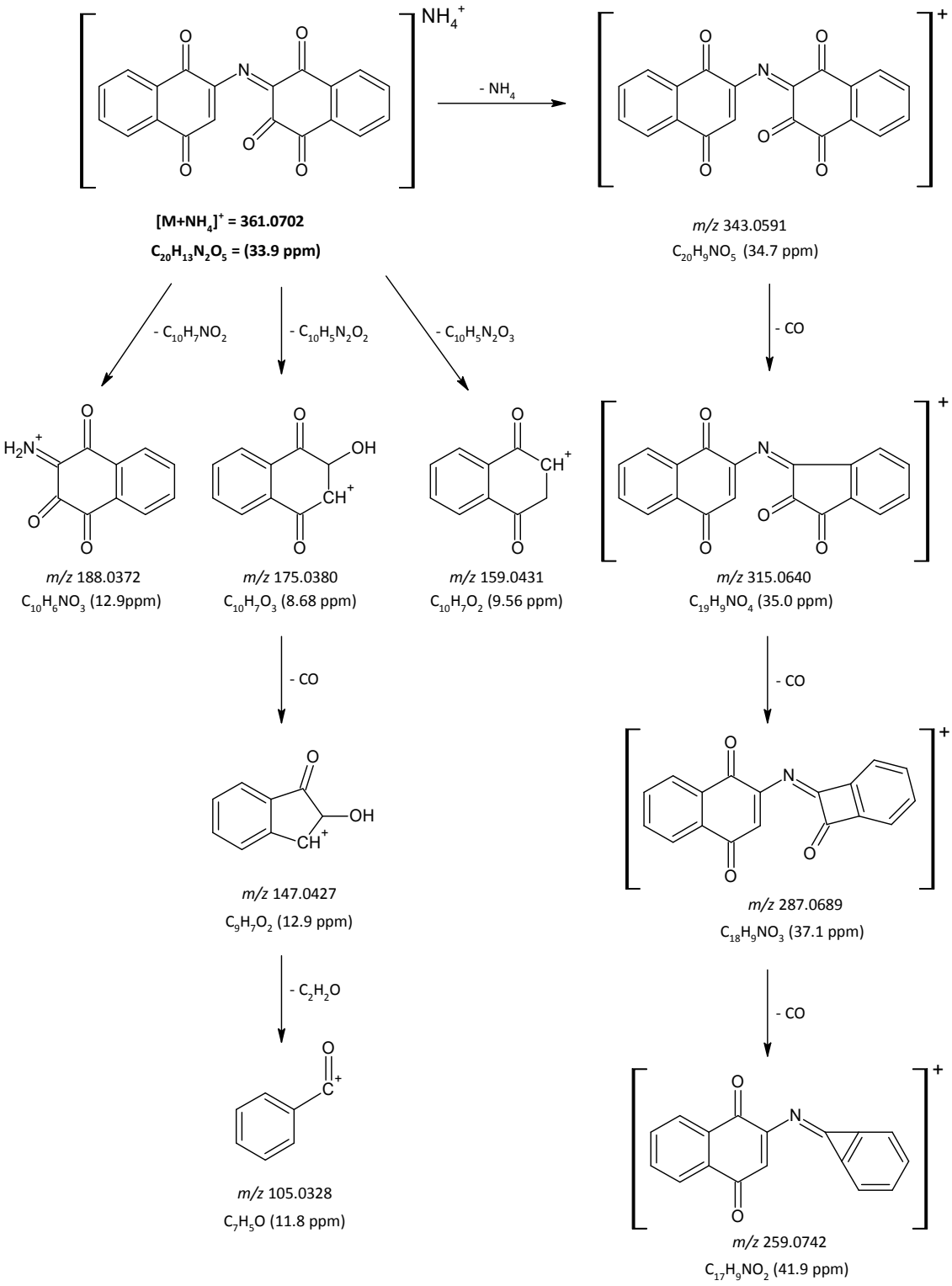


Figure 2-18 Proposed structure and MS fragmentation pathways for product $[M+NH_4]^+$ of 361.0702.

The protonated product, $[M+H]^+$, at *m/z* 246.0748, is proposed to be an intermediate formed from the reaction between lawsone and alanine (Figure 2-19). In the reaction, it is proposed that the NH_2 group of the α-amino acid (alanine) undergoes addition at C2 then eliminates H_2O to form the product, an imine derivative.

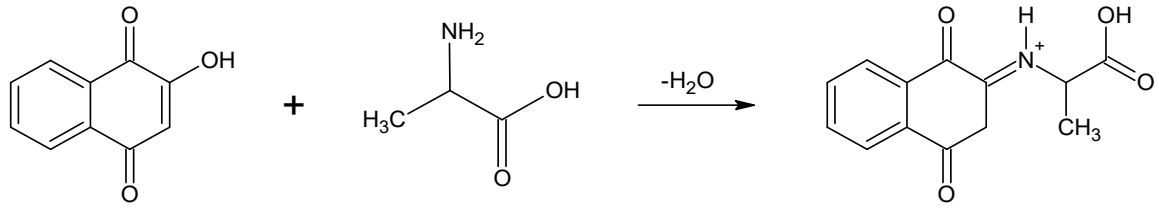


Figure 2-19 Reaction scheme for the reaction of lawsone and alanine.

If indeed the reaction of lawsone is analogous to that of ninhydrin with various amino acids, the reaction of which has been studied extensively (77; 139), then the product at *m/z* 246 is likely to be the intermediate that is formed prior to the decarboxylation step in the formation of the final product which is similar to that of RP. The literature describing the mechanism for the formation of RP (77) supports the proposed structure of the intermediate proposed in Figure 2-19 and its mass fragmentation pattern in Figure 2-17.

The protonated product, $[M+NH_4]^+$, at *m/z* 361.0702, corresponding to the formula $C_{20}H_{13}N_2O_5$ (33.9 ppm mass accuracy error) is proposed to have formed as a result of the reaction between lawsone and alanine in a two-to-one ratio, respectively. A possible mechanism for the formation of this product is illustrated in Figure 2-20. Comparison of the product ion scan spectrum obtained for the product at *m/z* 175 (Figure 2-15 (b)) and the fragmentation pattern belonging to the product at *m/z* 361 showed that it possessed the same prominent product ion at *m/z* 175 and 159. From these major fragmentation ions, it suggests that the product at *m/z* 361 shares a common molecular structure with its starting material.

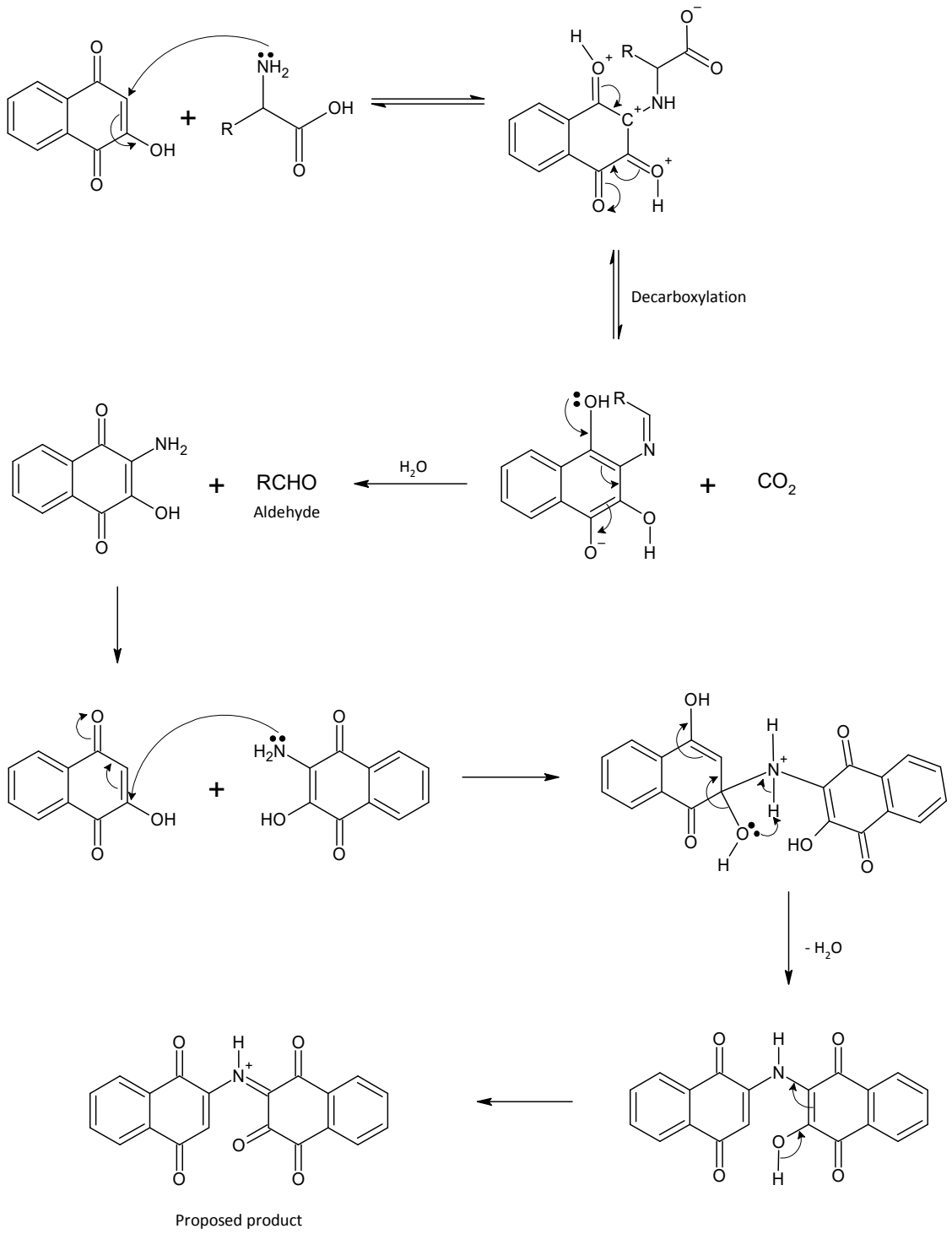


Figure 2-20 Proposed mechanism for the reaction of lawsone and α-amino acids.

The TIC chromatogram, Figure 2-21, of the lawsone and lysine reaction shows that there are potentially four compounds of interest.

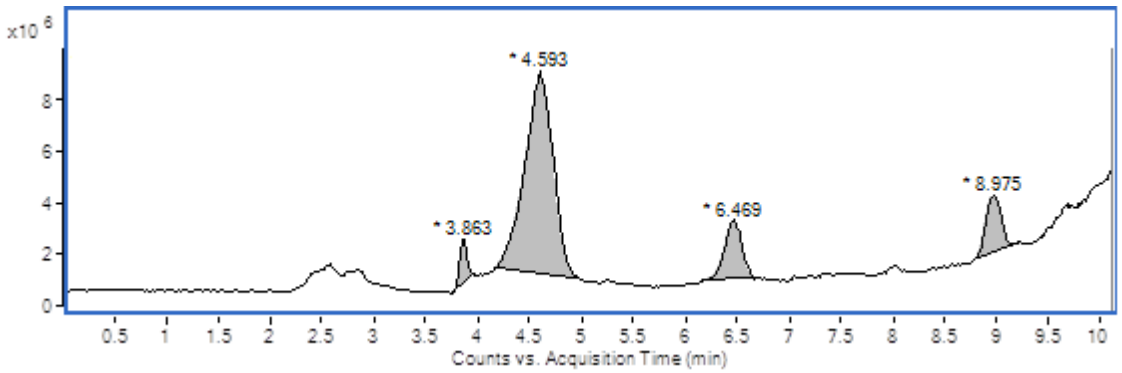


Figure 2-21 TIC chromatogram showing the lysine and lawsone reaction mixture (FE = 170 V).

The product ion scan spectra of the compounds with the elution time of 3.86 min and 8.98 min, appear to have a protonated molecular mass at *m/z* 175.0388 and *m/z* 361.0701, respectively. Closer inspection of the product ion scan spectrum for the protonated product at *m/z* 175.0388 revealed that it shared common product ions as the $[M+H]^+$ at *m/z* 175 from the lawsone and alanine reaction. Further, it also corresponded well to the formula $C_{10}H_6O_3$ (4.108 ppm mass accuracy error), which was previously identified as lawsone. It was determined that the product at *m/z* 361.0701 corresponded to the molecular formula $C_{20}H_{13}N_2O_5$. This compound was observed to have a fragmentation pattern consistent with the protonated compound at *m/z* 361 in the lawsone and alanine reaction. Therefore, it is likely that the same compound was formed in both reactions.

The protonated molecular mass for the compound at 4.59 min had a *m/z* 258.1124, with major product ions at *m/z* 240.1014, *m/z* 223.0764, *m/z* 213.0908, *m/z* 195.0802, *m/z* 175.0387 and *m/z* 105.0336 (Figure 2-22). It was hypothesised that the protonated molecule corresponded to the molecular formula $C_{15}H_{15}NO_3$ (2.40 ppm mass accuracy error). Fragmentation of the protonated molecule resulted in the loss of water to yield the prominent ion at *m/z* 240.1014. From this fragment, a mass difference of 17 Da was observed, which may be explained by a loss of NH_3. From *m/z* 240.1014, a loss of CHN was proposed, corresponding to a mass difference of 27 Da, at

product ion peak *m/z* 213.0909. The observation of product ion peak at *m/z* 195.0802 may be explained by the loss of H_2O from *m/z* 213.0909.

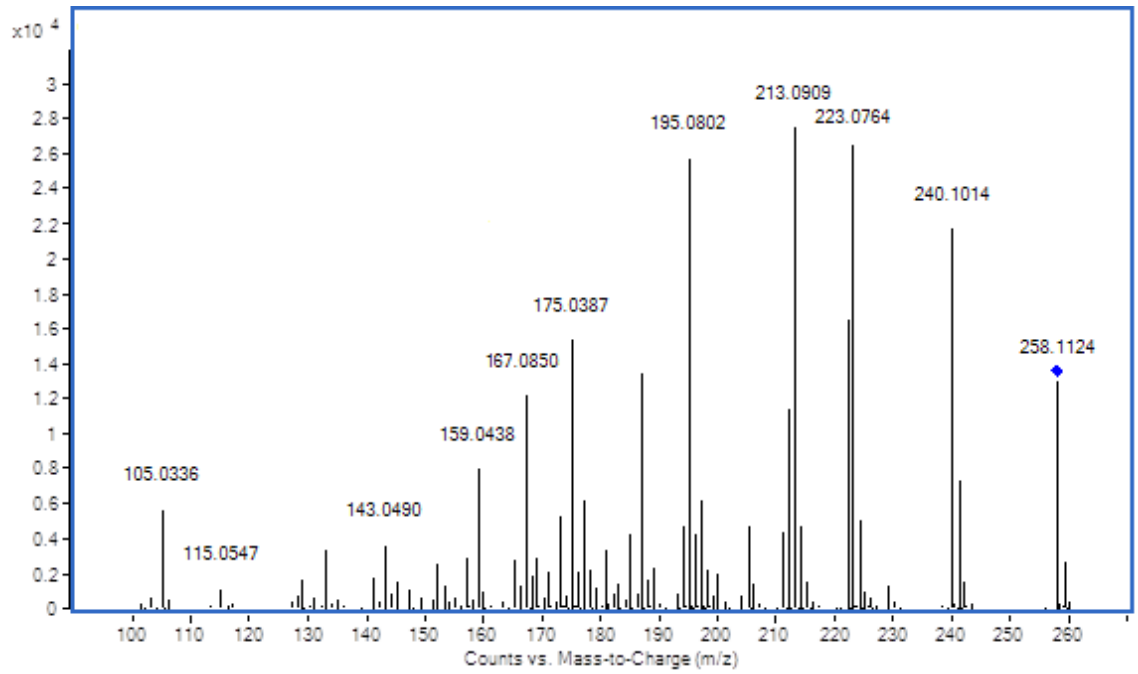


Figure 2-22 Product ion scan spectrum of the product with $[M+H]^+$ of 258.1124 (FE = 170 V, CE = 25 eV).

The product ion peak at *m/z* 175.0387, could be explained by the removal of C_5H_9N from the molecular ion. This peak corresponded to the formula $C_{10}H_7O_3$ (4.68 ppm mass accuracy error) and may have produced two product ion peaks, one at *m/z* 159.0438 and the other at *m/z* 105.0336. The latter product ion peak was also observed as a product ion peak for *m/z* 175, which was matched to the formula for lawsone. The product ion peak observed at *m/z* 175.0387 is highly indicative of the lawsone molecular structure within the compound at *m/z* 258. The tentative fragmentation pathway the proposed protonated product at *m/z* 258 (R_t = 4.59 min) is depicted in Figure 2-23.

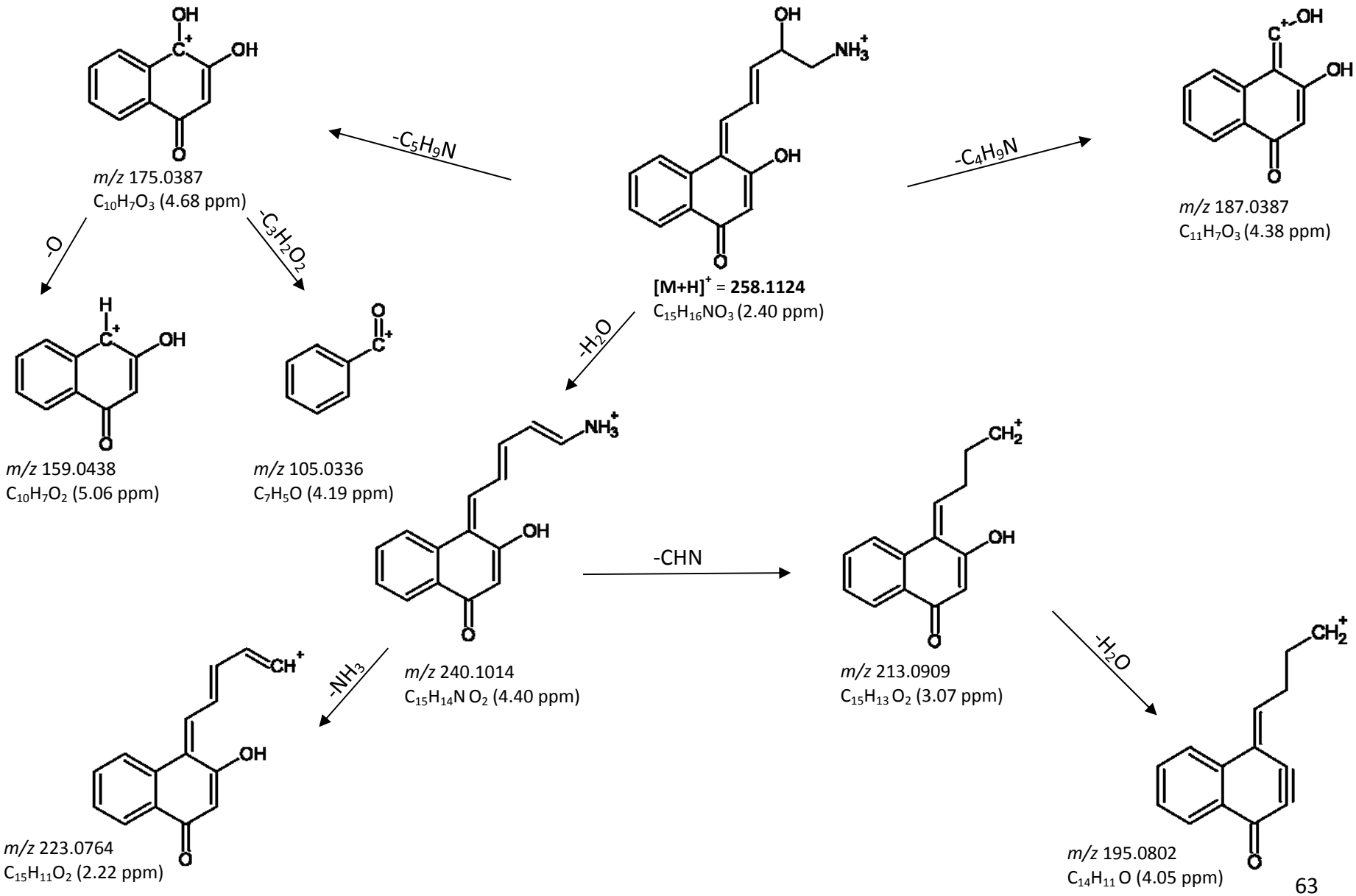


Figure 2-23 Proposed structure and MS fragmentation pathway of product with $[M+H]^+$ of 258.1124.

A relatively less intense peak, eluting at 6.47 min, was also observed in the TIC chromatogram of the lawsone and lysine reaction. The product ion scan spectrum, Figure 2-24, reveals that it has a protonated molecular mass at *m/z* 444.1437, with major product ions at *m/z* 258.1120, *m/z* 187.0387 and *m/z* 105.0349. The mass of the protonated molecule was tentatively assigned the molecular formula $C_{26}H_{22}NO_6$, with 2.28 ppm mass accuracy error.

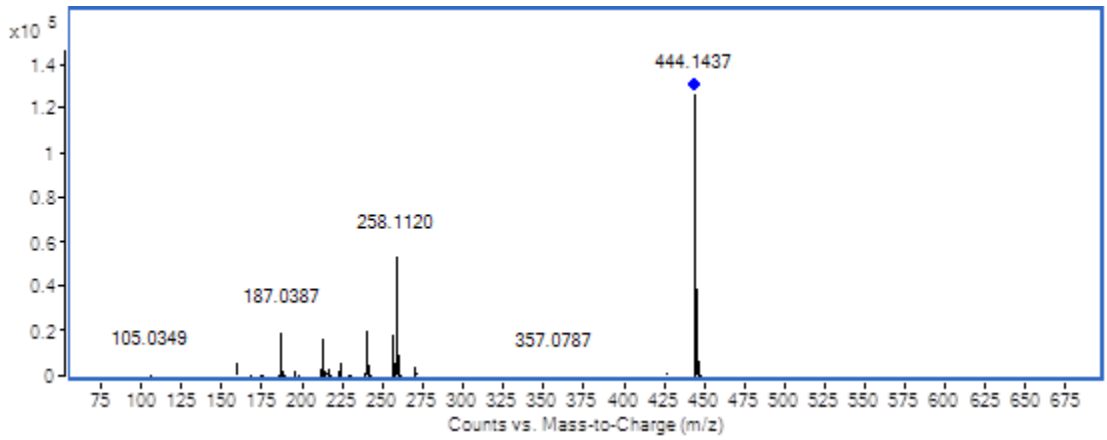


Figure 2-24 Product ion scan spectrum of the product with $[M+H]^+$ = 444.1437 (FE = 170 V, CE = 25.0 eV).

The protonated product, $[M+H]^+$ at *m/z* 444.1437, produced fragment ions at *m/z* 187.0387 and 105.0349. These fragments were also found in the protonated molecule at *m/z* 258.1124. This suggests that the compound at *m/z* 444 is likely to have a molecular structure resembling the protonated product at *m/z* 258, after a mass loss of 186 Da. The tentatively proposed structure and fragmentation pathway of the protonated compound at *m/z* 444 is presented in Figure 2-25. The two product ions at *m/z* 105.0349 and 187.0387, are proposed to have formed in a similar manner as the product ions from the protonated product at *m/z* 258, as depicted in Figure 2-23.

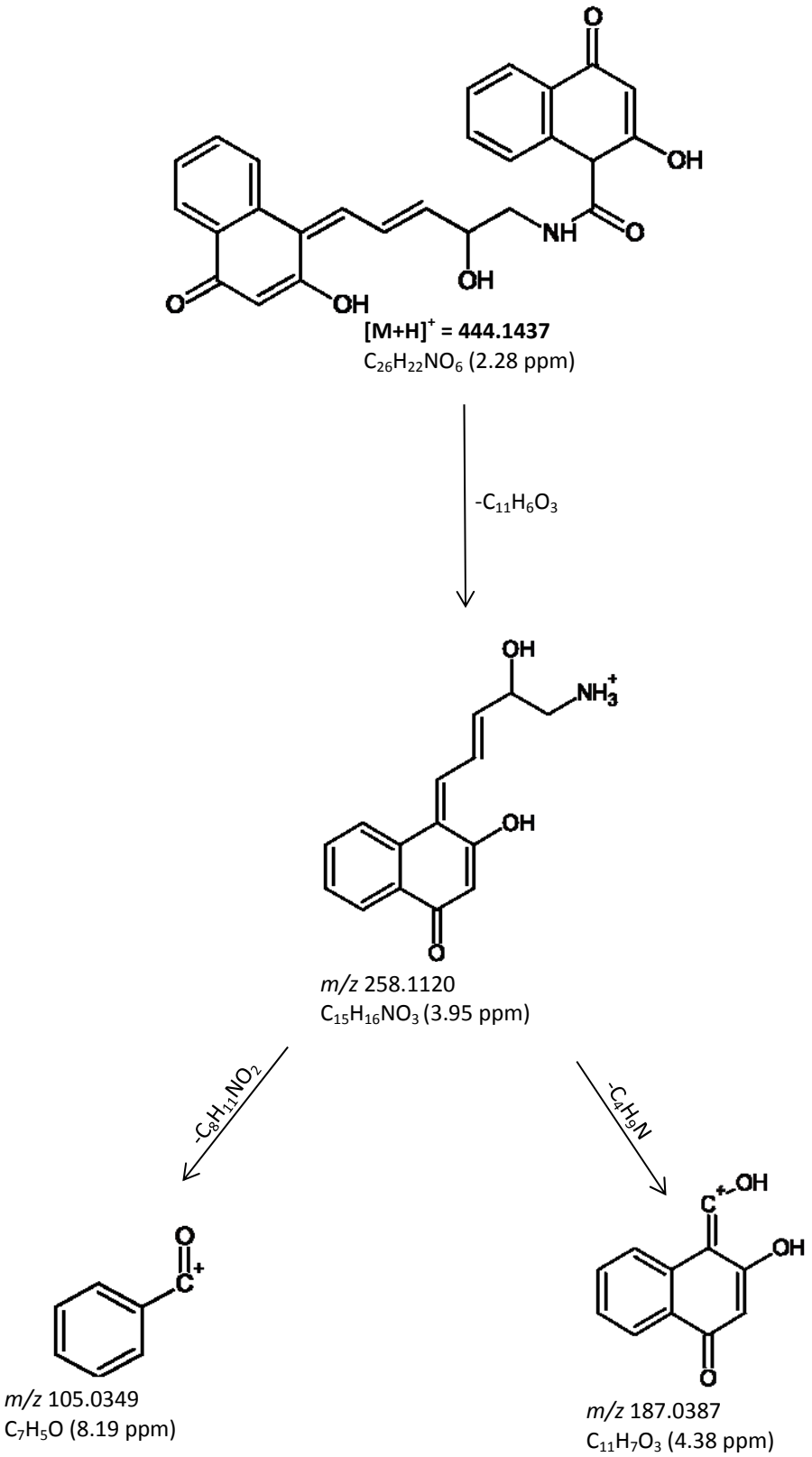


Figure 2-25 Proposed structure and MS fragmentation pathway of product with $[M+H]^+$ of 444.1437.

Analysis of the TIC chromatogram for the reaction of lawsone and serine appeared to have yielded three compounds of interest, eluting at retention times 3.71 min, 6.96 min and 8.74 min, depicted in Figure 2-26.

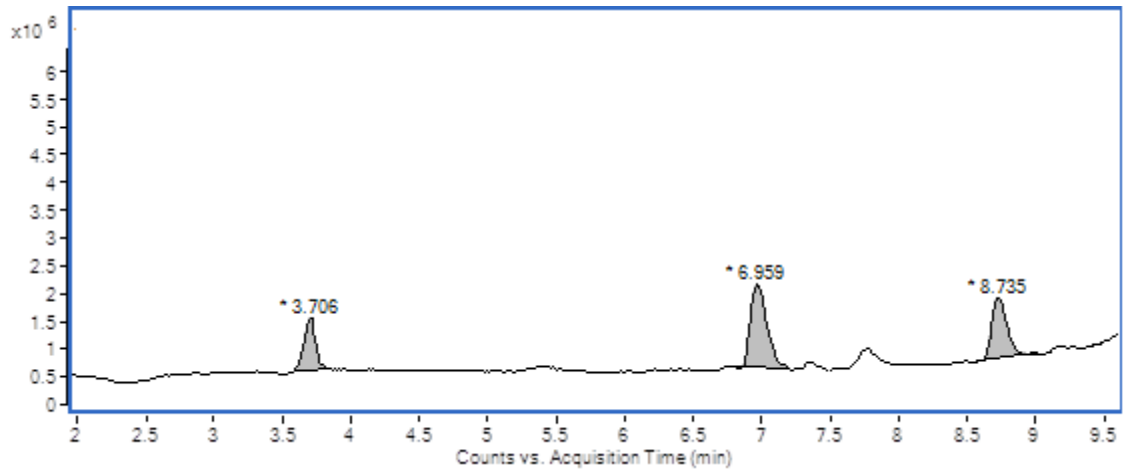


Figure 2-26 TIC chromatogram of the showing the lawsone and serine reaction mixture (FE 170 V).

The product ion scan spectra of the compounds with the elution time of 3.71 min and 8.74 min, appeared to have a protonated molecular mass at *m/z* 175.0389 and 361.0689, respectively. The two compounds were observed to have product ion peaks consistent with the protonated compounds at *m/z* 175 and *m/z* 361, in the lawsone and alanine and also the lawsone and lysine reactions. Therefore, it is likely that the same compound (*m/z* 361) was formed in both reactions.

The protonated molecule, eluting at 6.96 min, was observed at *m/z* 373.0698, corresponding well to the molecular formula $C_{22}H_{13}O_6$ (3.79 ppm mass accuracy error) (Figure 2-27). The main product ion peaks at *m/z* 355.0581, 327.0647, 299.0699 and 271.0745, were tentatively assigned structures with a proposed MS fragmentation pathway from the protonated molecular ion (Figure 2-29). In Figure 2-28 the first product ion at *m/z* 355.0581 indicated a neutral loss of water from the precursor ion. A loss of 28 Da was observed for the subsequent major product ions, which may be explained by the loss of CO from the proposed MS fragments.

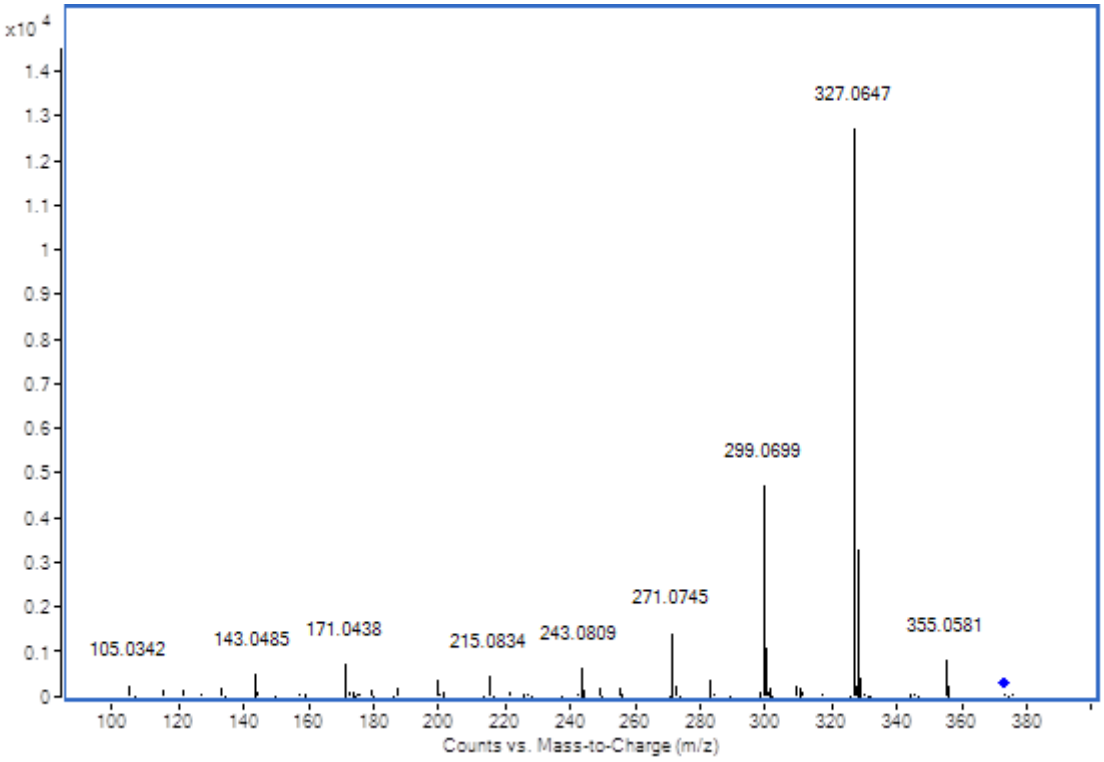


Figure 2-27 Product ion scan spectrum of product with $[M+H]^+$ of 373.0698 (FE = 170 V, CE = 25 eV).

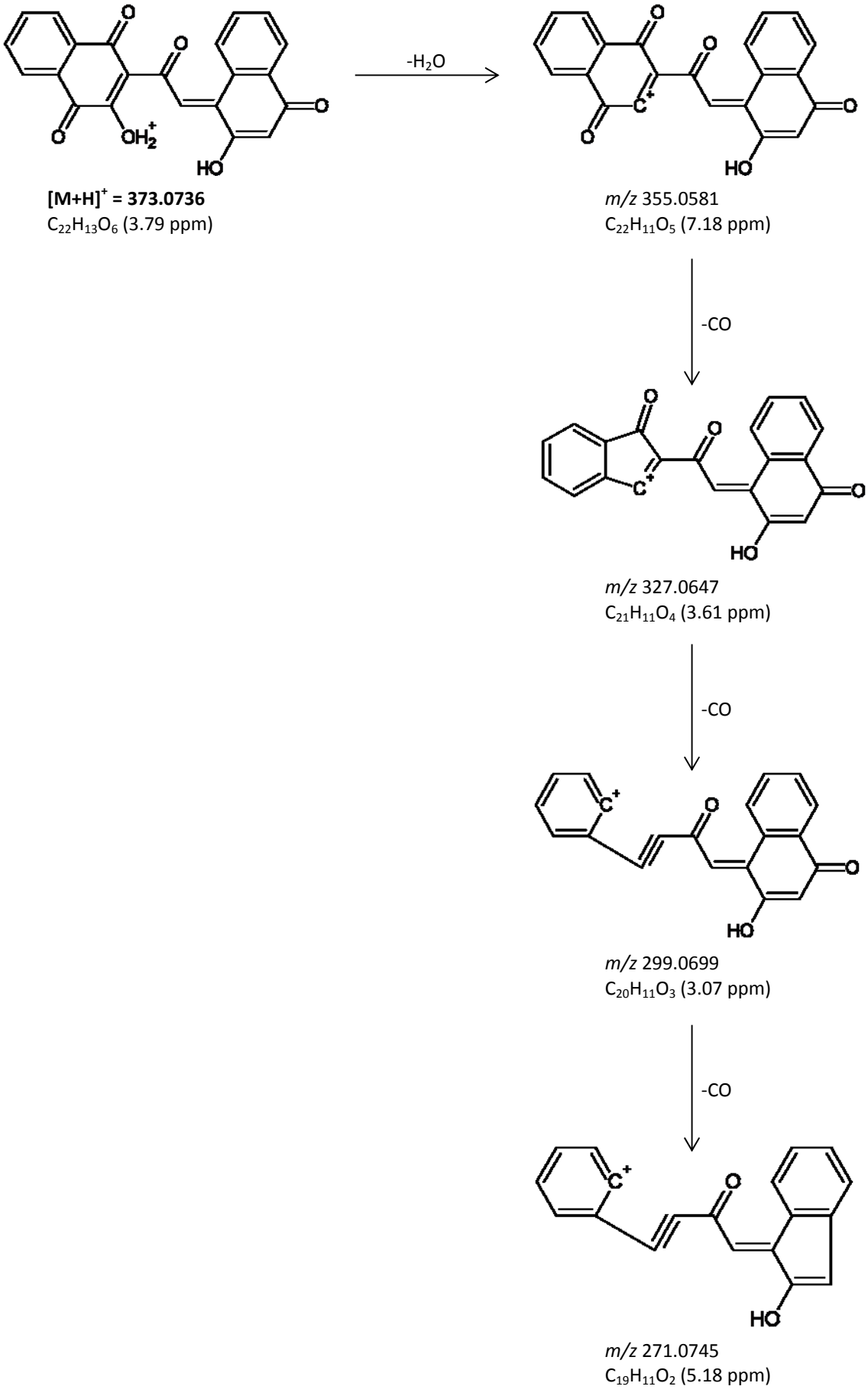


Figure 2-28 Proposed structure and MS fragmentation pathway of product with $[M+H]^+$ of 373.0736.

2.4 Conclusions

The reaction products of lawsone with alanine, lysine and serine were analysed using FTIR spectroscopy, NMR spectroscopy and LC-MS. HRMS data from LC-MS experiments show that the same product, *m/z* 361, was formed by three reactions. The fragmentation of the product suggests that the product formed comprised of two lawsone molecules linked by a nitrogen that is analogous to that of RP and the 1,2-indanedione-amino acid product. NMR and FTIR data further support the formation of the proposed product with the assignment of its functional groups within the correct regions. From these results, a mechanism for the reaction between lawsone and α-amino acids has been proposed. It is hypothesised to react in a similar manner to ninhydrin, where lawsone reactions with α-amino acids to form an imine intermediate following decarboxylation and hydrolysis. This intermediate then reacts with another molecule of lawsone to form the proposed lawsone-amino acid product in a 2 to 1 ratio (lawsone: amino acid).

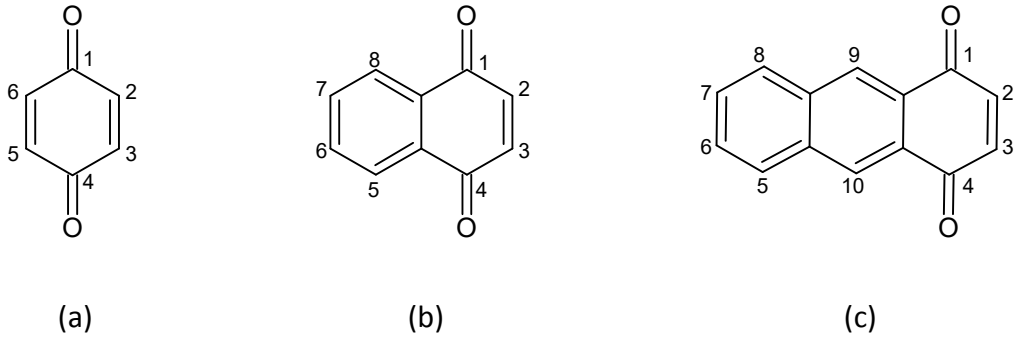
The use of a highly sensitive technique such as LC-MS showed that other products were also formed in the reactions. For example, in the lawsone and alanine reaction, an imine intermediate was found to have formed, suggesting either the reaction was incomplete or that the reaction to the lawsone-amino acid product is reversible. The reaction is likely to proceed in the same manner as 1,2-indanedione and ninhydrin toward α-amino acids and amines. In the reaction of lawsone with more complex amino acids, LC-MS separated a number of minor and/or by-products that were formed in low concentrations that were likely to be below the limit of detection of the NMR spectrometer. The formation of various by-products in the reaction of ninhydrin with lysine and longer chained amino acids have been well documented by Friedman (77).

Therefore, the use of a combination of techniques supports the formation of the proposed product that is depicted in Figure 2-5 (b). The separation of the imine intermediate in the lawsone and alanine reaction also suggests that the reaction mechanism proceeds in a similar manner as RP formation.

Chapter 3: Synthesis of quinones

3.1 History of quinone synthesis

Quinones consist of two carbonyl groups connected by conjugated double bonds in an aromatic system. In a polyaromatic system, the carbonyl groups may exist across different rings. Accordingly, quinones may be grouped depending on the aromatic system on which they are attached; for example, benzoquinones from benzene, naphthoquinones from naphthalene and anthraquinones from anthracene (140). Quinone chemistry is broad and wide ranging, 1,2-quinones such as 1,2-indanedione (Figure 1-9) also belong to the same family. This thesis will address the chemistry and synthesis of 1,4-quinones. The nomenclature for the different classes of quinones is shown in Figure 3-1.



(a) (b) (c)

Figure 3-1 Nomenclature for a) benzoquinones, b) naphthoquinones, and c) anthraquinones

Quinones are ubiquitous in nature and can be found in a number of plants, fungi and bacteria (141). As quinones are often the component that causes flora and fauna to be brightly coloured, it is likely that this characteristic has led to their use in various industries including the food, cosmetics and dyes and pigments industries. For centuries plants have been cultivated and used to produce different coloured dyes. For example, the Celts used woad plants to produce indigo (142; 143), and the Aztecs used the cochineal bug to produce red coloured dyes (144), while the ancient Egyptians and Romans used madder roots to obtain differed red-coloured dyes (145; 146).

One of the simplest quinones, 1,4-benzoquinone or hydroquinone, was first synthesised in the 19th century by the Russian chemist Alexander Voskresensky who oxidised stoichiometric amounts of quinic acid, from *Cinchona* bark, with manganese dioxide and sulfuric acid (147; 148) (Figure 3-2). It was later discovered that 1,4-

benzoquinone can also be synthesised by the oxidation of aniline using the same reagents (147).

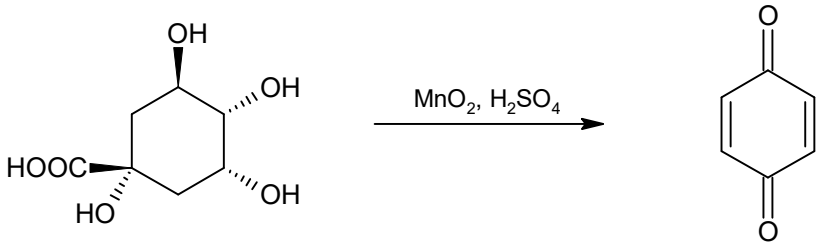


Figure 3-2 Reaction scheme showing the oxidation of quinic acid with manganese dioxide and sulfuric acid (147).

The successful synthesis of benzoquinone paved way for the synthesis of naphthoquinones and anthraquinones. In 1840, Laurent prepared anthraquinone by the oxidation of anthracene with nitric acid. The same product can also be formed by the oxidation of anthracene with chromic acid (149). Historically, the synthesis of quinones from anthracene was problematic due to the difficulties in the purification of anthracene from coal tar distillate and the recovery of reduced chromic acid liquors was commercially unviable (150). Despite advances in quinone chemistry, there was limited interest in these compounds until it was discovered that these processes could be adopted in the manufacture of dyes (151).

As mentioned previously, natural dyes can be extracted from plants and insects. However, as the extraction is often laborious, product purity cannot be ascertained and the popularity of certain colours historically meant that supply could not keep up with demand (145). These issues led to research into the structural elucidation of natural dyes in order to produce synthetic dyes.

In 1856, the colour mauveine (mauve) was synthesised by Sir William Henry Perkin through the oxidation of aniline sulfate with potassium dichromate, followed by methanolic extraction (151; 152). The first natural dye, produced from the madder root was the colour red derived from natural alizarin (Figure 3-3). Alizarin was so popular that its isolation investigated intensively. The compound was eventually isolated by Robiquet and Colin (145). However, the successful synthesis of alizarin and the elucidation of its structure were not accomplished until 1868 by Graebe and

Liebermann (145). The commercialisation of anthraquinones, such as alizarin, was made possible through the preparation of the intermediate *o*-benzoylbenzoic acid by the reaction of phthalic anhydride, benzene and aluminium chloride in a reaction discovered by Friedel and Crafts, which was later coined the Friedel-Crafts reaction (150). The ease of synthesis and market demand for alizarin led to research and production of other synthetic quinone dyes and as a result, the use of natural products in dyes declined dramatically. It also instigated the field of research termed 'anthraquinone chemistry' (149).

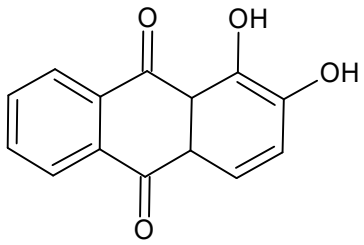


Figure 3-3 Structure of alizarin (1,2-dihydroxy-9,10-anthraqauinone), adapted from Caro *et al.* (153).

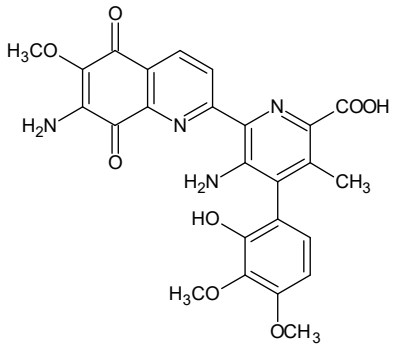
Increasing health and safety issues arising from harmful waste by-products, and a desire for environmental sustainability have led to a resurgence in research on the use and production of polyaromatic compounds (154), as well as safer alternatives for the production of precursor materials. For example, the current route of synthesis of 1,4-benzoquinone is by the Hock oxidation of 1,4-diisopropylbenzene and peroxide oxidation of phenol (155). Although this presents a vast improvement over the historic routes, with a reduced number of synthetic steps and the elimination of by-products, the precursor material still requires the use of a benzene analogue, which is harmful to the users, the environment and aquatic life. Research has therefore been conducted into using microbial synthesis as a 'green' approach to synthetic chemistry (155).

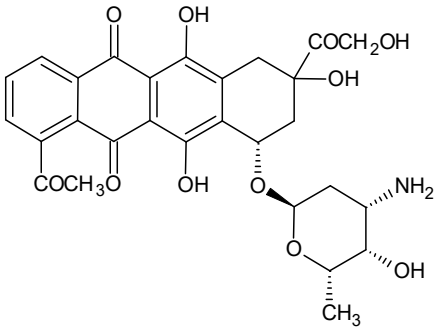
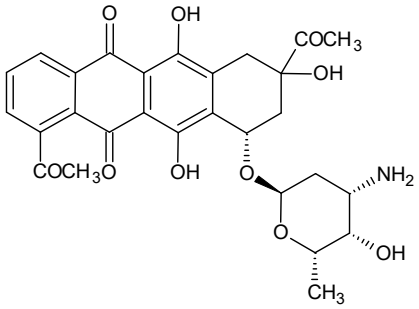
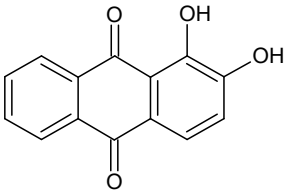
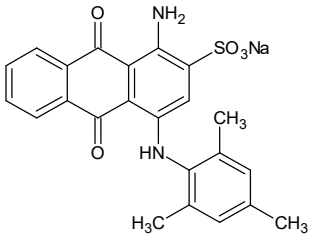
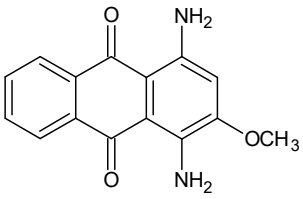
The extensive use of quinones in the dye industry culminated in considerable research on compounds in the naphthoquinone and 9,10-anthraquinone series. Advances in synthetic chemistry have provided a great number of uses for compounds with the quinone moiety. Despite these advances, the use and synthesis of 1,4-anthraquinones have not been explored as extensively.

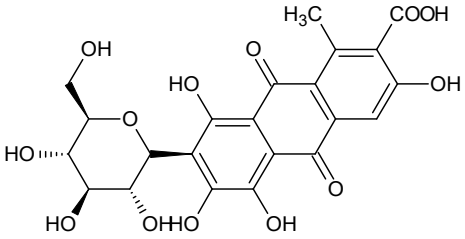
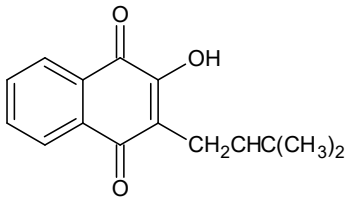
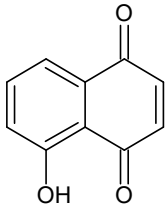
3.2 Uses of quinones

In Section 3.1, it was noted that quinones have been used in dyes and historically in traditional medicines. Further to their use as textile dyes, they have also been exploited as pigments in foods, as well as in cosmetics. Some quinones that have been employed for these purposes include juglone (5-hydroxy-1,4-naphthoquinone), lawsone (2-hydroxy-1,4-naphthoquinone) and alizarin and its analogues (156). Herbal preparations containing quinones have also been used for the treatment for acne, inflammation and various bacterial infections; e.g. plumbagin (5-hydroxy-3-methyl-1,4-naphthoquinone). Many drugs in western medicine including various antimalarial drugs, dermatological preparations and chemotherapeutic drugs (157-160) also contain the quinone structure or are derived from quinone intermediates (161). Quinones have also been used in chemistry as catalysts and chromatographic agents (162-165). Table 3-1 lists examples of some compounds and their various uses.

Table 3-1 Table of some compounds incorporating a quinone moiety and their uses.

Name	Structure of compound	Uses
Streptonigrin		- Antitumor drug that can be used to treat tumours found in a number of sites, including breast, lung, head cancers, lymphoma and melanoma (166). - Consists of *p*-benzoquinone moiety and is derived from *Streptomyces flocculus* (167). - Activity based on inhibition of various cell mechanisms, impairment of DNA and RNA syntheses and topoisomerase II inhibition (166).

Name	Structure of compound	Uses
Doxorubicin (Trade name Adriamycin® or Rubex ®)		- Chemotherapeutic drug for the treatment of cancers such as leukaemia, lymphoma, thyroid cancer (168). - Activity based on interactions with DNA, topoisomerase II inhibition and generation of free radicals (169). Due to toxic side effects (e.g. cardiotoxicity and inflammation) and resistance, it is used in conjunction with other agents to improve its efficacy (170).
Daunomycin		- Chemotherapeutic drug used for the treatment of various cancers. - Activity based on the intercalation of DNA base pairs with its aromatic planar ring system (171), thus inhibiting DNA replication and RNA transcription (172). Like doxorubicin, it is used in multidrug therapy.
Alizarin		- Derived from Madder (*Rubia tinctorum* and *Rubia cordifolia)*, extracted and used from as early as 1350 B.C (154; 173). - Orange-red coloured dye historically used as a food colourant for wakame, kelp, meat, fish and shellfish (174).
Acid blue 129		- Used to impart colour on wool, nylon, silks, inks, leather and paper (154) - Blue coloured dye (175).
Disperse red 11		- Used to impart colour on cellulose acetate, nylon and polyester (176). - Bright bluish violet coloured dye (177).

Name	Structure of compound	Uses
Carminic acid		- Natural colourant derived from insects of the genus *Dactylopius coccus Costa* that are found in Peru, Mexico, Bolivia, Chile and Spain (144). - Red coloured dye used in cosmetics, food, and for the colouration of textiles and plastics (178; 179).
Lapachol		- Yellow coloured pigment that is found in trees of the genus *Tecoma*, in the West Indies and South America (125). - Reported to have anti-inflammatory, antimicrobial and antineoplastic properties (180)
Juglone		- Derived from nut trees (Juglandaceae family) *Juglans cinerea*, *T. Regia*, *T. nigra* (125). - Brown coloured dye for the colouration of wool, cotton, food and cosmetics (181). - Incorporation of moiety in development of possible anticancer drugs (182; 183).

3.3 Synthesis of quinones

One of the aims of this thesis was to study the effects of changes to the molecular structure of fingermark reagents. For example, will the introduction of various substituents to a reagent and/or the extension of a conjugated system lead to increased fingermark sensitivity?

In the work described in this thesis, a number of quinones were synthesised from simple quinones such as 2-hydroxy-1,4-benzoquinone and (un)substituted 1,4-anthraquinones. The quinones were synthesised in order to compare substituent effects. One compound of interest, 2-hydroxy-1,4-naphthoquinone, was readily available and at low cost, so its synthesis was not necessary.

3.3.1 Benzoquinones

A literature search indicated that 2-hydroxy-1,4-benzoquinone can be synthesised by a few different methods using derivatives of benzene. The methods used in this thesis were chosen based upon the ease of synthesis, the number of steps involved to reach the final product (as this will heavily impact on its yield), and the availability and cost of the reagents. Most of the published methods involve the oxidation of 1,2,4-benzenetriol. These include:

- the oxidation of 1,2,4-benzenetriol in a phosphate buffer at pH 8.8, under a constant stream of oxygen (184; 185);
- the oxidation of 1,2,4-benzenetriol in an electrochemical set-up while purging with nitrogen (186);
- the oxidation of 1,2,4-benzenetriol using an enzyme, with the formation of the product monitored qualitatively using UV-spectrophotometry (187; 188);
- the oxidation of 1,2,4-benzenetriol using ferric chloride, in a reaction similar to that of 2-hydroxy-3,6-dimethylbenzoquinone from 3,6-dimethylbenzene-1,2,4-triol (189; 190).

These methods do not indicate the recovery of the product; therefore, alternate synthetic routes were sought. Two different methods were chosen for the synthesis of 2-hydroxy-1,4-benzoquinone; one involved the oxidation of 2,4,6-trihydroxybenzoic acid (191), while the other involved the oxidation of 1,2,4-benzenetriol using Fétizon's reagent, silver carbonate on Celite®.

Silver carbonate on Celite® is a mild oxidant used for the conversion of alcohols to aldehydes and ketones. Reactions using Fétizon's reagent are usually performed by refluxing a neutral solvent, such as benzene, with an excess amount of reagent (192; 193). The use of benzene is generally avoided due to safety issues, but other neutral solvents such as toluene, chloroform and dichloromethane can also be used. The reaction mechanism of Fétizon's reagent on alcohols is well documented. It is postulated that the alcohol in the reactant is reversibly adsorbed onto the surface of the oxidant and a covalent bond is formed between the oxygen and the silver ions

(192; 194). The literature indicates that this method provides excellent yield (195). The reaction scheme is depicted in Figure 3-4.

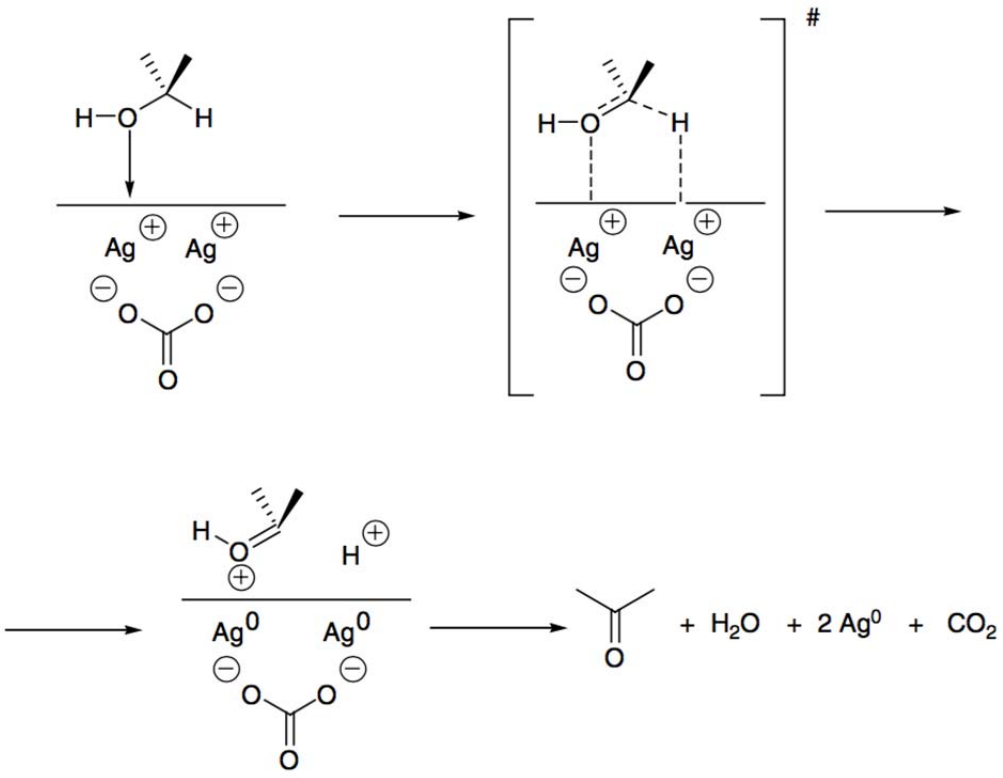


Figure 3-4 Reaction mechanism between silver carbonate and alcohols, adapted from Tojo *et al.* (193).

3.3.2 Anthraquinones

Anthraquinones, namely 9,10-anthraquinone and its analogues, are an oxidation by-product of anthracene and chromic acid (149). Historically, difficulties exist with this method of manufacture mainly due to the impurities that are intrinsically present within commercially available anthracene (150). As a result, the use of 1,4-dihydroxy-9,10-anthraquinone (quinizarin) was explored. This can yield 1,4-anthraquinone and substituted 1,4- and 9,10-anthraquinones (196).

A well-known route of synthesis using quinizarin and thionyl chloride as starting materials was developed by Green in 1926 (197). At the time of its publication the structure of the main product was proposed to be 10-chloro-1-hydroxy-4,9-

anthraquinone. This product was later elucidated as 9-chloro-10-hydroxy-1,4-anthraquinone (Figure 3-5). Other authors have noted the formation of analogous compounds arising from the reaction of thionyl chloride with 2-methyl-and 2-bromo-quinizarin (196).

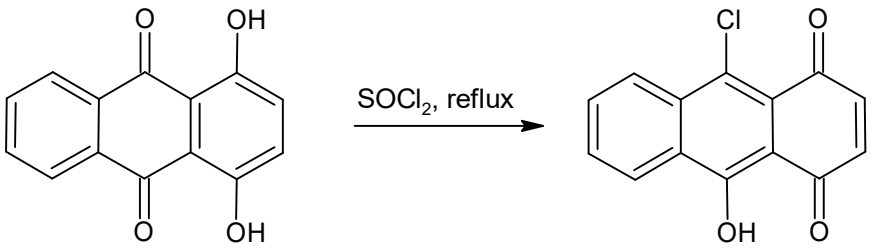


Figure 3-5 Reaction scheme depicting the formation of 9-chloro-10-hydroxy-1,4-anthraquinone from the reaction of quinizarin and thionyl chloride, adapted from Green (197).

The use of quinizarin as a starting material was also explored in the current work, mainly due to its availability and economic considerations. Quinizarin allowed for the introduction of different substituents within the molecule, thus providing an opportunity to compare their effects on the fluorescence of fingermarks treated with the compounds formed. Furthermore, it was a convenient method to obtain substituted 1,4-anthraquinones, as opposed to the 9,10-anthraquinones most commonly referred to in the literature.

Quinizarin can also yield 1,4-anthraquionone via reduction using sodium borohydride (198). 2-Hydroxy-1,4-anthraquinone may then be synthesised from 1,4-anthraquinone via Thiele-Winter acetoxylation.

The Thiele-Winter acetoxylation reaction was first described by Johannes Thiele in 1898. It involved the reaction of 1,4-benzoquinone with acetic anhydride in a catalytic quantity of concentrated sulfuric acid to form 1,2,4-triacetoxybenzene in high yield (199; 200). After extensive studies, Thiele and Winter showed that the same reaction can be applied to both 1,2-naphthoquinone and 1,4-naphthoquinone to form 1,2,4-triacetoxynaphthalene. Furthermore, the catalytic amount of sulfuric acid can be replaced by zinc chloride if it is used in equimolar amounts with the quinone in the reaction (201).

In the acetoxylation reaction, two of the acetoxy groups are derived from the oxygen of the carbonyl group in the quinone system and the other is added during the reaction (199). The proposed mechanism for the formation of 1,2,4-triacetoxyanthracene is illustrated in Figure 3-6. The Thiele-Winter reaction can be applied to a selection of benzoquinones, naphthoquinones and anthraquinones (189; 199; 202-207).

Other methods have also been developed to produce 2-hydroxy-1,4-anthraquinone. Fieser published a series of papers relating to the structural elucidation and synthesis of anthraquinones and naphthoquinones (208-213). In that research, Fieser (211) prepared 2-hydroxy-1,4-anthraquinone in three steps, starting with the treatment of 1,2-anthraquinone with sodium sulphite to form the sodium salt of 1,2-anthraquinone-4-sulfonic acid. This was followed by the hydrolysis and esterification of the sulfonic acid group. The tautomerism of the quinone structure allowed for the formation of 2-methoxy-1,4-anthraquinone. The 2-methoxy derivative was then used to synthesise 2-hydroxy-1,4-anthraquinone using basic hydrolysis without purification. Fieser stated that the three-step reaction was found to be "*the most satisfactory method found for the preparation of the 2-hydroxy-1,4-anthraquinone"* and that "*2-hydroxy-1,4-anthraquinone resembles the corresponding naphthoquinone to a marked degree"* (211). The reaction scheme for the three-step synthesis is depicted in Figure 3-7.

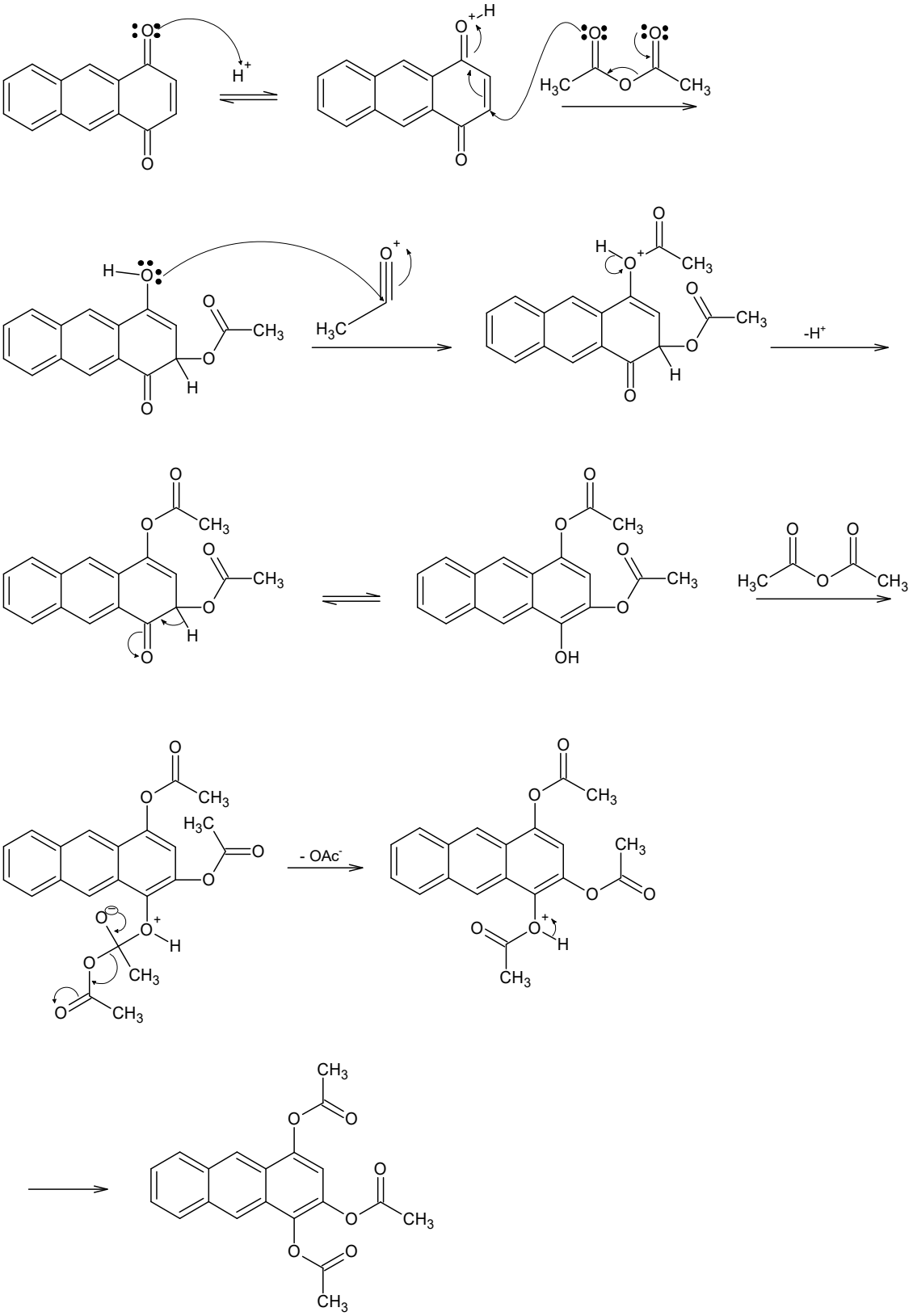


Figure 3-6 The proposed mechanism for the reaction of 1,4-anthraquinone with acetic anhydride in a Thiele-Winter acetoxylation reaction, adapted from Mundy, *et al.* (200).

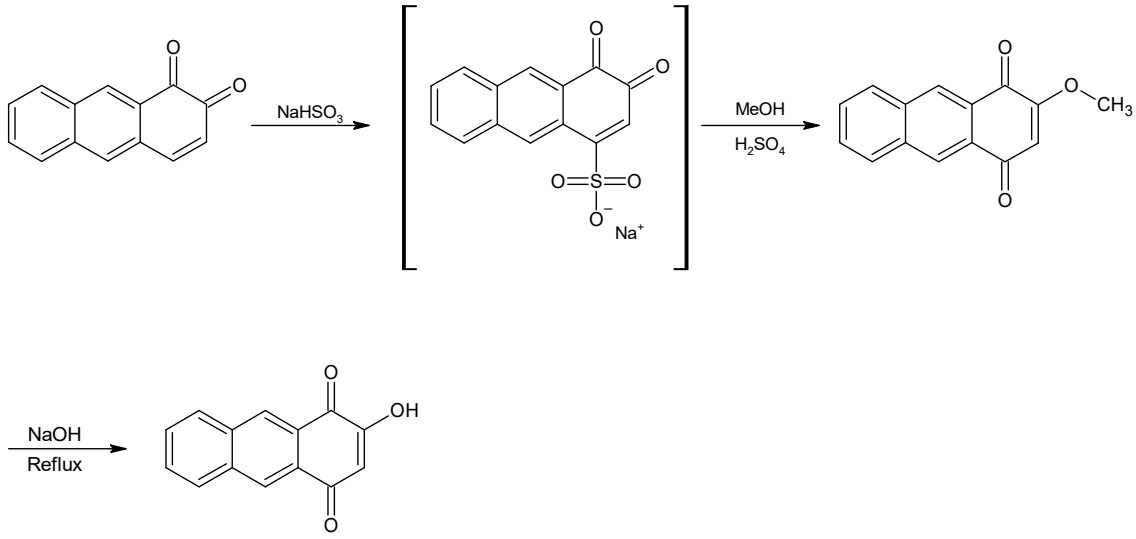


Figure 3-7 Reaction scheme depicting the reaction of 1,2-anthraquinone to 2-hydroxy-1,4-anthraquinone (211).

Utilising quinizarin as a starting material is ideal due to its low cost and it may be considered as a precursor for the anthraquinones studied in the current research. On the other hand, ammonium 1,2-anthraquinone-4-sulfonate, a similar starting material to that used by Fieser, is much more expensive. Table 3-2 provides an indication of the prices of some quinones from various chemical providers, such as Sigma-Aldrich[1], Thermo-Fisher Scientific[2] and TCI[3]. It should be noted that only the lowest quote is included in the table.

Table 3-2 Table comparing the relative price and weight of compounds from commercial vendors.

Compound	Mass (g)	Price (AUD) (approximate)	Price (AUD) per gram (g)
2-hydroxy-1,4-naphthoquinone[1]	10 – 100	34 – 228	2.3 – 3.4
2-methoxy-1,4-naphthoquinone[1]	5	~ 92	~ 18.4
1,4-naphthoquinone[1]	5 – 500	40 – 147	0.30 – 8
sodium 1,2-naphthoquinone-4-sulfonate[1]	10.0	~ 240	24

Compound	Mass (g)	Price (AUD) (approximate)	Price (AUD) per gram (g)
2-hydroxy-1,4-anthraquinone	-	-	-
2-methoxy-1,4-anthraquinone	-	-	-
1,4-anthraquinone[1,2,3]	1 – 25	89 – 355	14.3 - 89
1,2-anthraquininone-4-sulfonate acid ammonium salt[1]	0.100	~ 198	~ 1980
quinizarin[1]	5 – 100	29 – 50	0.5 – 5.8
1,2,4-benzenetriol[1]	1 – 5	96 – 337	67.4 – 96

3.4 Materials and methods

The following reagents were obtained from Sigma-Aldrich: 1,2,4-benzenetriol *ReagentPlus®* 99 % [CAS 533-73-3], quinizarin ≥ 96.0% [CAS 81-64-1], 1,2-anthraquinone -4-sulfonic acid, ammonium salt (Aldrich^{CPR}) , Celite® S (Fluka) [CAS 61790-53-2], potassium permanganate AR grade (Fluka) [CAS 7722-64-7], thionyl chloride *ReagentPlus®* ≥ 99 % [CAS 7719-09-7], and sodium borohydride ≥ 98 % [CAS 16940-66-2].

The following reagents and solvents were AR grade and obtained from Chem-Supply Pty Ltd, Australia unless stated otherwise: absolute ethanol [CAS 64-17-5], acetone [CAS 67-64-1], chloroform reagent grade ≥ 99.8 % [CAS 67-66-3], dichloromethane [CAS 75-09-2], diethyl ether HPLC grade [CAS 60-29-7], glacial acetic acid [CAS 64-19-7], hydrochloric acid [CAS 7647-01-0], methanol [CAS 67-56-1], silver carbonate (Acros) 99 % [CAS 534-16-7], sodium carbonate anhydrous [CAS 497-19-8], sodium hydroxide [CAS 1310-73-2], sodium methoxide anhydrous powder pure (Acros) [CAS 124-41-4], sodium sulfate anhydrous [CAS 7757-82-6], sulfuric acid 98 % [CAS 7664-93-9], and toluene [CAS 108-88-3].

Infrared spectra were recorded on a Nicolet Magna IR 760 or a Digilab FTS 7000 IR spectrometer. Samples were prepared as KBr disks or by direct sampling with an ATR crystal.

^{1}H and ^{13}C NMR spectra were obtained using an Agilent Technologies 500/54 (500MHz/54 mm bore) premium shielded NMR coupled to a 7510-AS autosampler operating at 500 MHz and 125 MHz, respectively. ^{1}H NMR spectra were also obtained using a Bruker DRX NMR spectrometer operating at 300.13MHz. Chemical shifts are reported in parts per million (ppm) relative to Me_4Si (TMS) as an internal reference. Spectral processing included proton integration, multiplicity assignment (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of a doublet), determination of coupling constants (Hz) and proton assignment.

Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) was performed on an Agilent 1200 series liquid chromatograph coupled to an Agilent 6500 series QTOF mass spectrometer. The column used was a Phenomenex C18 column (150 x 3 mm x 5 µm). MassHunter software (Agilent Technologies) was used for instrument control, data acquisition and data analysis.

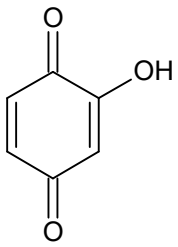
Melting points were obtained using a John Morris Electrothermal melting point apparatus and were corrected using benzoic acid (mp. 122 °C) as a reference.

Drying of organic residues was performed using AR grade anhydrous sodium sulfate (Na_2SO_4). Methanol was dried by storing over molecular sieves. For reactions requiring anhydrous reaction conditions, the glassware was flame-dried prior to use and (where stated) performed in an atmosphere of nitrogen.

3.4.1 Preparation of Fétizon's reagent

Fétizon's reagent was prepared following the method outlined by Tojo and Fernandez (193). Celite® S was washed with a solution of methanol containing concentrated hydrochloric acid (10 % v/v) and washed with deionised water. It was then dried at approximately 120 °C for 24 hours. The dried Celite® S (15 g) was added to a stirred solution of silver carbonate (100 mmol, 17.5 g) in 100 mL of deionised water. Sodium carbonate (52.5 mmol, 15 g) in 150 mL of deionised water was added to the solution of silver carbonate and Celite®. The mixture was stirred for a further 10 minutes. The product was filtered and dried under reduced pressure over night to remove residual water and used without further purification.

3.4.2 Synthesis of 2-hydroxy-1,4-benzoquinone, 1

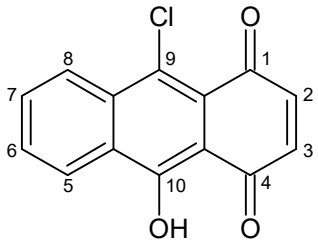


1

2-Hydroxy-1,4-benzoquinone, **1**, was synthesised by refluxing a stirred solution of 1,2,4-benzenetriol (0.416 mmol, 52.8 mg) with two equivalent moles of freshly synthesised 48.7 % silver carbonate (Ag_2CO_3) on Celite ® (Fétizon's reagent) (460 mg) in 33 mL of toluene for 20 minutes in a 100-mL single-necked round bottom flask. The resulting mixture was pale yellow in colour. It was cooled to room temperature and filtered. Excess toluene was used to wash the residue free of the product. The filtrate was concentrated by rotary evaporation. It was further dried under reduced pressure for at least five hours to remove residual solvent. The product obtained weighed 41.9 mg (80.6 %) and was used without purification for the detection of fingermarks on porous surfaces. mp. 127 °C ^{1}H NMR ($CDCl_3$): δ 6.14, 1H, H3, d (J = 2.4 Hz); 6.76, 1H,

H5, dd (J= 10.0, 2.4 Hz); 6.84, 1H, H6, d (J = 9.9); 6.95, 1H, s, OH. ^{13}C NMR ($CDCl_3$): δ 170.9, 160.1, 95.3, 60.5, 20.8.

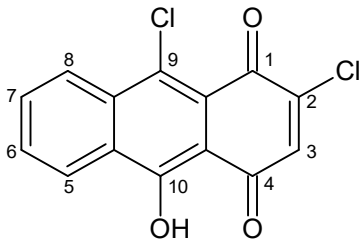
3.4.3 Synthesis of 9-chloro-10-hydroxy-1,4-anthraquinone, 2



2

The following method is an adaptation from the reaction of quinizarin with thionyl chloride as described by Green (197). Quinizarin (10.0 g, 0.416 mol) and freshly distilled thionyl chloride (80 mL) were added into a 250-mL single-necked round bottom flask. The mixture was stirred and kept at reflux for approximately 20 hours, resulting in a dark red solution. The mixture was allowed to cool to room temperature, where dark red needle-like crystals were precipitated from the solution. The product, **2**, was filtered and washed with toluene. It was then recrystallised using dichloromethane and placed under reduced pressure for a minimum of eight hours to ensure complete solvent removal. The product weighed 8.22 g (76 %). mp: 225 - 227 °C (Lit. 225 - 226 °C). ^{1}H NMR ($CDCl_3$): δ 7.01, 2H, H2,3, s; 7.89, 2H, H6,7, dt (J = 22.5, 8.7 Hz); 8.67, 2H, H5,8, dd (J = 20.4, 7.8 Hz); 8.64, 1H, H6, s; 14.73, 1H, O**H**, s.^{13}C NMR ($CDCl_3$): δ 187.7, 183.0, 163.0, 142.0, 137.5, 135.1, 132.4, 129.9, 128.0, 127.5, 127.5, 125.0, 122.8, 109.3. HRMS (ESI) calcd for $C_{14}H_8ClO_3$: $[M+H]^+$ = 259.0161; found: $[M+H]^+$ = 259.0111.

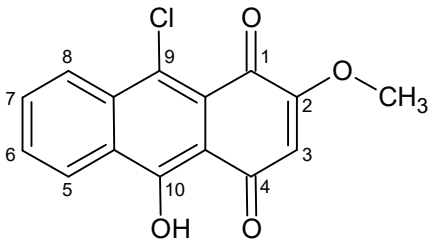
3.4.4 Synthesis of 2,9-dichloro-10-hydroxy-1,4-anthraquinone, 3



3

Excess concentrated hydrochloric acid (32 %) (10 mL) and potassium permanganate (5.0 g, 0.316 mol) were added to a dropping funnel attached to side-arm flask. The side-arm flask was attached to two consecutive Drechsel flasks, containing concentrated sulphuric acid and glass wool, respectively, to facilitate the removal of water. This was set up in a gas generation configuration. Dry chlorine gas was bubbled through a solution of chloroform and acetic acid (3:1, 70 mL) containing **2** (50.0 mg, 0.193 mmol) via a needle inlet attached to a 250-mL two-neck round bottom flask. The mixture was stirred at room temperature for approximately three to five hours and a colour change was observed from orange to a pale yellow-orange. Absolute ethanol (70 mL) was then slowly added to the mixture. The reaction was refluxed for one hour. The mixture was then cooled and concentrated at reduced pressure, which resulted in an orange solid suspended in the remaining acetic acid. The mixture was filtered and washed with acetic acid (2 x 10 mL) and dried under reduced pressure. The product, **3**, was recrystallised from dichloromethane forming orange needles that weighed 44.3 mg (78 %). mp: 215 - 218 °C. ^{1}H NMR ($CDCl_3$, 500Mz): δ 7.27, 1H, H_3, s; 7.94, 2H, H6,7, dt (J = 23.1, 8.4 Hz); 8.66, 2H, H5,8, dd (J = 16.5, 7.9 Hz); 8.64, 1H, H6, s; 14.45, 1H, O**H**, s.^{13}C NMR ($CDCl_3$): δ 181.8, 180.3, 163.3, 144.4, 139.1, 135.2, 132.9, 130.3, 128.5, 127.7, 127.6, 125.2, 122.5, 108.6. IR $\bar{\upsilon}_{max}$ (cm^{-1}): 3435, 1653, 1628, 1599, 1569, 1489, 1432, 1399, 1327, 1267, 1238, 1139, 1105, 1039, 1018, 945, 877, 848, 818, 773, 721, 650, 626, 549. LC-MS (+ESI): 292.9751, 264.9802, 257.0000, 173.0132, 201.0103. HRMS (ESI) calcd for $C_{14}H_7Cl_2O_3$: $[M+H]^+$ = 292.9772; found: $[M+H]^+$ = 292.9751.

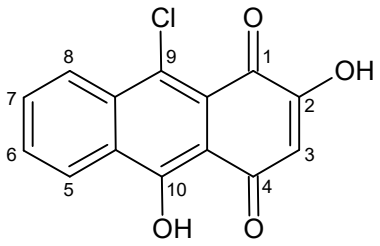
3.4.5 Synthesis of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone, 4



4

A 50-mL two-necked round bottom flask with a magnetic stirrer bar was charged with a solution of **3** (0.222 mmol, 65 mg) and four mole equivalents of fresh sodium methoxide in dry methanol (10 mL). Upon the addition of sodium methoxide, the solution turned purple. The reaction progress was monitored and stopped after 48 hours. The reaction mixture was acidified by adding hydrochloric acid (3 %, 20 mL) dropwise to pH 2, precipitating a red product. The product was filtered, washed with water, and air dried to yield 57.4 mg (90 %). The crude product formed was determined by NMR spectroscopy to be a mixture of approximately 85 % *2-methoxy* and 15 % *3-methoxy* isomers. Purification of the crude **4** using column chromatography was performed; however, it only resulted in slightly higher purity, approximately 90 % *2-methoxy* and 10 % *3-methoxy* isomer. Based on this, the yield was taken to be approximately 51.7 mg 2-methoxy isomer (**4a**) and approximately 5.7 mg 3-methoxy isomer (**4b**). ^{1}H NMR ($CDCl_3$, 500Mz): δ 3.94, 3H, s, C**H**$_3$; 6.27, 1H, s, H3; 7.89, 2H, H 6,7, td (J = 49.5, 8 Hz); 8.65, 2H, $H_{5,8}$, dd (*J*=38, 8.5 Hz), 14.44, 1H, s, O**H**. ^{13}C NMR ($CDCl_3$): δ 183.7, 182.6, 163.4, 157.8, 135.7, 132.6, 131.8, 129.7, 127.5, 125.1, 113.6, 56.6. IR ν_{max} (cm^{-1}): 3115, 3071, 2943, 2928, 2855, 1676, 1645, 1614, 1599, 1576, 1493, 1433, 1406, 1364, 1329, 1306, 1287, 1267, 1230, 1219, 698**.** LC-MS (+ESI) 289.0280, 262.0331, 246.0100, 205.0131. HRMS (ESI) calcd for $C_{15}H_{10}ClO_4$: $[M+H]^+$ = 289.0268; found: $[M+H]^+$ = 289.0280.

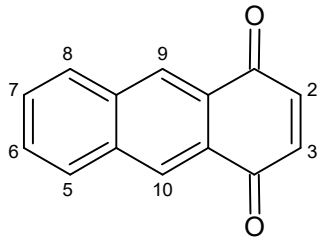
3.4.6 Synthesis of 9-chloro-2,10-dihydroxy-1,4-anthraquinone, 5



5

A 10-mL two-necked round bottom flask was charged with **4** (0.050 mg, 0.172 mmol) in sodium hydroxide solution (3 – 5 % w/v). The reaction was kept under reflux and was monitored at 30 minute intervals using TLC for approximately two hours. When no further reaction could be observed, the reaction mixture was filtered while hot. Upon cooling in an ice-bath, the mother liquor was acidified to pH 2 using hydrochloric acid (6 M), which resulted in an orange-red precipitate. The mixture was left to stir for an additional two hours. The product, **5**, was then collected by filtration and washed with water (2 x 2 mL) and dried at reduced pressure for a minimum of five hours. The weight of the product **5** was 41.4 mg (87 %). mp: 239 – 240°C. ^{1}H NMR ($CDCl_3$, 500Mz): δ 6.46, 1H, s, H3; 7.32, 1H, s, C2-O**H**; 7.903, 2H, H 6,7, td (J = 50, 7.5 Hz); 8.67, 2H, H5,8, dd (*J* = 45.5, 8.5 Hz), 14.44, 1H, s, C10-O**H**. ^{13}C NMR ($CDCl_3$): δ 181.9, 180.3, 163.4, 139.2, 135.4, 132.9, 130.3, 128.5, 127.8, 127.6, 125.3, 122.7, 117.2, 108.7. IR ν_{max} (cm^{-1}): 3438, 1666, 1641, 1592, 1552, 1500, 1438, 1410, 1369, 1082, 861, 760, 668. LC-MS (+ESI): 275.0021, 231.0162, 203.0213, 168.053, 195.0400, 139.0515. HRMS (ESI) calcd for $C_{14}H_8ClO_4$: $[M+H]^+$ = 275.0111; found: $[M+H]^+$ = 275.0021.

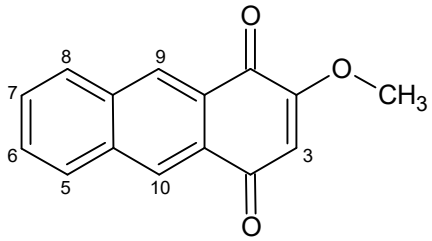
3.4.7 Synthesis of 1,4-anthraquinone (AQ), 6



6

The following reaction was adapted from the experimental method outlined by Hua *et al.* (198). A 50-mL two-neck round bottom flask was charged with nitrogen and a solution of quinizarin (1.00 g, 0.0042 mol) in dry methanol. The reaction mixture was cooled in an ice-salt water bath until it reached 0 °C. Active sodium borohydride (78.7 mg, 0.0208 mol) was then added with stirring. The reaction mixture was monitored at 30 minute intervals using TLC and stopped accordingly. The temperature of the reaction mixture was maintained at 0 °C throughout the duration of the reaction. The resulting red solution was then acidified, dropwise, with 3 % hydrochloric acid over a period of approximately 10 minutes. After the orange precipitate was filtered and washed with water, it produced a mixture of 1,4-anthraquinone and quinizarin that did not readily separate with recrystallisation from an acetone-ether mixture. This mixture was then separated by vigorously stirring in aqueous sodium hydroxide (3 – 5 % w/v), which resulted in a purple solution. The mixture was then extracted three times with dichloromethane, with the organic phase collected and solvent-stripped using rotary evaporator. The product, **6**, was collected as yellow needles upon drying under reduced pressure for approximately five hours to yield 77.9 mg (89.9 %). mp: 202 – 203 °C (204 – 206 °C); ^{1}H NMR ($CDCl_3$): δ 7.08, 2H, H2,3, s; 7.73, 2H, H6,7, dd (J = 6.5, 3.3 Hz); 8.10, 2H,H5,8, dd (J = 6.2, 3.3 Hz); 8.60, 2H, H9,10, s. ^{13}C NMR ($CDCl_3$): δ 184.7, 140.1, 134.8, 130.2, 129.6, 128.9, 128.4. HRMS (ESI) calcd for $C_{14}H_9O_2$: $[M+H]^+$ = 209.0603; found: $[M+H]^+$ = 209.0668.

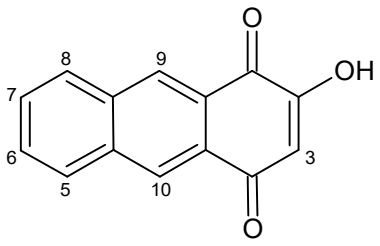
3.4.8 Synthesis of 2-methoxy-1,4-anthraquinone (MAQ), 7



7

The following method is an adaptation of the synthesis of 2-hydroxy-1,4-naphthoquinone (214). Methanol (1 mL) in a 10-mL two-necked round bottom flask was cooled in an ice-salt bath for approximately 10 minutes. Concentrated sulfuric acid (0.08 mL) was slowly added with stirring to the methanol. The mixture was stirred for an additional 15 minutes in the ice-salt bath. The flask was then removed from the ice-salt bath and 1,2-anthraquinone-4-sulfonic acid, ammonium salt (100 mg, 32.7 mg) was slowly added to the solution. The mixture was stirred for an additional 30 minutes at room temperature. It was then refluxed and monitored by TLC over two hours. No distinct colour change was noted, but the solution did change slightly from a buff to a medium brown colour. After refluxing, dry methanol (0.5 mL) was added to the flask and left stirring for another 30 minutes. A mixture of ice-water was then added to quench the reaction. The resulting compound was filtered and washed with iced water. The product, **7**, was vacuum dried at reduced pressure and formed light yellow coloured needles that weighed 63.2 mg (82 %). mp: 217 – 218 °C (Lit. 217 °C). ^{1}H NMR ($CDCl_3$, 500Mz): δ 3.94, 3H, OCH_3, s; 6.30, 1H, H3, s; 7.74, 2H, H6,7, m; 8.08, 2H, H5,8, d (J = 9.5 Hz); 8.64, 1H, H6, s; 8.69, 1H, H7, s. ^{13}C NMR: δ 187.2, 184.2, 159.0, 135.3, 130.3, 130.2, 130.1, 129.3, 128.3, 111.6, 56.5. HRMS (ESI) calcd for $C_{15}H_{10}O_3$: $[M+H]^+$ = 239.0708; found: $[M+H]^+$ = 239.0631.

3.4.9 Synthesis of 2-hydroxy-1,4-anthraquinone (HAQ), 8



8

A 10-mL two-necked round bottom flask, was charged with **7** (50 mg, 0.210 mmol) and sodium hydroxide solution (3 % w/v). The mixture was refluxed for approximately 30 minutes or until the reagent had dissolved. The light yellow coloured solution was filtered while hot. Upon cooling in an ice-bath, it was acidified with 6 M hydrochloric acid, producing a deep orange-red suspension. It was left to stir for an additional two hours. The product, **8**, was then collected by filtration and washed with water. It was dried at reduced pressure to produce a pale terracotta powder that weighed 40.1 mg (85 %). mp: 240 – 242 °C (Lit. 243 °C). ^{1}H NMR ($CDCl_3$): δ 6.48, 1H, H3, s; 7.49, 1H, O**H**, s; 7.73, 2H, H6,7, m; 8.08, 2H, H5,8, d (J = 8); 8.64, 1H, H_6, s; 8.69, 1H, H7, s.^{13}C NMR ($CDCl_3$): δ 187.3, 184.2, 160.2, 138.3, 137.0, 133.0, 133.0, 132.9, 132.3, 132.2, 131.6, 131.5, 128.8, 111.1. HRMS (ESI) calcd for $C_{14}H_9O_3$: $[M+H]^+$ = 225.0552; found: $[M+H]^+$ = 225.0673.

3.5 Results and discussion

3.5.1 2-Hydroxy-1,4-benzoquinone, 1

The first method that was attempted for the synthesis of **1** involved the use of a strong oxidant, methylene blue, on 2,4,6-trihydroxybenzoic acid (191). The reaction was performed under a stream of oxygen using methanol as solvent. The reaction mixture was then irradiated with a mercury arc lamp (254 nm) stirring at room temperature and monitored over a period of three days. The method was unsuccessful; the NMR spectrum of the extracted product did not indicate the formation of the desired compound. In order to promote the formation of the product, different solvents and reaction times were trialled. These included protic solvents, such as ethanol, propanol and butanol as some researchers suggested that solvent effects on the reaction can lead to vast differences in product formation (215). Variation of the amount of oxidant was also attempted but these attempts were unsuccessful. These results were contrary to the ones achieved by the authors, who reported yields of approximately 20 %. These autoxidation types of reaction, however, could yield **1**, as well as other undesirable by-products and/or decomposition products such as dimers, dihydroxybenzoquinones, open-chain acids and cyclopentane derivatives (189).

In these experiments, **1** was successfully synthesised from freshly prepared silver carbonate on Celite®. The product was characterised and its properties corresponded to literature values (216). However, the percentage yields for different reactions varied greatly from approximately 50 to 90 %. This is likely due to slight changes in reaction conditions (e.g. refluxing of solvent, stirring speeds of the magnetic stirrer) between each experiment (193). These conditions can affect the reaction greatly as the reaction is dependent upon a chemisorption process between the –OH group in the reactant and the silver carbonate. Therefore, reaction conditions need to be identical in order to have reproducible results. Figure 3-8 shows the ^{1}H NMR spectrum of **1** in $CDCl_3$.

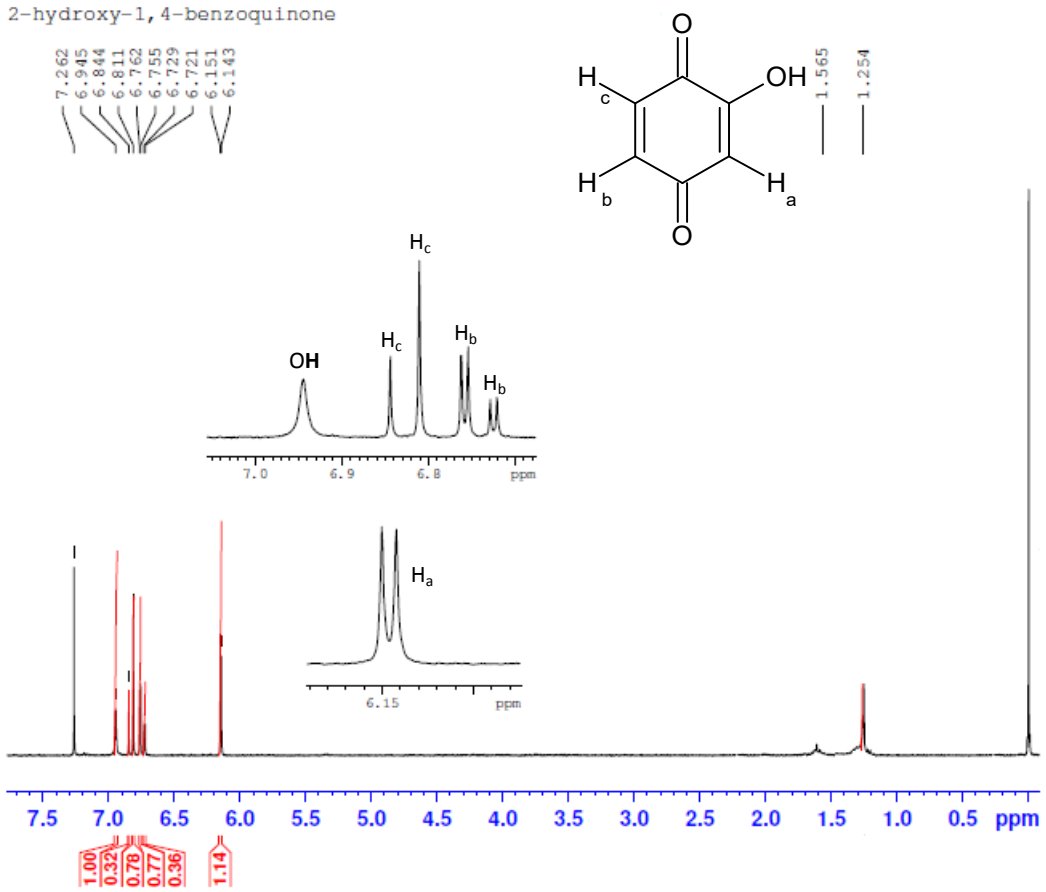


Figure 3-8 ^{1}H NMR spectrum ($CDCl_3$) of 2-hydroxy-1,4-benzoquinone.

The proton of the hydroxy group is a singlet appearing at δ6.94 ppm, H_a is a doublet appearing at δ6.15 ppm, H_b is a doublet of a doublet appearing at δ6.72 – 6.76 ppm and H_c is a doublet appearing at δ6.84 – 6.94 ppm. The chemical shifts of the protons are observed to be in the correct region and correspond to the structure of the compound.

3.5.2 9-Chloro-10-hydroxy-1,4-anthraquinone, 2

2 was successfully synthesised following the original method described by Green (197). The compound was synthesised in high yield (89 %) using freshly distilled thionyl chloride. Although this compound has been previously reported in various literature (196; 197), little analytical data has been provided. Furthermore, Green described the use of benzene or alcohols such as methanol or ethanol for the recrystallization and

washing of the product. For the experiments performed in this work, the use of alcohols proved unsatisfactory as the NMR spectrum of the product indicated that trace amounts of starting material remained in the final product even after two recrystallisations. Benzene was not used due to its carcinogenic effects; instead, dichloromethane was used as a solvent for recrystallisation. The product obtained was of sufficient purity, as evidenced by the analytical data presented. The product was found to have a melting point of 226 - 227 °C (1 mmHg), which corresponds to the literature value (197). This product was characterised by its ^{1}H and ^{13}C NMR, LC-MS and FTIR. The data correlates with the structure of the compound and the known values from literature (196).

Sutter and Weis (196) noted that for the chlorination of quinizarin, the difference in yield when using freshly distilled thionyl chloride and 'older' thionyl chloride was negligible. The reaction was repeated in the current work in order to confirm this theory and it was found that when using 'older' thionyl chloride, the product was contaminated with residual amounts of quinizarin. In these instances, the overall yield of the reaction was quite poor (approx. 40 %). This shows that the 'age' of thionyl chloride can affect the yield of the product. This may be due to water absorption by the reagent, causing degradation that can have a negative impact on the yield.

3.5.3 2,9-Dichloro-10-hydroxy-1,4-anthraquinone, 3

Some previous studies have indicated that the chemistry of 1,4-anthraquinone is similar to that of 1,4-naphthoquinone (149; 211). Therefore, the method reported by Wheeler and Scott (217), for the synthesis of 3-chloro-5-hydroxy-1,4-naphthoquinone from 5-hydroxy-1,4-naphthoquinone (juglone), was adapted in the next step of the synthesis.

The synthesis involved the chlorination of **2** to form **3**. The reaction proceeded the via chlorine gas produced by the oxidation of concentrated hydrochloric acid using potassium permanganate. Water was removed from the gas by passing it through a series of Drechsel flasks that contained concentrated sulfuric acid and glass wool.

However, instead of using cold glacial acetic acid, a mixture of cold chloroform and glacial acetic acid was used as solubility was increased in this solvent system. The glassware configuration for the synthesis of **3** is depicted in Figure 3-9.

Figure 3-9 Reaction configuration in which chlorine gas is generated and bubbled into a solution containing 9-chloro-10-hydroxy-1,4-anthraquinone.

The reaction mixture was continually stirred at room temperature for 5 to 6 hours and TLC was used to monitor the reaction. Various repeated experiments indicated that the longer that the reaction was stirred (up to 6 hours), the higher the yield of the product obtained. This may be because the chlorine gas is trapped within the vessel, thus allowing it to interact with the compound for longer periods of time, indicating that the reaction is slow. The average yield of these experiments was approximately 80 %.

As with the naphthoquinone reactions, the intermediate formed from this reaction would yield a trihalide addition product, that is, 2,3,9-trichloro-10-hydroxy-1,4-anthraquinone. The hydroxy group at C-10 acts as a Lewis acid and hydrogen bonds are formed with the adjacent carbonyl group. The elimination of the more acidic hydrogen

and the adjacent chlorine atom is made possible by refluxing in ethanol, consistent with the results reported by Wheeler and Scott (217).

Increasing the scale of the reaction was also attempted, but these experiments produced low overall yield (approx. 30 %). Furthermore, the product was often contaminated with residual amounts of starting material, which were difficult to remove by recrystallisation alone. The starting material could be removed by column chromatography. This, however, was time-consuming with larger scale reactions. Therefore, multiple smaller-scaled experiments were performed, as increasing the scale of starting material negated any increases in yield. The products from these experiments were characterised by using NMR spectroscopy, FTIR spectroscopy and LC-MS.

The ^{1}H NMR spectrum of the product in Figure 3-10 shows two singlets, at δ7.27 ppm and δ14.45 ppm, attributed to C3-H and C10-OH, respectively. The doublet of doublets at δ8.66 ppm, attributed to C5-H and C8-H, is also at the expected region for aromatic protons. The triplet of doublets at δ7.94 ppm, attributed to C6-H and C7-H, is further upfield in comparison with the doublet of doublets, as they are not as affected by the electronegative effects of the chloro and hydroxy groups, which are attached to C-9 and C-10, respectively. These protons are also observed to be in the expected region.

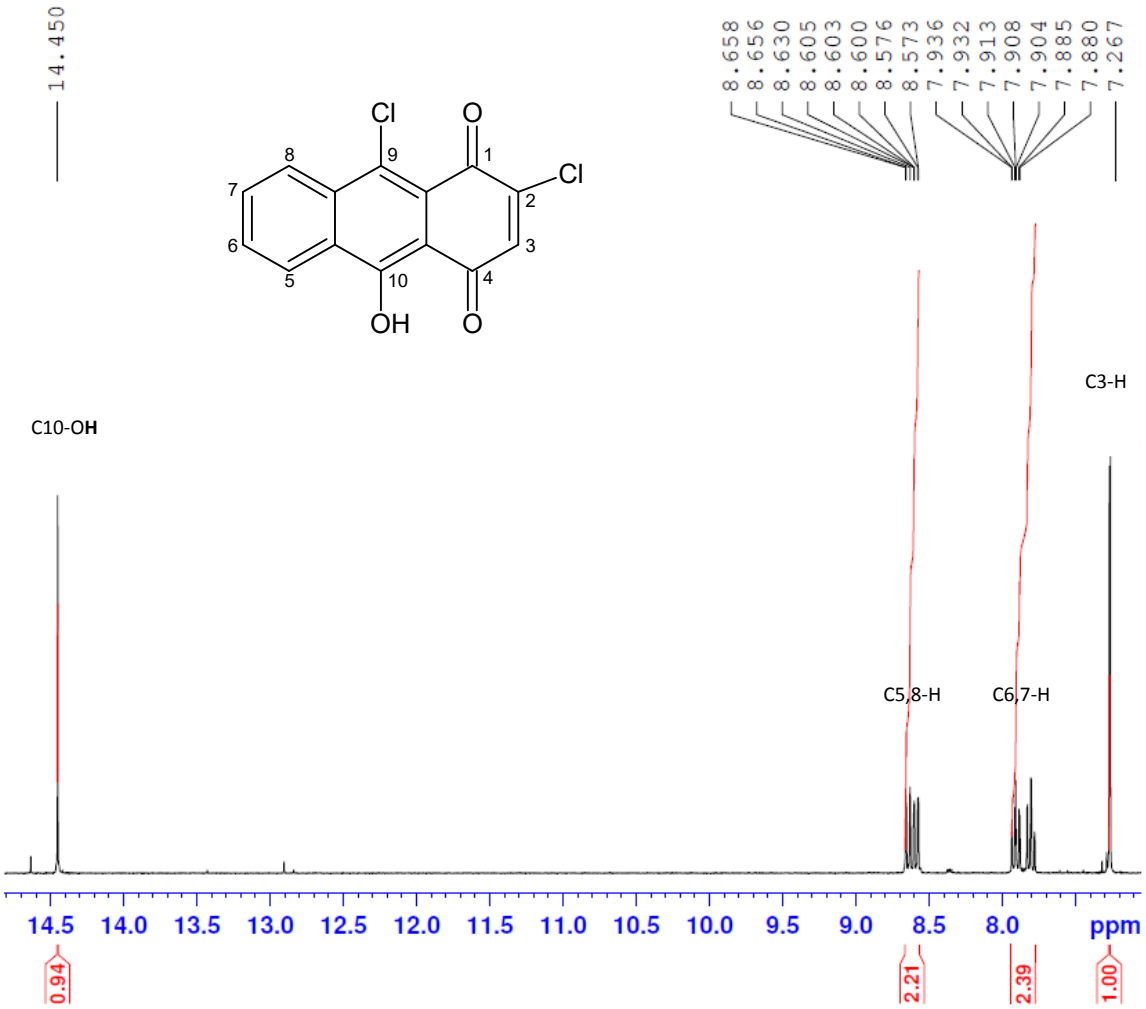


Figure 3-10 ^{1}H NMR spectrum of 2,9-dichloro-10-hydroxy-1,4-anthraquinone.

LC-MS data provides further confirmation of the structure of the compound. The HRMS full scan spectrum of **3** conducted in positive mode (Figure 3-11) shows the $[M+H]^+$ to have *m/z* = 292.9751, which corresponds to the molecular formula $C_{14}H_7Cl_2O_3$ (7.2551 ppm mass accuracy error). This correlates with the expected protonated product **3**. The sodium adduct of **3** ($[M+Na]^+$) is also present with *m/z* = 314.9533, which corresponds to the molecular formula $C_{14}H_6Cl_2O_3Na$.

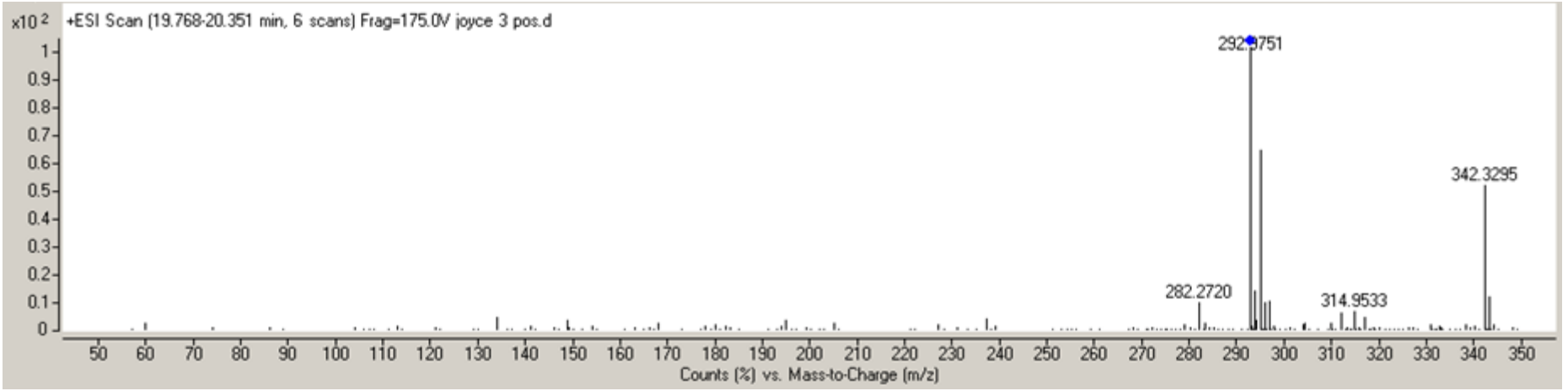


Figure 3-11 High resolution mass spectrometry full scan spectrum of 2,9-dichloro-10-hydroxy-1,4-anthraquinone (FE = 175.0 V, CE = 20eV), with $[M+H]^+$ of 292.9751 and $[M+Na]^+$ of 314.9533.

3.5.4 9-Chloro-10-hydroxy-2-methoxy-1,4-anthraquinone, 4

After the successful synthesis of **3**, **4** was synthesised. The reaction was predicted to be straightforward, as the halide ion attached to C-2 is considered a good leaving group. The reaction was performed by stirring the starting material in a solution of sodium methoxide and cold methanol under a stream of nitrogen (N_2) gas. Early attempts at this reaction produced unsatisfactory yields (approx. 14 %). In order to improve the yield, the reaction was repeated with changes to sequence in which the reactants were added, but this approach had no effect on the yield. Improvement of the yield (approximately 72 – 90 %) was achieved upon using a different batch of sodium methoxide. This indicated that the original sodium methoxide was the source of the problem as all other factors remained constant. The reaction was likely to have failed as a result of moisture contamination that reduced the effectiveness of the nucleophile.

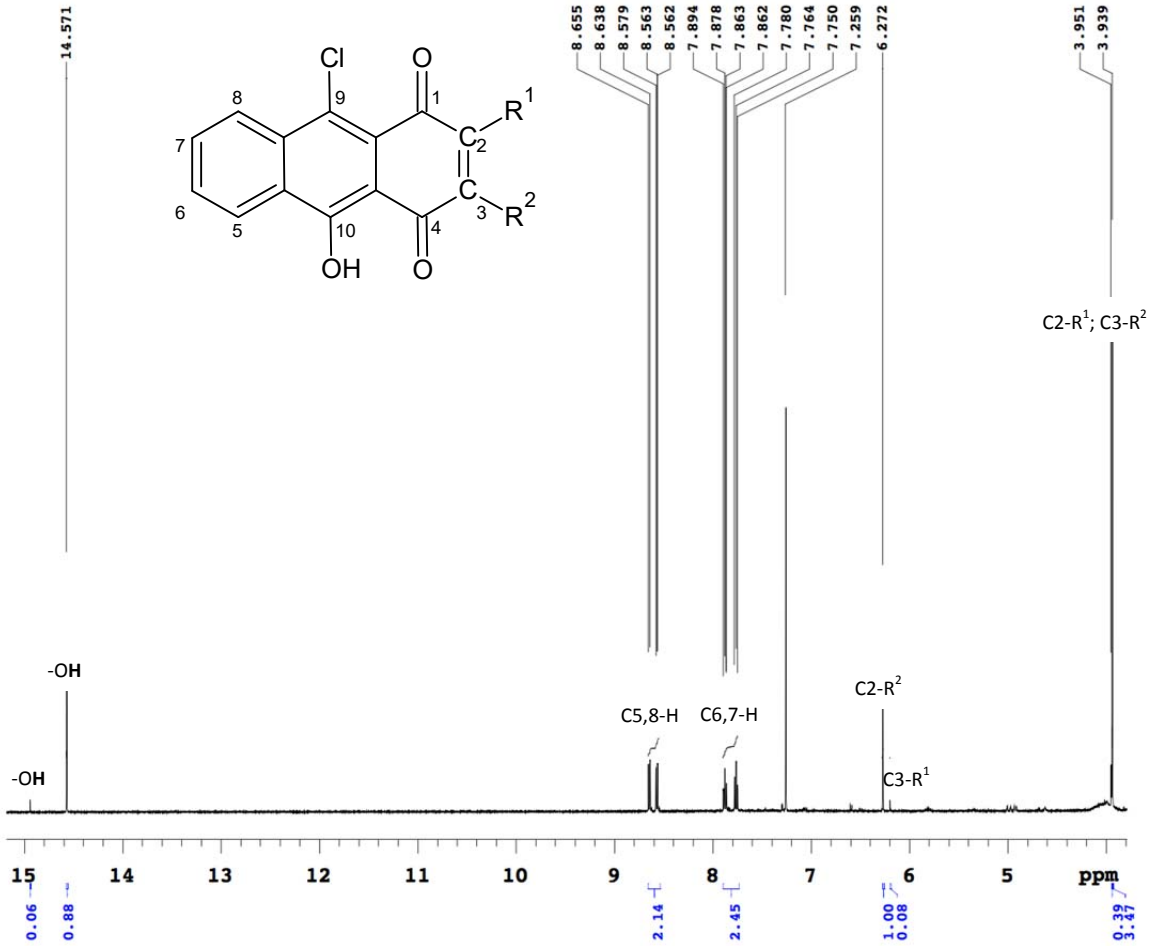


Figure 3-12 ^{1}H NMR (500Mz) spectrum, expanded from region 4.0 ppm to 15.0 ppm, isomers of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone where C2-R^1 = -OCH_3 and C2-R^2 = H and C3-R^1 = H and C3-R^2 = -OCH_3.

The ^{1}H NMR spectrum (Figure 3-12) of the methoxy compound showed that there are two singlets, both occurring at approximately δ3.9 ppm. These are both consistent with the chemical shift arising from -OCH_3 groups. In addition, two singlets are also present at approximately δ6.3 ppm, one of which is attributed to the proton attached to the alpha carbon of the 2-methoxy group, and the other to the 3-methoxy group. Similarly, two singlets are also present at approximately δ14.6 and δ14.9 ppm.

These irregularities were of particular interest, as the proposed compound was expected to contain only one methoxy group at the C-2 position. It is proposed that two different reactions occurred during the synthesis of the compound, one being an addition-elimination reaction in which the methoxy group is functioning as the nucleophile attacking the carbon (C-2) that is attached to the chlorine. The

intermediate is then stabilised by the resonance of the quinone structure. The other (less likely) possibility is that, instead of attacking the carbon that is attached to the chlorine, the methoxy group is attacking the carbon (C-3) that is attached to the hydrogen. This results in the formation of a 3-methoxy analogue, the minor product in the reaction. The two possible mechanisms for the formation of the methoxy analogues are depicted in Figure 3-13 and Figure 3-14.

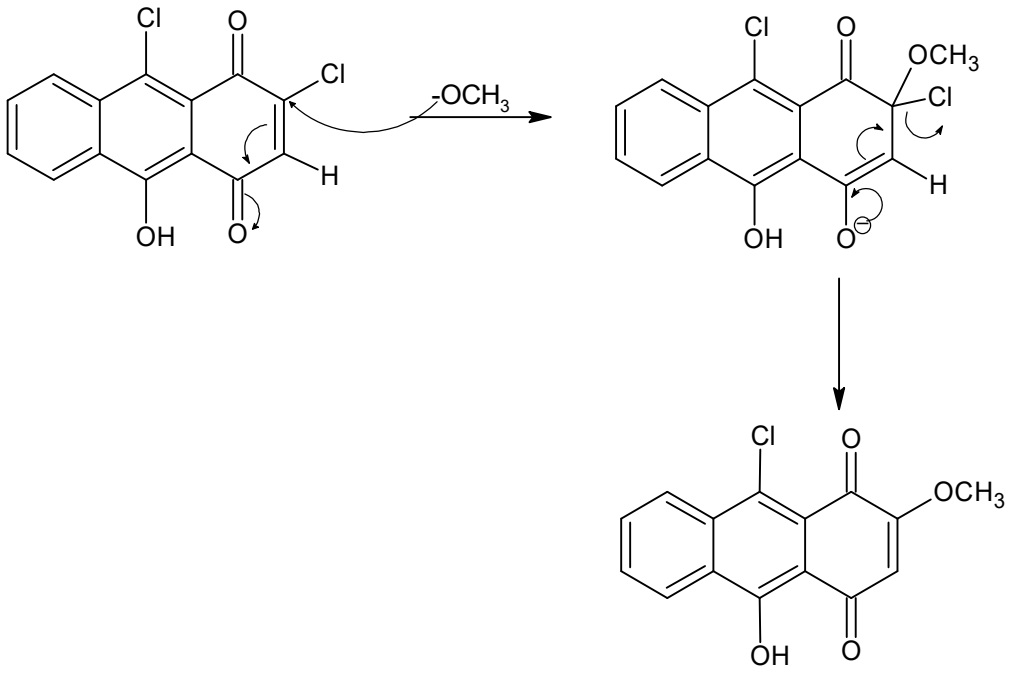


Figure 3-13 Possible mechanism for the formation of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone from 2,9-dichloro-10-hydroxy-1,4-anthraquinone and sodium methoxide.

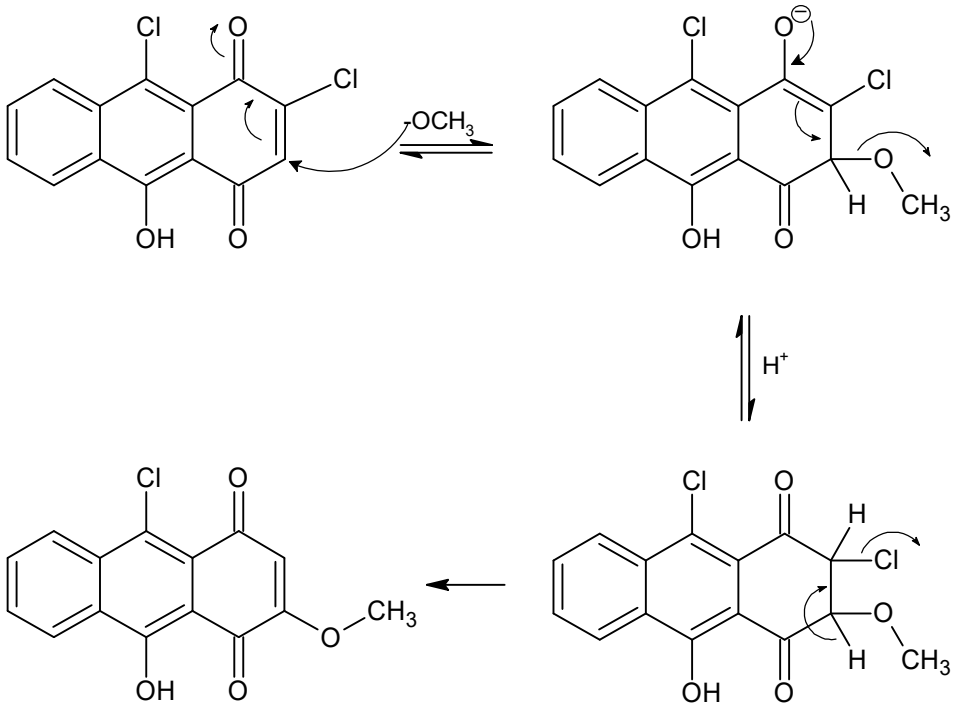


Figure 3-14 Proposed mechanism of the 3-methoxy analogue formed from the reaction of 2,9-dichloro-10-hydroxy-1,4-anthraquinone and sodium methoxide.

Attempts were made to separate the two compounds using recrystallisation, but this led to a decrease in the yield, as the crude product had to undergo multiple recrystallisations in order to achieve a somewhat pure compound. Therefore, in order to increase the yield, the use of column chromatography was utilised as it allowed for greater separation of the two compounds. This was evident in the NMR spectrum of the purified product, where the singlet attributed to the 3-methoxy compound, occurring at the chemical shift of approximately δ3.9 ppm, is significantly smaller than the peak arising from the 2-methoxy isomer. Figure 3-15 shows the separation of the two isomers using column chromatography. Two distinct bands can be observed, corresponding to the two isomers separated by the column.

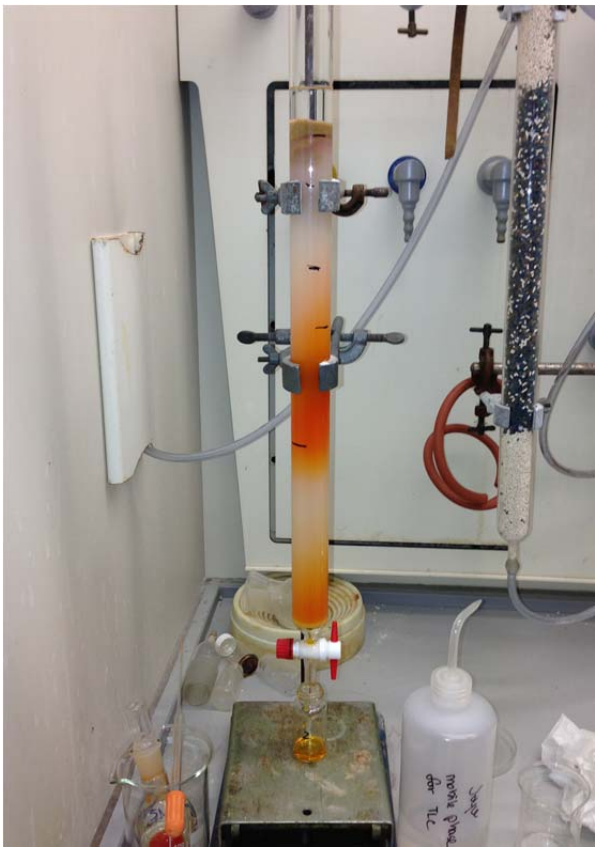


Figure 3-15 The column used for the separation of 2- and 3-methoxy analogues.

Further confirmation of the formation of the methoxy group was provided by the ^{13}C NMR spectrum, in which a peak was present at δ56.56 ppm. The chemical shift of this peak is characteristic of a carbon in an ether and this peak was clearly absent in the starting material **3**. In addition, the 2D-NMR (HSQC) spectrum of the product (Figure 3-16) shows correlation between the protons in the ether and the carbon to which the protons are attached. The spectrum also shows the presence of a peak at δ157.80 ppm, attributed to C2 to which the ether substituent is attached.

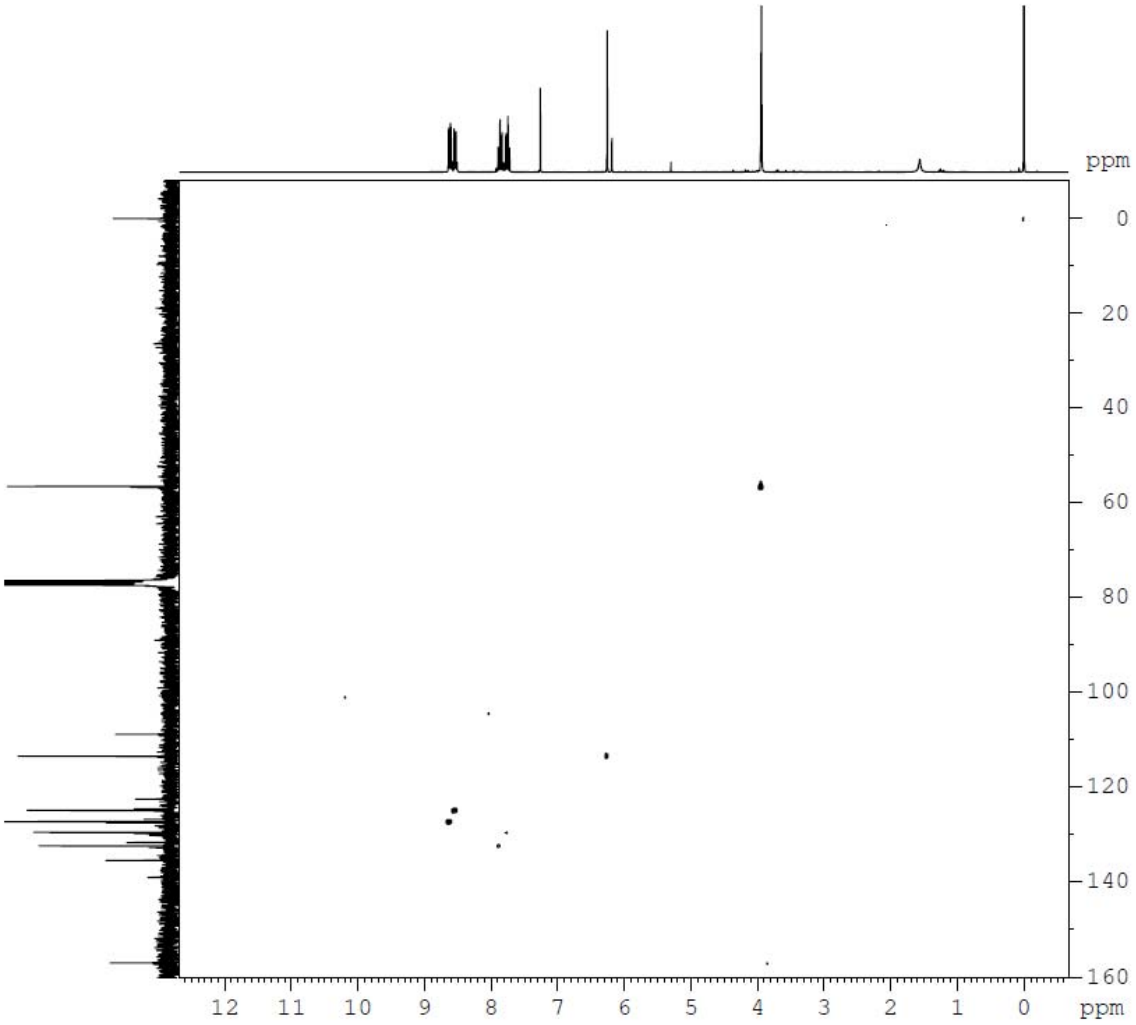


Figure 3-16 2-D NMR (COSY) spectrum of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone.

The HRMS full scan spectrum (Figure 3-17) of the product indicated the $[M+H]^+$ at *m/z* = 289.0280 was found to correlate with the protonated molecular formula for the expected product, **4** ($C_{15}H_{10}ClO_4$, 4.285 ppm mass accuracy error). When the product was analysed in negative mode, the molecular ion, $[M-H]^-$ was found at *m/z* = 287.0132. This was found to correlate with the molecular formula for the deprotonated form of the expected product, **4** ($C_{15}H_8ClO_4$, mass accuracy error 7.277 ppm). The combination of these results supports the formation of the expected product from the synthesis.

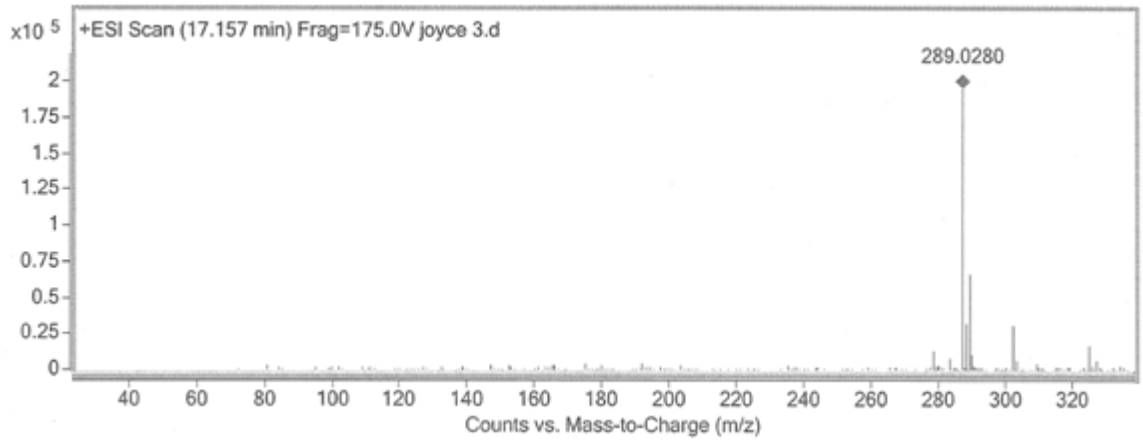


Figure 3-17 High resolution mass spectrometry full scan spectrum of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone (FE = 175 V, CE = 20 eV), with $[M+H]^+$ of 289.0280.

3.5.5 9-Chloro-2,10-dihydroxy-1,4-anthraquinone, 5

The final product from this multi-step reaction was **5**. This was synthesised by refluxing **4** in sodium hydroxide for approximately two hours. Figure 3-18 illustrates the reaction scheme for the formation of 9-chloro-2,10-dihydroxy-1,4-anthraquinone.

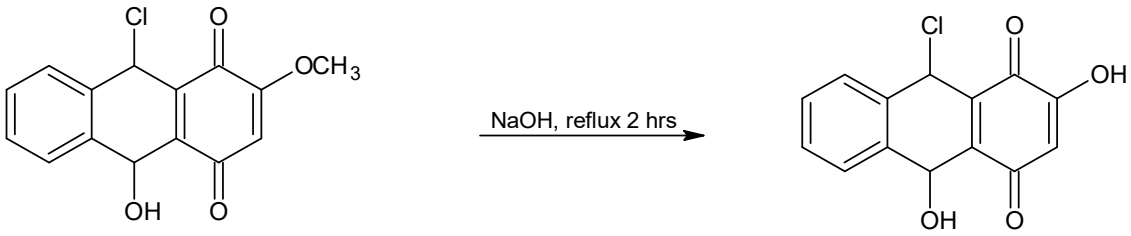


Figure 3-18 Reaction scheme depicting the demethylation of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone.

This product was characterised by its melting point and FTIR spectrum. Analysis by ^{1}H and ^{13}C NMR and LC-MS was also performed. The ^{1}H NMR (500Mz) spectrum (Figure 3-19) shows three singlets at δ6.45, 7.32 and 13.75 ppm due to the protons C3-H, C2-OH and C10-OH, respectively. The resonances for the protons C5-H and C8-H appear as doublet of doublets at δ8.57 and δ8.68 ppm. The resonances of the protons C6-H and C7-H appear as a triplet of doublets at δ7.78 and δ7.90 ppm. These resonances are in the expected regions and appear to have similar chemical shifts to the corresponding

protons in the substituted anthraquinones that were synthesised in the experimental work in Section 3.4.3 to 3.4.5. Furthermore, the singlet due to the protons of the methoxy group was also absent in ^{1}H NMR spectrum. The ^{13}C NMR spectrum of the product demonstrates the disappearance of a peak at approximately δ56 ppm, indicating the absence of the methoxy group in the final product.

The HRMS full scan (Figure 3-20) indicated the product $[M+H]^+$ was found to have a *m/z* of 275.0121. This correlates with the molecular formula for the expected protonated product, **5** ($C_{14}H_8ClO_4$, 3.595 ppm mass accuracy error). In negative mode, the $[M-H]^-$ was found to have a *m/z* of 272.9963, corresponding with the molecular formula for the expected deprotonated **5** ($C_{14}H_6ClO_4$, 3.072 ppm mass accuracy error). The combination of data gathered from LC-MS, NMR and FTIR analyses confirm that the synthesised compound is as expected.

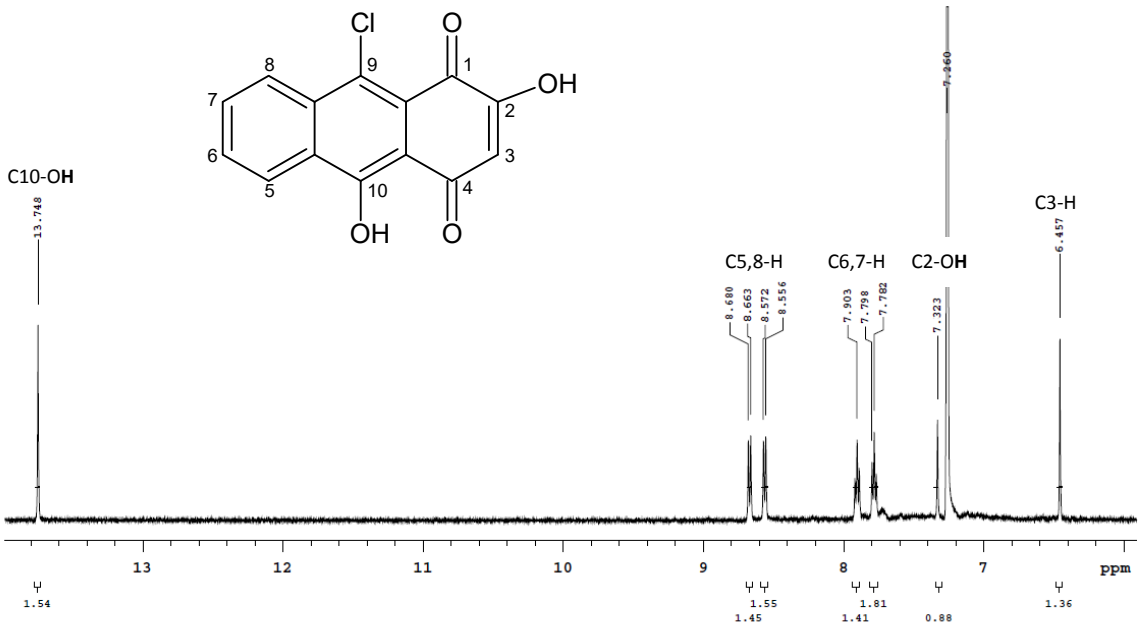


Figure 3-19 ^{1}H NMR ($CDCl_3$) of 9-chloro-2,10-dihydroxy-1,4-anthraquinone from 6 – 14 ppm.

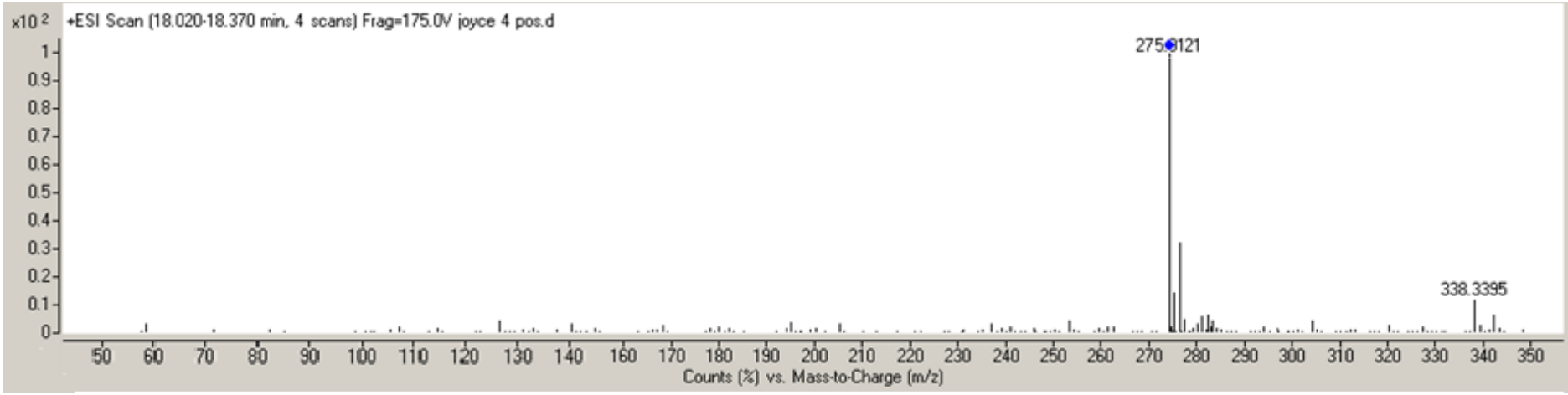


Figure 3-20 High resolution mass spectrometry full scan spectrum of 9-chloro-2,10-dihydroxy-1,4-anthraquinone (FE = 175.0 V, CE = 20.0 eV) $[M+H]^+$ of 275.9121.

3.5.6 1,4-Anthraquinone, 6

Due to tautomeric equilibrium between the middle ring and the external quinone ring (218), it was possible to form **6** from the reduction of quinizarin using sodium borohydride in dried methanol at 0 °C under nitrogen (198). Sodium borohydride reduces quinizarin in two steps. The first step of the reaction involves the addition of a hydride ion from sodium borohydride to C-9 and C-10, forming a C-H bond and breaking the C=O bond. The negatively-charged oxygen is protonated during the acidification step in the reaction and water is eliminated. Figure 3-21 illustrates the reaction.

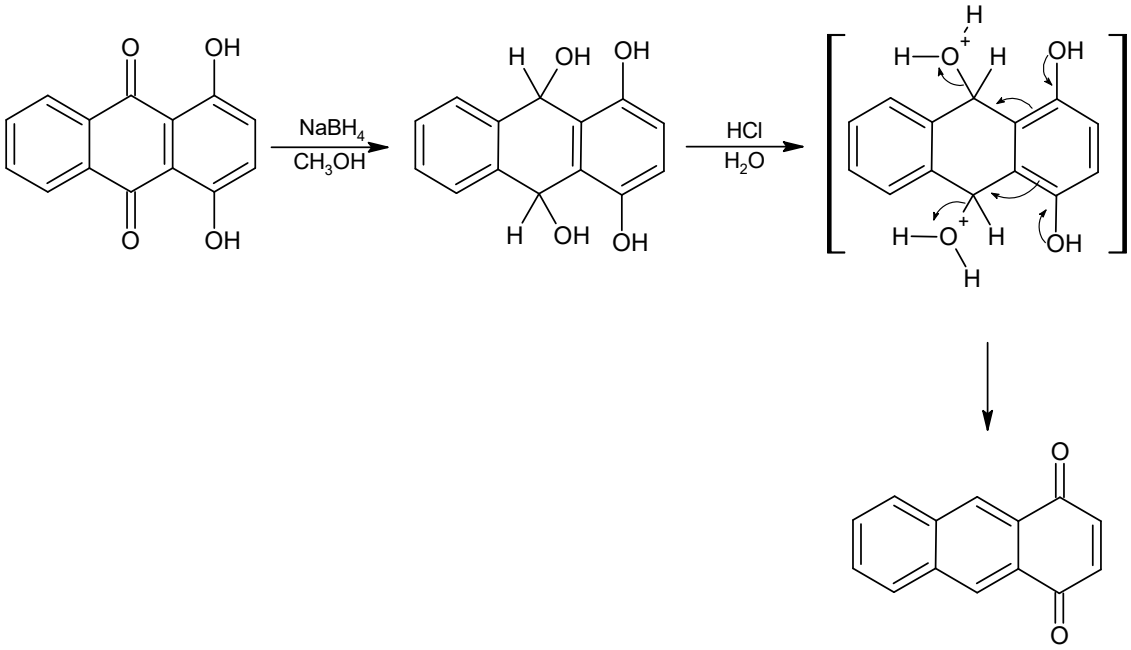


Figure 3-21 Reaction scheme of quinizarin in sodium borohydride, adapted from Lou, K. (219).

The progress of the reaction was monitored using TLC and was quenched using hydrochloric acid when no further reaction was observed. The NMR spectrum indicated a large amount of starting material remained in the reaction mixture. Multiple attempts to recrystallise the crude product were made. These repeated recrystallisations meant that the yield of the reaction was compromised. Figure 3-22

shows the impure product after two consecutive recrystallisation attempts. The spectrum indicates that a large amount of starting material remained in the mixture. The red crosses indicate the signals arising from the protons are attributed to quinizarin.

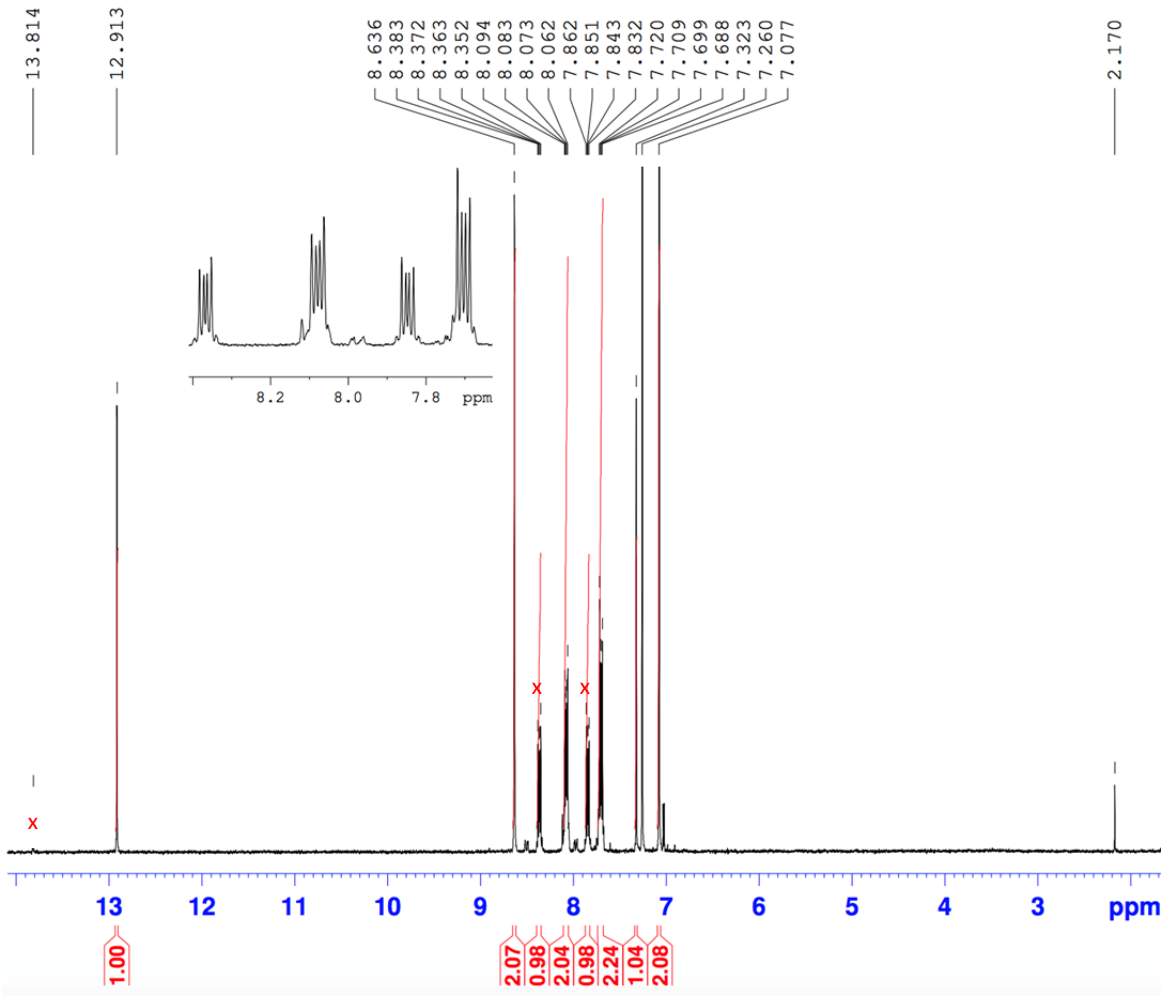


Figure 3-22 Impure product, 6, where 'x' marks residual quinizarin.

Another method was pursued in order to separate the starting material, quinizarin, from the product. The chemistry of these two compounds differs mainly by the presence of the two hydroxy groups that are present on quinizarin, shown in Figure 3-23.

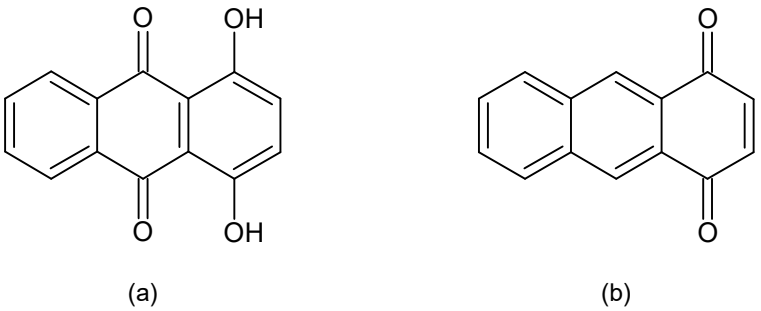


Figure 3-23 Structures of (a) quinizarin, (b) 1,4-anthraquinone

These two compounds were able to be separated with the use of a sodium hydroxide solution (3 – 5 % w/v), which ionised the quinizarin and rendered it soluble in water, while **6** remained insoluble. It was then separated using liquid-liquid extraction, where three consecutive dichloromethane washes were used to clean the mixture.

The reaction illustrated by Figure 3-24 proved to be a simple yet effective method for sample clean-up. This is evident in Figure 3-25, which shows the ^{1}H NMR spectrum, expanded from 9.0 ppm to 4.0 ppm, of the purified **6**. Note the differences in the aromatic region that differentiate the impure and pure sample in Figure 3-22 and Figure 3-25, respectively. The multiplets arising from the quinizarin protons are not present in Figure 3-25.

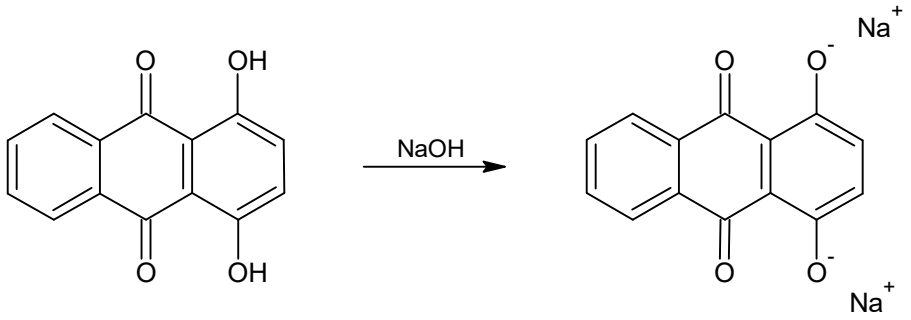


Figure 3-24 Reaction scheme illustrating the ionisation of quinizarin in an aqueous sodium hydroxide solution

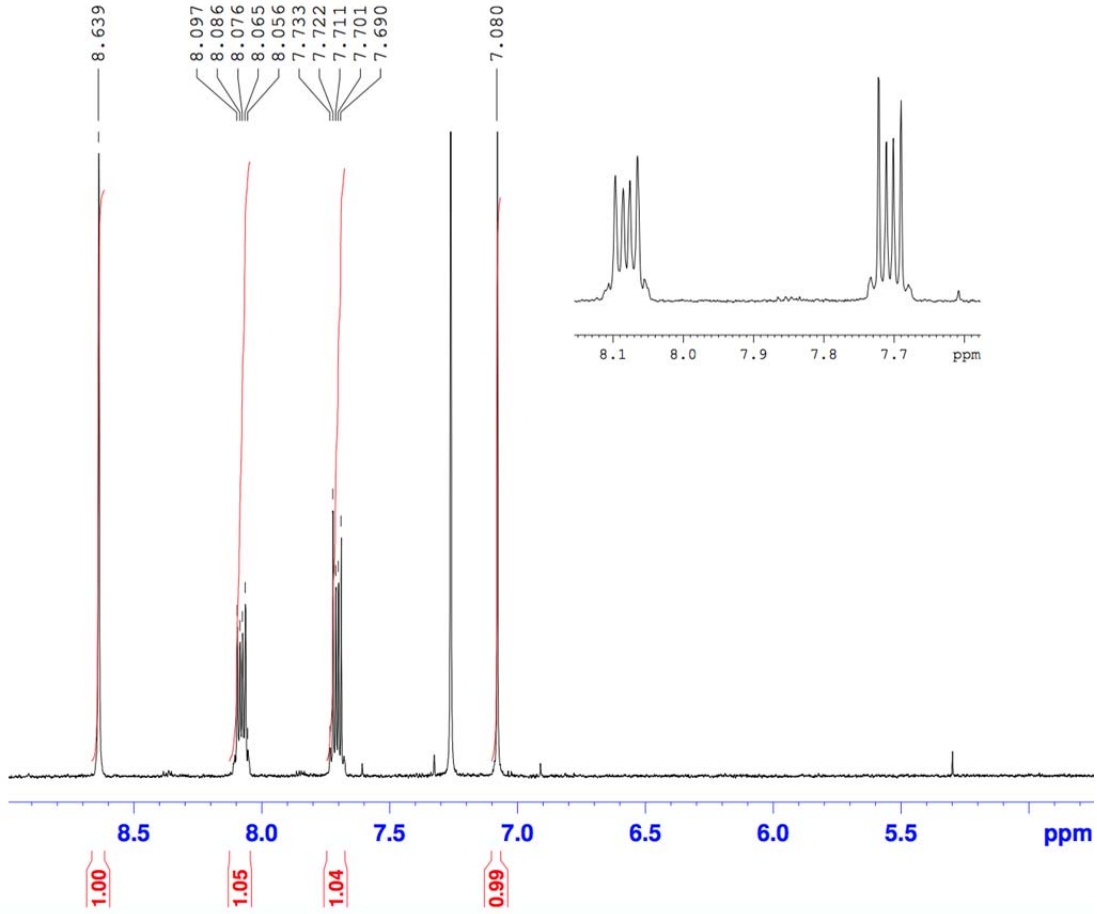


Figure 3-25 ^{1}H NMR spectrum of the expanded region from 4.0 ppm to 9.0 ppm highlighting the absence of peaks attributed to quinizarin of product after washing with a NaOH (3 % v/v) solution.

During these experiments, it was also noted that the number of moles of sodium borohydride used for the reduction also impacted the yield of the product. At 1:1 mole equivalents of sodium borohydride to quinizarin, only a small amount of **6** was synthesised and an optimum yield was not achieved. Increasing the amount of sodium borohydride in the reaction resulted in higher product yield. This eventually reached a maximum yield and any increases in the moles of sodium borohydride used thereafter had negative impacts on the yield of the product. Table 3-3 demonstrates the relationship between sodium borohydride in the reaction and the yield of the product obtained.

The effects were also noted by Nor *et al.* who postulated that **6** and 4-hydroxyanthracene-1,10-dione were formed when low concentrations of sodium

borohydride were used in the reaction (220). This was likely to have contributed to the low yield of **6** that could be formed. The reaction scheme for the formation of these impurities is depicted in Figure 3-26. Characterisation of the impurities formed in this reaction was not within the scope of this project. Regardless, if 4-hydroxyanthracene-1,10-dione was a by-product, it would have been separated during the purification step with the sodium hydroxide solution.

Table 3-3 Moles of sodium borohydride used in the reaction and its corresponding yield

Sodium borohydride used (moles)	Approximate yield of product (%)
1	10
4	80
10	50

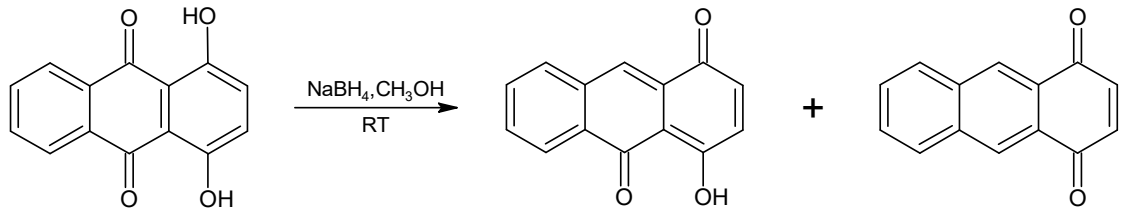


Figure 3-26 Impurities formed in the reaction of quinizarin and sodium borohydride with methanol. Adapted from Nor *et al.* (220).

3.5.7 2-Methoxy-1,4-anthraquinone, 7

The reaction was performed following the method set out by Fieser and Martin (221) and Fieser (211). The reaction was monitored by TLC, and the reaction times were substantially longer than the literature values. Fieser noted that the reaction mixture formed a paste, but this was not the case in the experiments that were carried out. This was likely due to the increase in the volume of solvents used – excess solvent was required to dissolve the starting material. Despite differences in observations, when the crude mixture was analysed by ^{1}H NMR spectroscopy (Figure 3-27), the chemical shifts in the ^{1}H NMR spectrum indicated that **7** had been formed. The singlet at

approximately δ3.94 ppm indicates the presence of a methoxy group. Figure 3-27 showed that quantities of **8** were also formed. This was indicated by the presence of a broad singlet occurring at approximately δ7.45 ppm and a singlet at approximately δ6.48 ppm. The presence of two compounds within the crude product would also explain the lack of a defined splitting pattern that would have resulted from the protons in the aromatic rings.

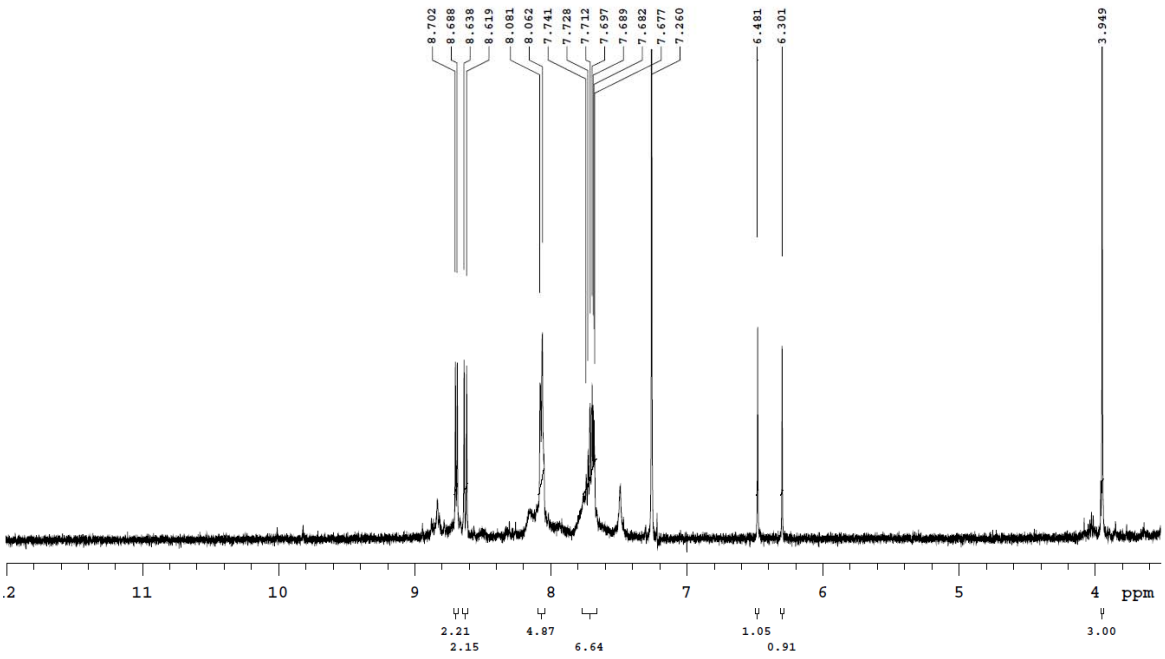


Figure 3-27 Crude reaction mixture containing 7 and 8.

The compound was purified by recrystallisation using ethanol followed by analysis using melting point, FTIR, NMR and LC-MS. The results of the melting point analysis corresponded to the literature value provided by Fieser (211). The other experimental data also corresponded with the proposed structure. In some instances, the crude product was used in the next step of the reaction, since the formation of **8** in this step would not affect the synthesis of the final product.

3.5.8 2-Hydroxy-1,4-anthraquinone, 8

Two different methods for the synthesis of **8** were evaluated. The first method involved a two-step reaction. The first step involved the synthesis of 1,2,4-

triacetoxyanthraquinone from **6** via Thiele-Winter acetoxylation, followed by basic hydrolysis in either a solution of sodium hydroxide or sodium methoxide (208).

The acetoxylation reaction was attempted and the product had a tar-like consistency. Attempts at purification were unsuccessful, with the NMR spectrum of the product lacking the characteristic $-CH_3$ peak that would arise from the acetoxy groups. Failure of the Thiele-Winter acetoxylation has been well-documented, likely due to the formation of side-chained reaction products rather than the acetoxylation product (199). As the first step of the reaction was unsuccessful, alternate plans were investigated for the synthesis of **8**.

Previous research conducted by Fieser indicated that **8** could be synthesised from 1,2-anthraquinone-4-sulfonic acid, sodium salt (211) (Figure 3-7). Due to the availability of starting materials, 1,2-anthraquinone-4-sulfonic acid, ammonium salt (AQS) was purchased. AQS was used for the synthesis of **7**, as discussed in Section 3.5.7.

The formation of **8** was possible by refluxing **7** in a weakly basic solution in which a hydrolysis reaction was performed successfully. Melting point data for **8** corresponded to those previously reported by Fieser (211). The other experimental data was also consistent with the expected structure.

3.6 Conclusions

One of the aims of this project was to compare the effects of changes to the molecular structure of fingermark reagents (e.g. increasing conjugation and altering the position of substituent groups, etc). In order to do this, a number of quinones from 2-hydroxy-1,4-benzoquinone to more complex 1,4-anthraquinone systems were synthesised.

A literature review indicated that 2-hydroxy-1,4-benzoquinone (**1**) could be formed via oxidation of 1,2,4-benzenetriol or 2,4,6-trihydroxybenzoic acid. The synthesis of **1** was attempted using 2,4,6-trihydroxybenzoic acid in the presence of a strong oxidant, methylene blue. These attempts were unsuccessful and the oxidation of 1,2,4-

benzenetriol using silver carbonate on Celite® was evaluated. This method proved successful and **1** was produced in high yield (approximately 80 %).

Various anthraquinones were synthesised in the current work, with the use of quinizarin as a starting material proving effective, as it allowed for the synthesis of 1,4-anthraquinones with relative ease and high yields. The chlorination of quinizarin with freshly distilled thionyl chloride produced 9-chloro-10-hydroxy-1,4-anthraquinone (**2**). Chlorination of **2** was carried out using a stream of chlorine gas to produce 2,9-dichloro-10-hydroxy-1,4-anthraquinone (**3**). **3** was reacted with sodium methoxide in an addition-elimination reaction, the major product being 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone (**4a**). A minor product, 9-chloro-10-hydroxy-3-methoxy-1,4-anthraquinone (**4b**) was formed . The final product, 9-chloro-2,10-hydroxy-1,4-anthraquinone (**5**), was synthesised via hydrolysis of **4**.

Other simple 1,4-anthraquinones that were successfully synthesised in this work include: 1,4-anthraquinone (**6**) from the reduction of quinizarin using sodium borohydride, 2-methoxy-1,4-anthraquinone (**7**) and 2-hydroxy-1,4-anthraquinone (**8**) .

These quinones were all successfully synthesised and characterised using a variety of analytical techniques in order to confirm their structure. The synthesised quinones vary in their substituents and the effects of these substituents on amino acids and fingermark development are evaluated in the next chapter.

Chapter 4: Use of quinones for latent fingermark detection

4.1 Introduction

As discussed in Chapter 3, quinones have diverse applications across a wide range of industries. Naphthoquinones in particular, were of interest as they have been shown to react with amines and amino acids to produce coloured and fluorescent compounds that have been utilised in chromatography and spectroscopy (131; 132; 222-226). For example, sodium 1,2-naphthoquinone-4-sulfonate has been used in chromatography for the determination of aminomethylbenzoic acid to produce a pink chromophore (227). It has also been used as a derivatising agent for the detection of primary, secondary and aromatic primary amino groups (130; 228). In addition, quinones formed during the oxidation of phenols in biological systems have been known to produce coloured adducts when reacted with proteins (229). These results indicate that there is potential for the use of naphthoquinones in the detection of amino acids that are inherently present in fingermarks. The use of naphthoquinones in the detection of latent fingermarks was previously investigated by Jelly *et al.* (127).

In this chapter, the synthesised quinones discussed in Chapter 3 were used for the development of amino acids and fingermarks. It was hypothesised that anthraquinones are more reactive than naphthoquinones towards amino acids that are found in fingermarks due to an increase in π conjugation. Furthermore, the comparison of these compounds is not only limited to ring conjugation but also the substituents that are present. The reaction of these anthraquinones on amino acids may lead to the formation of coloured products which are also fluorogenic. The aim of this chapter is to assess and compare the ability of the synthesised compounds in developing latent fingermarks. The working solutions of each of the quinones are then assessed against IND-Zn, a well-established and the primary technique used by Australian law enforcement agencies for the detection of latent fingermarks on porous surfaces.

4.1.1 Considerations in reagent optimisation

A number of factors should be considered in the optimisation of a reagent formulation. These factors include reactivity towards amino acids, the solvents and concentration effects, pH, metal salts and the development techniques.

Reactivity of a fingermark reagent towards amino acids is an important parameter in reagent optimisation. Amino acids (serine, alanine, and lysine) that were chosen in this study are abundant and representative of the natural secretions found in fingermark deposits. The initial work was conducted with amino acids spotted on filter paper and fingermarks deposited on a range of porous surfaces. Filter paper is made up of cellulose fibres without added optical brighteners or fillers, which are sometimes present in paper (230). The elimination of these factors indicates whether the reagent is in fact reacting with the amino acids rather than the paper. The use of cellulose may also act as a surface catalyst and aid in the reaction by stabilising any intermediates and products that are formed (106). The use of amino acids on filter paper also eliminates variations in donor secretions. Therefore, these 'standard' test strips can objectively demonstrate the sensitivity/selectivity of the compound tested.

The quality and fluorescence of the developed fingermarks can be affected by a reagent's formulation (99). The choice of solvents and concentration of reagent can influence the stability and solubility of the reagent in the stock and/or the working solutions. Operational needs such as work health and safety, ease of use, costs and background discolouration are also factors when selecting solvents. The diffusion of inks and fingermarks on the substrate should also be taken into consideration when formulating the stock and/or the working solution (74).

The reaction of conventional fingermark reagents such as ninhydrin, DFO and 1,2-indanedione commonly proceeds in an acidic environment (98). The additives in paper for example carbonate fillers, can lead to a basic substrate (231). As a result, current working solutions contains small amounts of acetic acid to neutralise the substrate (49). Furthermore, acetic acid can also act as a catalyst for the reaction between the

reagent and amino acids in fingermarks. Therefore, the effects of pH may be crucial in the optimisation of fingermark reagents.

Improvements in fingermark ridge details can be achieved through the addition of metal salts either in solution or as a post treatment. For example, ninhydrin forms a metal complex following treatment with a zinc or cadmium metal salt that is fluorescent when cooled with liquid nitrogen (99). The formation of a metal complex also occurs with DFO developed fingermarks, however, secondary metal salt treatment only has a minimal effect on the fluorescence of DFO treated prints (88). The addition of zinc chloride to an indanedione solution serves as a Lewis acid catalyst, which stabilises the formation of an intermediate during the rate limiting hydrolysis step (106). Since naphthoquinones and anthraquinones are expected to react with amino acids in a similar manner, the addition of metal salts may also be beneficial to the reaction. However, Jelly *et al.* did not observe improvements when zinc chloride solution was added to the lawsone working solution (127).

Most fingermark reagents require the use of heat to develop latent fingermarks (49). Some of these development techniques require the use of humidity in conjunction with heat treatment in order to accelerate the rate of reaction between the eccrine secretions present in fingermarks, such is the case with ninhydrin and genipin (39; 74). There are exceptions in which heat treatment can be omitted, for example, fingermarks on thermal paper developed with IND-Zn is developed in the dark as it reduces discolouration of the paper substrate (232; 233). For some reagents, such as DFO and IND-Zn, the use of a heat press (no humidity) is sufficient to produce highly fluorescent fingermarks. Stoilovic noted that articles treated with DFO when subjected to heat treatment provided by a domestic iron versus that provided by commercial ironing press produced negligible differences in results (234). Previous research on development techniques for fingermark reagents used a domestic iron (234), commercial dry ironing press (94; 98) and an oven (95; 235; 236). The use of a heat press is substituted in the following experimental method.

Latent fingermarks undergo rapid changes post deposition. These changes are dependent on a number of factors such as surface, temperature and humidity (30).

Changes in these factors can be accounted for by using fingermarks of different ages. However, operationally it is unlikely that fresh fingermarks will be collected and treated in a 24 hour period.

4.2 Materials

4.2.1 Reagents

All reagents were obtained from Sigma-Aldrich Australia unless otherwise stated: 1,2,4-trihydroxybenzene *ReagentPlus®* 99 % [CAS 533-73-3]; 1,2-naphthoquinone-4-sulfonate, sodium salt (NQS) [CAS 521-24-4]; 2-hydroxy-1,4-naphthoquinone (lawsone) 97 %[CAS 83-72-7]; 1,4-dihydroxy-9,10-anthraquinone (quinizarin) ≥ 96.0% [CAS 81-64-1]; and 1,2-anthraquinone-4-sulfonate, ammonium salt (AQS) (Aldrich^{CPR}). Reagent grade cetrimonium bromide (CTAB) [CAS 57-09-0], L-alanine ≥ 98 % [CAS 56-41-7], L-lysine ≥ 98 % [CAS 56-87-1], L-serine *ReagentPlus®* ≥ 99 % [CAS 56-45-1], citrate acid ACS reagent ≥ 99.5 % [CAS 77-92-9], cetrimonium bromide (CTAB) ≥ 98 % [CAS 57-09-0], anhydrous zinc chloride ≥ 99.995 % [CAS 7646-85-7] and zinc nitrate ≥ 99.0 % [CAS 10194-18-6] were used as supplied.

All reagents and solvents used were obtained from Chem-Supply Pty Ltd, Australia and were AR grade unless otherwise state: 1,4-dioxane [CAS 123-91-1], 1-butanol [CAS 71-36-3], 1-propanol [CAS 64-17-5], acetonitrile [CAS 75-05-8], absolute ethanol [CAS 64-17-5], ethyl acetate [CAS 141-78-6], ethylene glycol [CAS 107-21-1], dichloromethane [CAS 75-09-2], diethyl ether [CAS 60-29-7], dimethyl sulfoxide [CAS 67-68-5], methanol [CAS 67-56-1], petroleum ether [CAS 8032-32-4], glacial acetic acid [CAS 64-19-7], sodium phosphate dibasic anhydrous [CAS 7558-79-4], and sodium dihydrogen orthophosphate dihydrate [CAS 13472-35-0].

Methyl nonafluoroisobutyl ether (HFE7100) was obtained from Novec TM, Australia.

The following reagents were obtained through Ajax Finechem Pty Ltd, Australia: anhydrous sodium acetate hydrate 99.9985 % [CAS 41484-91-7], trisodium citrate

dihydrate 99 % [CAS 6132-04-3], and Triton X-100 [CAS 9002-93-1] were used as supplied.

Sodium dodecyl sulfate (SDS) biotechnology grade [CAS 151-21-3] was obtained through BDH Prolabo Chemicals, Australia and used as supplied.

1,2-Indanedione [CAS 16214-27-0] was obtained through Optimum Technology, Canberra (Australia) and used as supplied.

All quinones, **1-8**, were synthesised and purified as stated in Chapter 3.

4.2.2 Instrumentation

A Rofin Poliview IV system (AF Micro Nikkor 60 mm lens) fitted with a Polilight PL-500, using V++ imaging software was used for observation and recording of fingermarks.

A Foster + Freeman VSC6000/HS and VSC Suite version 6.6 were used to visualise and obtain fluorescence emission spectra of the amino acids treated with the quinone solutions. It was also used to record fingermark fluorescence.

A JENCO 672 Digital pH/MV/temp meter was used for measure the pH of all quinone solutions.

A Memmert UM 400 Oven and Singer Magic Steam Press 7 were used for development for fingermark samples.

4.3 Methods

4.3.1 General Approach

One of the aims of this study is the application of the synthesised quinones to amino acids and fingermarks on porous surfaces and to investigate their effectiveness and suitability as possible fingermark reagents for forensic casework. Once the reagents

were determined to be selective to fingermark components, the working solutions of these reagents were then optimised. This was achieved through the development of fingermarks from a small number of donors on a range of porous surfaces. While it was noted that a small sample size of donors and substrates does not reflect reality, it is reasonable in this preliminary study as it can demonstrate the ability of the novel reagents to develop fingermarks (237). The fingermarks that were examined were aged over a period of up to one month. A number of preliminary comparisons were made during the study using the synthesised quinones. Some of the comparisons made included the measurements of the fluorescence produced by the products between the amino acids and fingermarks developed by the reagents, in order to determine the effects of substitution and conjugation. In addition, comparisons with IND-Zn for the development of fingermarks on porous surfaces were also conducted.

4.3.2 Reactivity with amino acids

Stock solutions of 0.1 M of each of the three amino acids (alanine, lysine, and serine) were made up in Milli-Q water (18.2 mΩ). The subsequent concentrations were made using serial dilution (0.01 M to $1x10^{-7}$ M). Using a pencil (2B) 1 cm^2 grids were drawn onto Whatman Ashless No. 42 filter paper and labelled 0.1 M to $1x10^{-7}$ M across the row (Figure 4-1). A 3 µL aliquot of each amino acid solution was spotted onto the corresponding grid, with the last grid spotted with blank Milli-Q water. The filter paper was air dried for at least 24 hours prior to use to prevent the diffusion of the amino acids across the filter paper. The filter paper was cut up into strips and the fingermark reagent was applied to the strips by dipping. The strips were air-dried, subjected to various development techniques and viewed with the appropriate excitation and filter in luminescence mode.

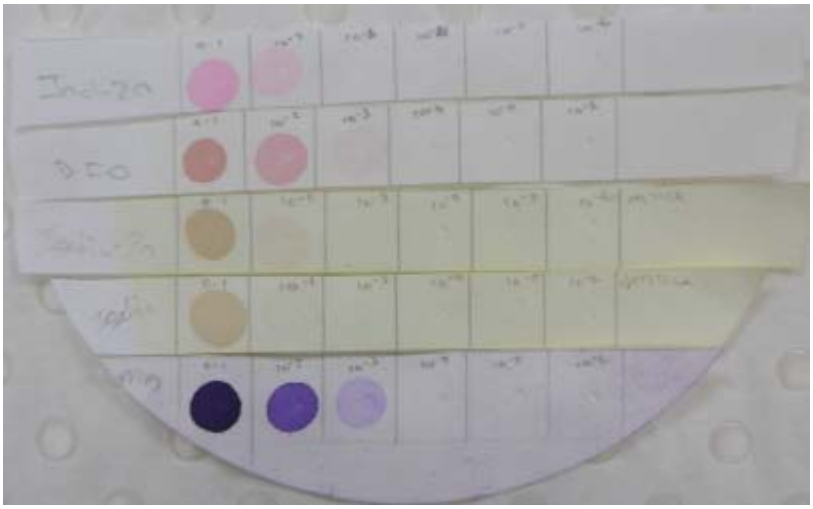


Figure 4-1 Filter paper spotted with amino acids in decreasing concentration (0.1 M to 0.1 x 10^{-6} M). Each strip of filter paper was developed with a fingermark reagent. Top to bottom: IND-Zn, DFO, NQS-Zn, isatin and ninhydrin.

The fluorescence emission of amino acid spots on filter paper was measured using a Foster + Freeman VSC6000/HS which provided an indication regarding the effectiveness of the reaction and therefore the limit of detection. The wavelengths for excitation were chosen based on a preliminary scan using the VSC6000 that then allows for the maximum emission wavelengths to be found. All synthesised quinones were imaged in luminescence mode with the VSC6000 using a 485 – 535 nm excitation and a 590 nm filter. The maximum emission spectra were obtained from ten different sites on the developed amino acid spot and an average was obtained. It was further normalised by subtracting from the blank (background). This was repeated across different reagent/solvent concentrations, pH, metal salts and development techniques.

4.3.3 Fingermarks

A sample group of six fingerprint donors (three males and three females) were used throughout this study for some degree of donor variability (good, average and poor donors). A small group was devised due the availability of donors and the preliminary nature of the work. The fingermarks evaluated ranged from fresh (deposited immediately prior to treatment), 1 day, 7 days, 1 month and 3 months. These

fingermarks were stored under laboratory conditions (approximately 20 – 22 °C and 30 – 40 % relative humidity) with protection from light.

The fingermarks were deposited on locally sourced blank Reflex® A4 paper (white, pink and purple), white lined paper and white recycled lined paper. The first approach to the deposition of fingermarks involved generating eccrine fingerprints where donors were asked to wash their hands thoroughly with soap and water. Their hands were then dried with a paper towel. The donors were then asked to place their hand in a powder-free nitrile glove for no more than 15 minutes and to remove the glove and rub their hands together. The second approach to deposition was based on uncharged/natural fingermarks (94; 98; 99; 238; 239). In both of these approaches, the donors were asked to deposit their fingerprints four times on a substrate sequentially (depletion series). They were instructed to place their hands down using medium force with a contact time of no more than three seconds.

Similarly to the method approached by Sears *et al.* (110), Roux *et al.* (94) and the guidelines outlined by the International Fingerprint Research Group (237), all the deposited fingermarks were then cut in half prior to treatment and each half was developed by a different reagent formulation (Figure 4-2). The split print approach was also used to compare each optimised quinone formulation against IND-Zn.

All comparisons were performed with split fingermarks on alternating sides of the mark. Each treated half was visualised under its respective optimal imaging conditions and digitally placed together for comparison. The developed fingermarks were then viewed using the VSC6000 and also recorded using a Rofin Poliview IV fitted with a Polilight PL-500 system. All anthraquinones, and NQS were imaged in luminescence mode using a 530 nm excitation and 610 nm barrier bandpass filter. Lawsone was imaged in luminescence mode using a 555 nm excitation and 610 nm barrier bandpass filter. IND-Zn was imaged in luminescence mode using a 505 nm excitation and 550 nm barrier bandpass filter.

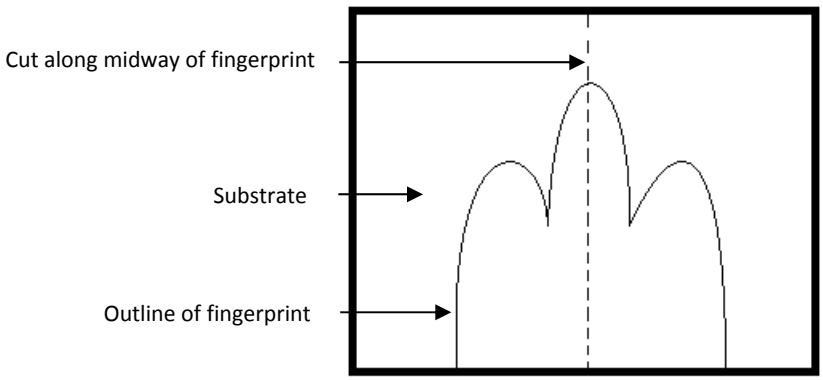


Figure 4-2 Schematic diagram of a split fingermark (three fingers) where each half is treated with a different formulation and subsequently viewed using the VSC6000 and the Polilight/Poliview system.

4.3.4 Development techniques

A number of development techniques were evaluated on amino acids on filter paper and fingermarks:

1. Heat press for 10 s and 20 s at 165 °C and 180 °C (Figure 4-3).
2. Heat press with the application of steam for 10 s and 20 s at 165°C and 180 °C.
3. Oven development at 100 °C and 150 °C with samples taken out at 15 minutes intervals up to 1.5 hours (Figure 4-3).
4. Room temperature with low light conditions.

Figure 4-3 Left: Memmert UM400 Oven, right: Singer Magic Steam Press 7.

4.3.5 Optimisation of solvents and concentration

For each reagent, the solubility of the reagents was evaluated by first diluting the reagent in a small volume of a relatively polar solvent then further diluting in a non-polar solvent to form the bulk of the working solution. A number of solvents were evaluated including methanol, ethanol, propanol, butanol, ethyl acetate, dichloromethane, and petroleum spirits. The non-polar carrier solvent used was HFE7100.

Each naphthoquinone (1 to 0.1 mg/mL) was dissolved in the aforementioned polar solvents to form a stock solution to determine its solubility. All anthraquinones (1 to 0.1 mg/mL) were first dissolved in a small volume of ethyl acetate to form a stock solution. Each of the stock solutions was subsequently diluted in a variety of solvents and HFE7100 to form the working solutions. Amino acid strips and fingermarks were dipped in each of the working solution and air dried prior to development with the techniques outlined in Section 4.3.4. The amino acid strips and fingermarks were viewed in luminescence mode using the VSC6000 and recorded using a Rofin Poliview IV fitted with a Polilight PL-500 system. Depending on the luminescence emissions measured for the amino acid strips and fingermarks, a lower concentration may be used as long as these readings did not produce adverse results. The concentration of the reagent was optimised concurrently for each solvent formulation.

4.3.6 Effects of pH

A pH range from 4 to 7, was analysed for each optimised fingermark reagent. The pH was adjusted as required by the addition of acetic acid or a phosphate buffer until the desired pH was achieved. The effects of pH were assessed for each fingermark reagent by measuring the fluorescence emission of the amino acid test strips and by qualitative analysis of the fingermarks developed by each formulation.

4.3.7 Effects of metal salt

Two zinc salts, zinc chloride and zinc nitrate, were chosen for the purposes of the study. Three different molar ratios (1:2, 1:10, and 1:25) of quinone to zinc metal were evaluated. The effects of metal salts were investigated by:

- pre-staining where the amino acid test strips and fingermarks are dipped in an ethanolic metal salt solution and air dried prior to treatment with the working solution,
- the incorporation of a small amount of ethanolic zinc salt solution into the working solution,
- post-treatment where the developed amino acid test strips and fingermarks are subjected to secondary treatment using an ethanolic zinc salt solution.

When the metal salt is added to the working solution the test substrate is heat treated in its optimised manner. Post-treatment with a metal salt solution involved treating the substrate with the working solution followed by the application of heat in its optimised manner. The substrate is then dipped in a metal salt solution and air-dried. Secondary heat treatment was subsequently applied using the laundry press under the following conditions:

1. Heat press for 10 s and 20 s at 165 °C and 180 °C.
2. Heat press with the application of steam for 10 s and 20 s at 165°C and 180 °C.

3. Development at room temperature in a low light environment.

4.3.8 Surfactant preparation

Three different surfactants were assessed on only NQS. The surfactants trialled were cetrimonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Triton X 100. For each surfactant, 0.1 M stock solutions were prepared; these were then diluted to working solutions (0.01 M and 0.005 M), then mixed with the optimised NQS solution and applied to the amino acid test strips and fingermarks by dipping and the appropriate development technique was applied. The amino acid test strips and fingermarks were viewed and evaluated in luminescence mode using the VSC6000 and recorded using a Rofin Poliview IV fitted with a Polilight PL-500 system.

4.4 Results and Discussion

4.4.1 Benzoquinones

4.4.1.1 2-Hydroxy-1,4-benzoquinone, 1

Attempts were made to use the synthesised product, **1,** to develop a fluorescent compound when reacted with amino acids. It was found that when placed in polar systems, oxidation of the quinone occurred rapidly where the colour changed rapidly from light yellow to light brown. The resulting product was a light brown precipitate. This colour change occurred slower in non-polar systems, within approximately 30 minutes of making up the solutions. However, no precipitate was noted to have formed prior to the addition of HFE7100. During the investigation into the photolysis of *p*-benzoquinones in aqueous solutions, Kurien and Robins (184) observed the formation of a brown precipitate in solutions of **1** that are above pH 2. It was determined that **1** polymerises into humic acid. Kurien and Robins also noted that it was insoluble in a wide range of organic solvents such as chloroform, petroleum ether, diethyl ether and benzene. The polymerised product was soluble in alcohol and acetone. Other research into the kinetics of hydroxyquinones also found that at high

pH, diquinones, cyclopentanones and other dimers were also formed (189). Due to difficulties relating to solubility and the instability of the reagent in various organic solvents, further experimental work with **1** was abandoned.

4.4.2 Naphthoquinones

Another goal of this study was to compare the effects of increasing conjugation from one quinone to another. As mentioned in Section 1.1.6.4, Jelly *et al*. have discussed the use of various naphthoquinones as potential fingermark detection reagents (97; 127). The results of 1,2-naphthoquinone-4-sulfonic acid, sodium salt (NQS) and 2-hydroxy-1,4-naphthoquinone (lawsone) were of particular interest to the work conducted in this project. Initial attempts at reproducing their results, specifically the development of fingermarks on porous surfaces, were unsuccessful. At the reported concentration (1mg/mL) NQS was insoluble in ethyl acetate and lawsone caused the oversaturation of the amino acid test strips and the substrate with the fingermark deposit. Furthermore, the results by Jelly *et al*. used only four formulations containing lawsone. Other formulations were not based upon the working solutions of 1,2-indanedione, DFO or ninhydrin (133).

A number of tests were conducted in order to find the optimum concentrations and solvent systems for the aforementioned compounds. The results are discussed in the subsections that follow.

4.4.2.1 1,2-Naphthoquinone-4-sulfonic acid, sodium salt (NQS)

As mentioned in Section 4.4.2, attempts were made to dissolve NQS in ethyl acetate following the method by Jelly *et al.*, however, this was unsuccessful and NQS was insoluble as it is a salt. In this current study, a number of solvents were used to solubilise NQS with the prepared concentration of 1 mg/mL (3.84×10^{-3} M).

It was determined from this study that the solubility of NQS could be greatly improved in polar solvents such as alcohols or water. The results are documented in Table 4-1. However, the use of polar solvents is not ideal for the formulations of fingermark reagents, as they tend to cause inks to diffuse through the paper and also the migration of eccrine secretions from within the paper (6). Generally fingermark reagents are dissolved in the minimum volume of a polar solvent for the stock solution and subsequently further diluted with a relatively non-polar solvent to make up the working solution (98).

Table 4-1 Solubility of NQS in a variety of solvents.

Solvents	Concentration (mg/mL)	Solubility
Acetone	1	Insoluble
95% Ethanol	1	Slightly
Absolute ethanol	1	Insoluble
Ethyl acetate	1	Insoluble
	0.5	Insoluble
1,4-Dioxane	1	Slightly
Methanol	1	Moderately
	0.5	Soluble
Dimethyl sulfoxide	1	Insoluble
Ethylene glycol	0.5	Insoluble
Ethylene glycol + ethyl acetate	0.5	Insoluble
Dichloromethane	1	Insoluble
Water	1	Soluble

At 1 mg/mL, NQS was moderately soluble in methanol, and thus it was thought that a reduced concentration could increase its solubility. At 0.5 mg/mL, NQS was completely soluble. Concentrations ranging from 0.5 to 0.05 mg/mL in mixed solvent systems were

tested using the amino acid test strips in order to determine the limit of detection. The results indicated that there was little to no gain in recorded fluorescence between concentrations of 0.5 mg/mL and 0.1 mg/mL. Therefore, work was concentrated on optimising solvent conditions using NQS working solution at 0.1 mg/mL. Table 4-2 shows the composition of the mixed solvent systems.

Table 4-2 Solvents evaluated in optimisation.

Stock solution (%)	HFE7100 (%)	Methanol (%)	Ethyl acetate (%)	Acetonitrile (%)	Propanol (%)	Butanol (%)
20	20	60	-	-	-	-
20	20	-	60	-	-	-
20	20	-	-	60	-	-
20	20	-	-	-	60	-
20	20	-	-	-	-	60

Viewing and recording of the fluorescence spectra of the NQS developed fingermarks and amino acids was conducted on the VSC6000 with an excitation range of 485 – 535 nm and a 590 nm filter. These conditions corresponded well with those found by Jelly *et al.* (135). The spectra in Figure 4-4– Figure 4-6 show the fluorescence emission detected when NQS was reacted with alanine, lysine and serine, respectively. The figures show that there are significant differences in fluorescence emission intensity depending on the solvents that were used. Alcohols may have an effect on the embedding of the final product into the substrate which can affect the fluorescence of the product. The polarity and volatility of the solvents can also dictate the amount of the time that the reagent has to react with the amino acid.

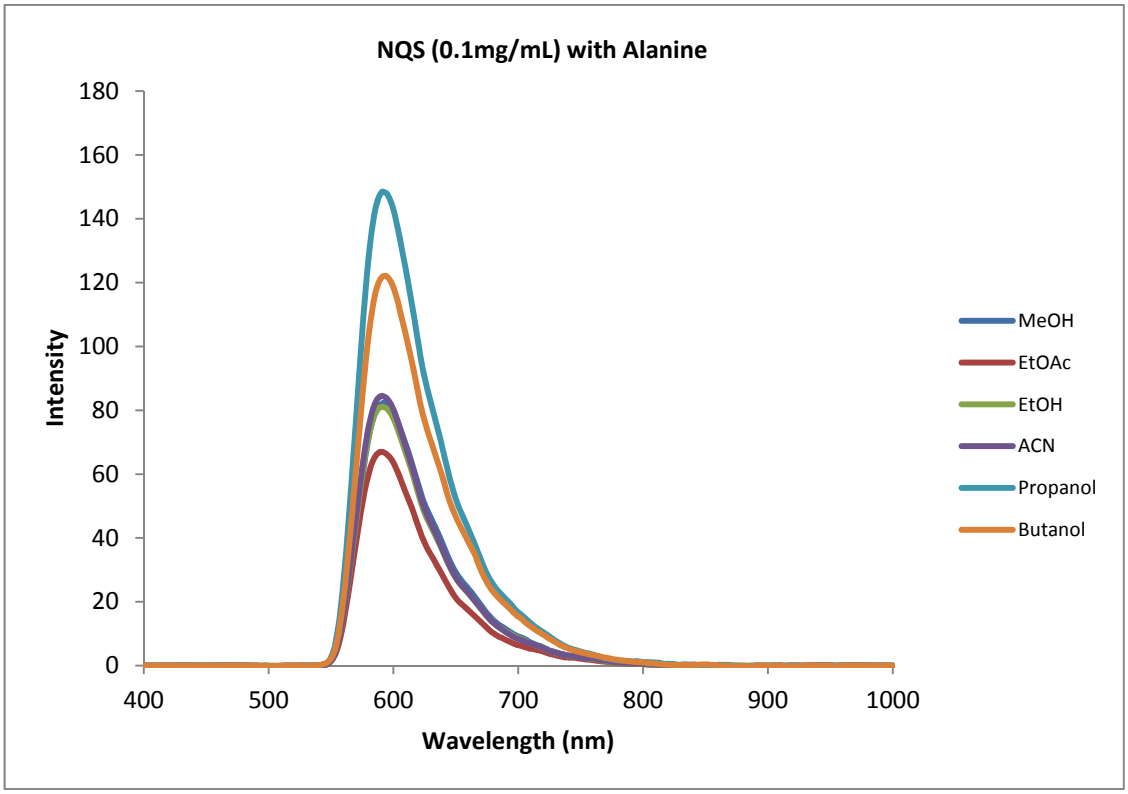


Figure 4-4 Fluorescence emission spectra for NQS reacted with alanine (excitation 485 - 535 nm, 590 nm filter).

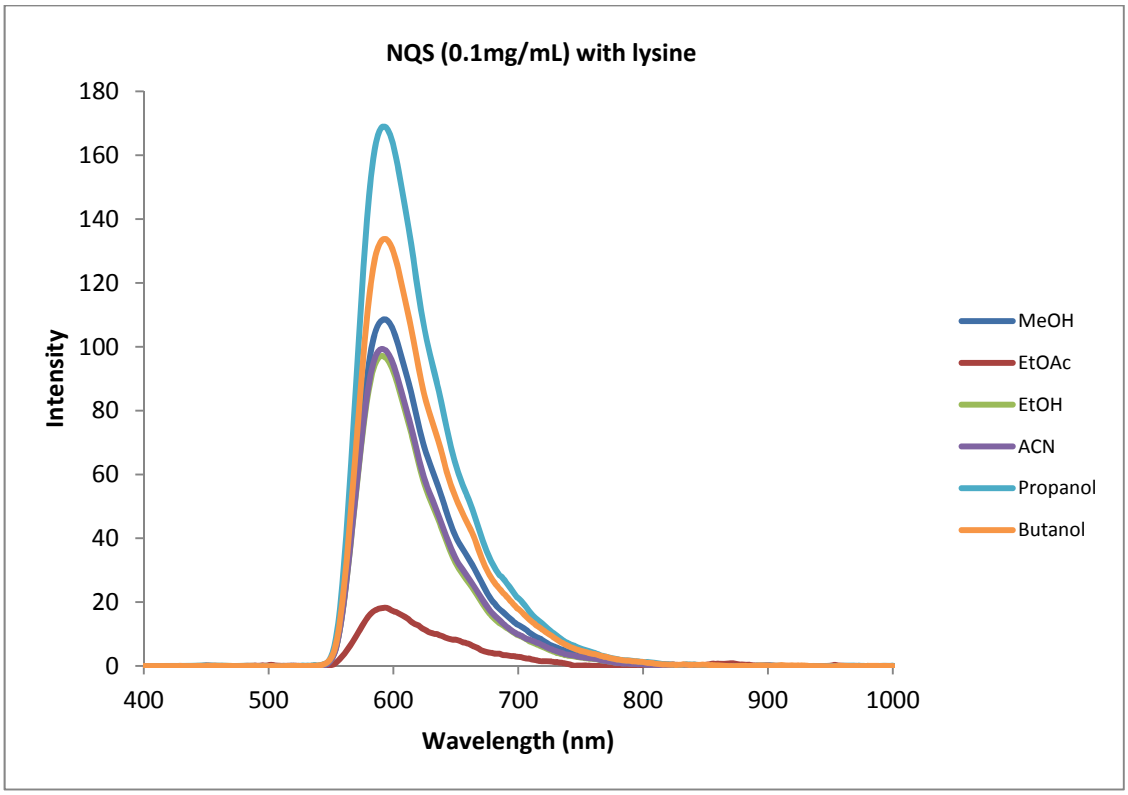


Figure 4-5 Fluorescence emission spectra for NQS reaction with lysine (excitation 485 - 535nm, 590 nm filter).

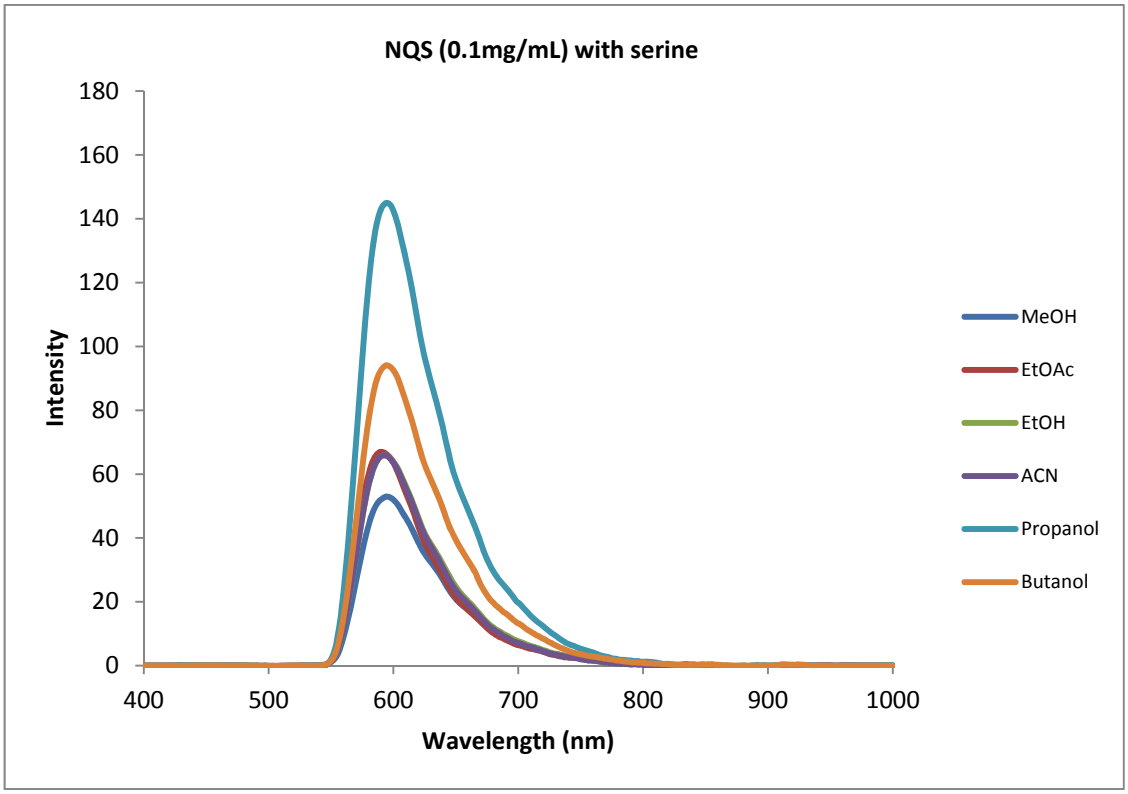


Figure 4-6 Fluorescence emission spectra of NQS reaction with serine (excitation 485 - 535 nm, 590 nm filter).

The above figures show that the use of propanol produced the most fluorescent amino acid spots. Attempts were made to reduce the volume of propanol in the working solution. This is because a non-polar solvent (such as HFE7100) should make up the bulk of the volume in a fingermark working solution due to fast evaporation and diffusion of inks and fingermarks can be eliminated.

The spectra (Figure 4-7 to Figure 4-9) show the fluorescence effects of altering the concentration of the working solution and volume of HFE7100. It can be seen that there is little to no difference in the fluorescence produced by the three amino acids when the volume of HFE7100 was decreased from 20 % and 10 % while keeping the concentration of the working solution at 0.1 mg/mL. However, the fluorescence intensity differed dramatically between 0.2, 0.1 and 0.05 mg/mL of NQS. The results indicate that higher concentrations of HFE7100 have negative effects on the fluorescence of the amino acids as solubility of NQS was an issue and the solution was unstable (precipitation of the reagent occurs after 15 to 30 minutes of being prepared). Therefore, to account for solubility and concentration effects, it was

determined that 0.1 mg/mL of NQS in a mixture of propanol (60 %) and HFE7100 (20 %) provided maximum fluorescence intensity. Table 4-3 show the concentration of NQS assessed during the study.

Table 4-3 Concentration of NQS assessed.

Concentration (mg/mL)	Working solution (% v/v)	HFE7100 (% v/v)	Propanol (% v/v)
0.2	40	20	40
0.05	10	20	70
0.1	20	30	50
0.1	20	40	40
0.1	20	20	60
0.1	20	10	70

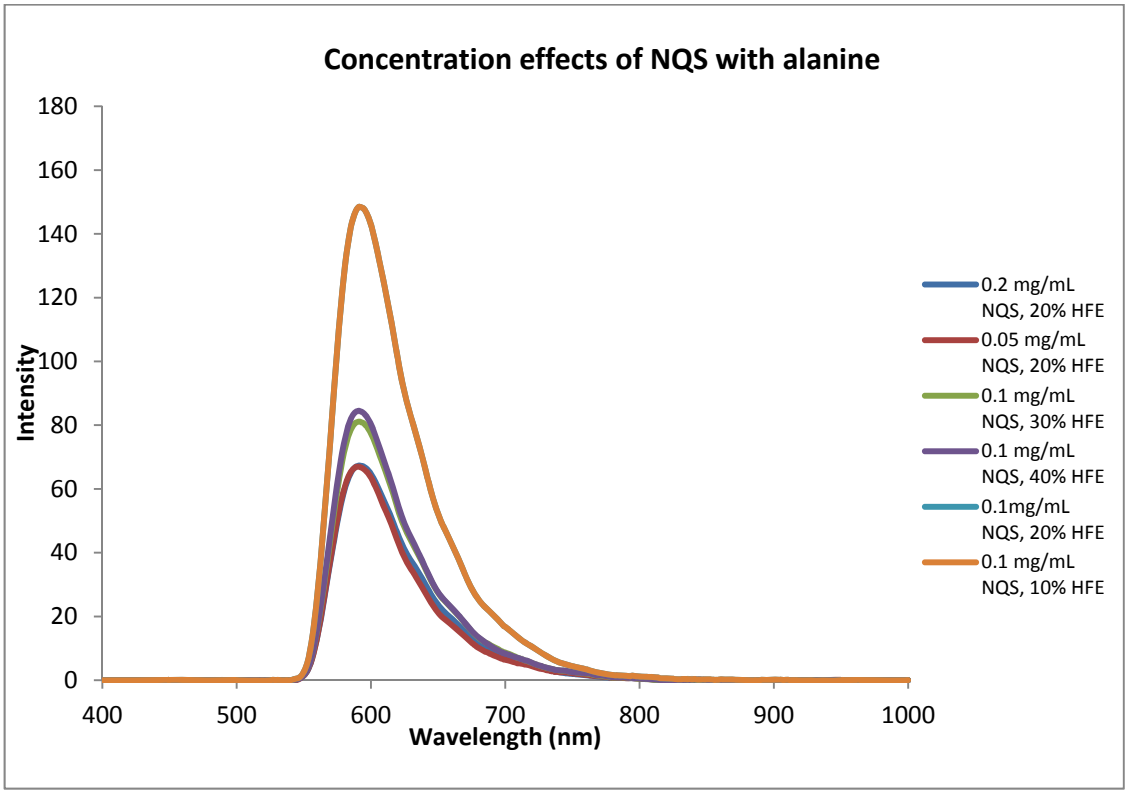


Figure 4-7 Effects of NQS concentration on its reaction with alanine (excitation 485 - 535 nm, 590 nm filter).

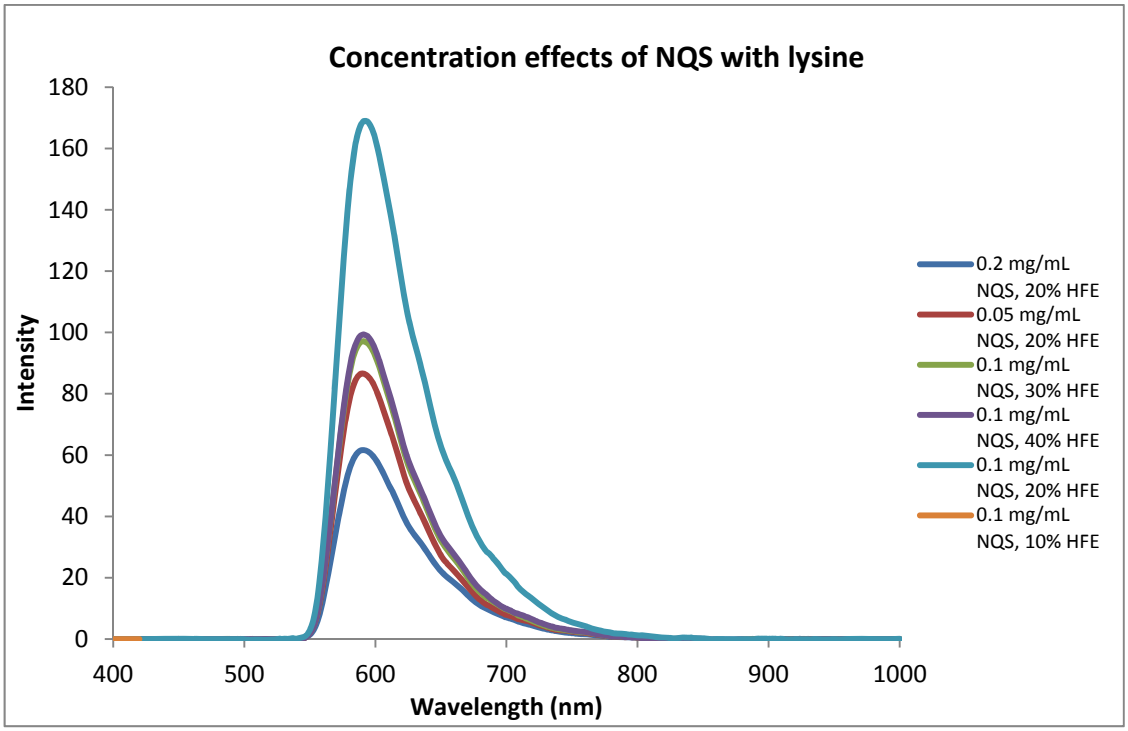


Figure 4-8 Effects of NQS concentration on its reaction with lysine (excitation 485 - 535 nm, 590 nm filter).

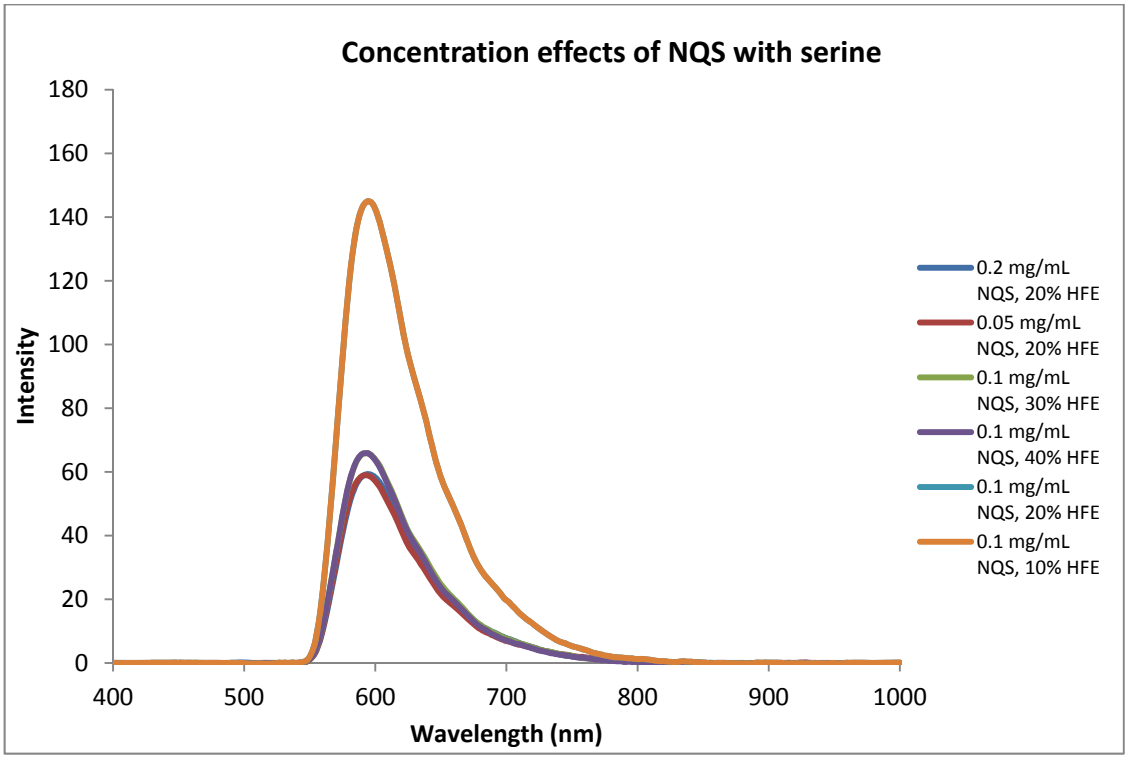


Figure 4-9 Effects of NQS concentration on its reaction with serine (excitation 485 - 535 nm, 590 nm filter).

Li and Zhang (228) used NQS in an aqueous solution for the determination of lysine in pharmaceutical and biological samples. It was found that solvents such as dioxane and ethanol had minimal effect on the determination of lysine. However, by increasing the volume of methanol, the UV-Vis absorbance of the product decreases dramatically until it approaches zero. The authors suggested the cause of this effect is likely due to be pH effects where at higher pH (pH 9.6), methanol forms methoxide which competes with lysine for NQS. A product (Figure 4-10) was proposed in which two moles of NQS reacted with one mole of lysine. The nucleophilic amino groups can attack C-4 due to the delocalisation of the electrons in of the ketone system.

The research by Li and Zhang indicated that the quantum yield of the product also increased with the use of a surfactant, especially with the use of an anionic surfactant. Li and Zhang noted that the positively charged amine group in benzyldimethyltetradecylammonium chloride reacts with the negatively charged oxygen at C-2 from the NQS moiety leading to a larger conjugated system.

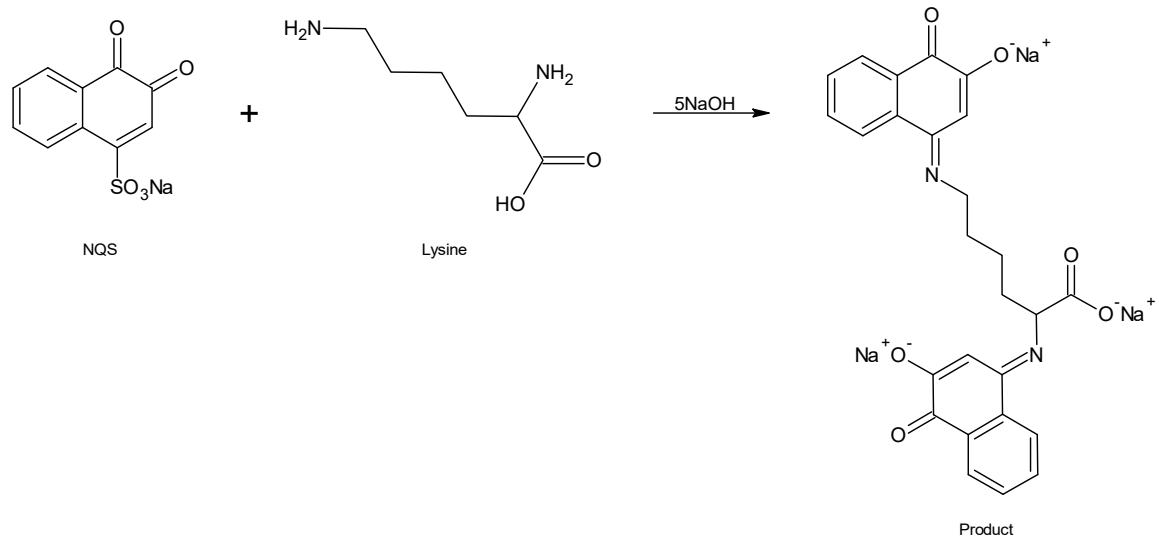


Figure 4-10 Schematic diagram depicting the formation of a product arising from the reaction between NQS and lysine, adapted from Li and Zhang (228)

The effects of different surfactants were assessed, cetrimonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Triton X 100. These were chosen due to their ionic nature (Table 4-4).

Table 4-4 Surfactants used in this study.

Surfactant	Ionic Nature
CTAB	Cationic
SDS	Anionic
Triton x 100	Non-ionic

These experiments using surfactants did not produce fruitful results and fluorescence across the three amino acids decreased significantly. Furthermore, the results showed poor development of latent fingermarks. A potential reason for this observation is that the time required for the interactions between NQS and the amino acids may be insufficient. The reaction described by Li and Zhang (228) was conducted in solution, the results of which cannot accurately predict the reaction on a porous surface. The reaction relies on the product (azomethine ylide) to have formed prior to its reaction with the surfactant to form a secondary product in solution. In addition, the azomethine ylide may be bound to the substrate, which inhibits further reactions due to steric hindrance. Although the three surfactants had a detrimental effect on the fluorescence, CTAB had a relatively higher intensity than SDS and Triton X 100. This indicated that CTAB has an effect on the NQS-amino acid product under the development conditions (heating in the oven for 1 hour). Due to the lack of improvements in fluorescence, further experimental work with surfactants was abandoned.

Previous research on the optimisation of fingermark reagents showed that pH levels of the working solutions can have an effect on the quality of fingermarks developed on porous surfaces. The pH effect on fingermarks was evaluated by the addition of acetic acid or a phosphate buffer. The results indicate that there is little to no difference in the fluorescence or colour produced at pH 4 – 7. At neutral pH, reduced contrast due to background staining was observed. For ease of use, the working solution was maintained at pH 5, which is the pH of NQS in solution.

The effects of metal salts in pre-staining, addition to the NQS solution and as a secondary post treatment were evaluated. When a metal salt solution was used on the substrate prior to and after development with the NQS solution, the developed fingermark ridges and amino acid spots appeared to be diffused. This is likely due to the ethanolic solution having a 'washing' effect on the amino acids (Figure 4-11), therefore pre-staining the substrate with a metal salt solution was abandoned. In an attempt to avoid this 'washing' effect, zinc chloride was added into the solution of NQS. However, the developed amino acids did not show great improvements in terms of colour or fluorescence. The fingermarks that were developed also showed no improvements, and in most cases did not yield any visible fingermarks under white light or in luminescence mode.

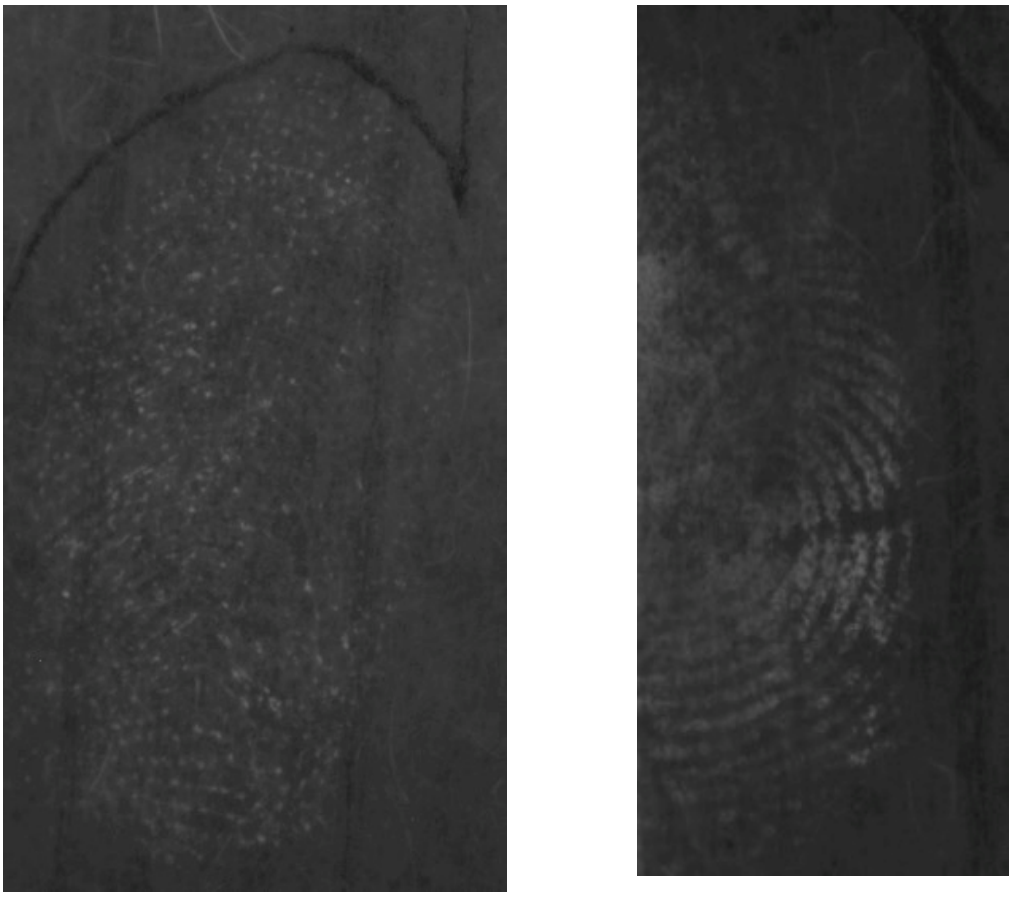


Figure 4-11 Two different fingermarks deposited on lined paper demonstrating the 'washing' effect of the ethanolic zinc chloride solution (photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s).

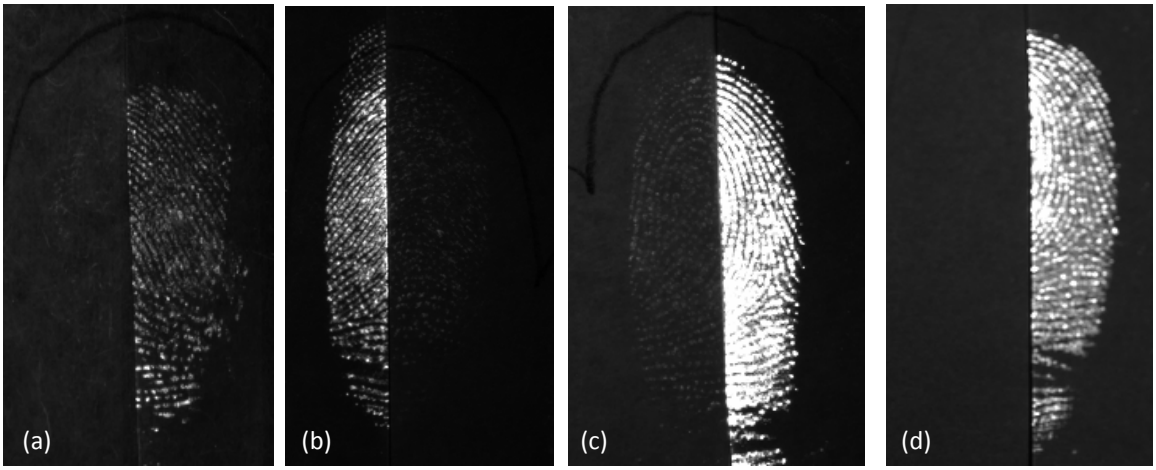


Figure 4-12 (a) Fingermark on lined paper; left: NQS, right: IND-Zn, (b) fingermark developed on lined paper, left: IND-Zn, right: NQS, (c) fingermark developed on white Reflex® paper, left: NQS, right: IND-Zn, (d) fingermark developed on pink Reflex® paper, left: NQS, right: IND-Zn (NQS: Photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s. IND-Zn: Photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

Generally, the NQS developed fingermarks were not very fluorescent when compared to the IND-Zn developed fingermark across all the surfaces that were tested in this the current study. Figure 4-12 shows some fingermarks that were developed with NQS. The fingermarks that could be developed with the NQS working solution were all freshly deposited eccrine charged fingerprints. The NQS working solution developed showed evidence of contact but no ridge details on lined paper. Development of fingermarks on white paper fared slightly better, Figure 4-12 (c) shows at least six minutiae that can lead to identification by fingerprint experts. On coloured paper (pink), no fingermark ridges/details could be observed. An example is shown in Figure 4-12 (d). Attempts were made to develop fingermarks that were aged (more than 1 week old), but all attempts were unsuccessful with no observable fingermark ridges.

4.4.2.2 2-Hydroxy-1,4-naphthoquinone (lawsone)

Jelly *et al*. (127) indicated lawsone was used at 1 mg/mL in a mixed solvent system that consisted of ethyl acetate and HFE7100 in a ratio of 1: 4 for the development of latent fingermarks on porous surfaces. The substrate was then heated in the oven for 1 hour at 140 – 170 °C to accelerate the reaction between lawsone and the fingermark. Jelly *et al.* found that the optimum fluorescence was achieved using an excitation

wavelength at 555 nm and a cut-off filter ranging from 550 – 610 nm. Attempts were made to reproduce these results without success. When the lawsone working solution was made in the manner as described, it was found that the solution was unstable at 1 mg/mL and lawsone precipitated after approximately 1 hour. The rapid deterioration of the solution would mean that the reagent would not be a viable option in forensic casework. Furthermore, when the working solution was applied to the substrate a large amount of the reagent precipitated on its surface indicating that the solution is over-saturated. When using the VSC6000, optimum fluorescence was achieved using the same conditions as NQS. While using the Polilight, illumination can be achieved using an excitation wavelength at 555 nm and a 610 nm filter. Due to the issues that were encountered in this initial experimental work, it was determined that a re-evaluation of the solvent systems and reagent concentration was required in order to compare the reactivity with the reagents that were synthesised for this thesis.

Concentration effects were examined by varying the concentration of lawsone in the working solutions from 0.75 mg/mL, 0.50 mg/mL, 0.25 mg/mL and 0.1 mg/mL. Development methods were also optimised concurrently. The effects of solvent systems on the fluorescence emissions obtained were also examined. It was necessary to dissolve lawsone in a small volume of polar solvent followed by dilution with HFE7100. The solvents that were examined were methanol, ethanol, propanol, butanol, ethyl acetate, petroleum spirits, dichloromethane and acetonitrile. It was found that at higher concentrations, from 0.75 mg/mL to 0.50 mg/mL, the lawsone-lysine product shows an intense fluorescence emission maximum at 640 nm and a less intense peak 590 nm. At lower concentrations, the lawsone-lysine product displays a less intense second peak occurring at 640 nm. Figure 4-13 shows the fluorescence emission spectra of the lawsone-lysine product at four different concentrations of lawsone that was examined. Figure 4-14 shows the reaction product between lawsone and serine or alanine exhibit one fluorescence emission maximum at 590 nm at all concentrations tested. These differences suggest that the lawsone-lysine reaction product is different to that of alanine and serine.

Based on these results, it was determined that the maximum fluorescence emission was achieved at 0.25 mg/mL lawsone in 25 % ethyl acetate, 25 % butanol and 50 % HFE7100. Similar to the results found by Jelly *et al.*, oven development of the substrates at 160 °C for one hour provided maximum fluorescence of the amino acid test strips and fingermarks.

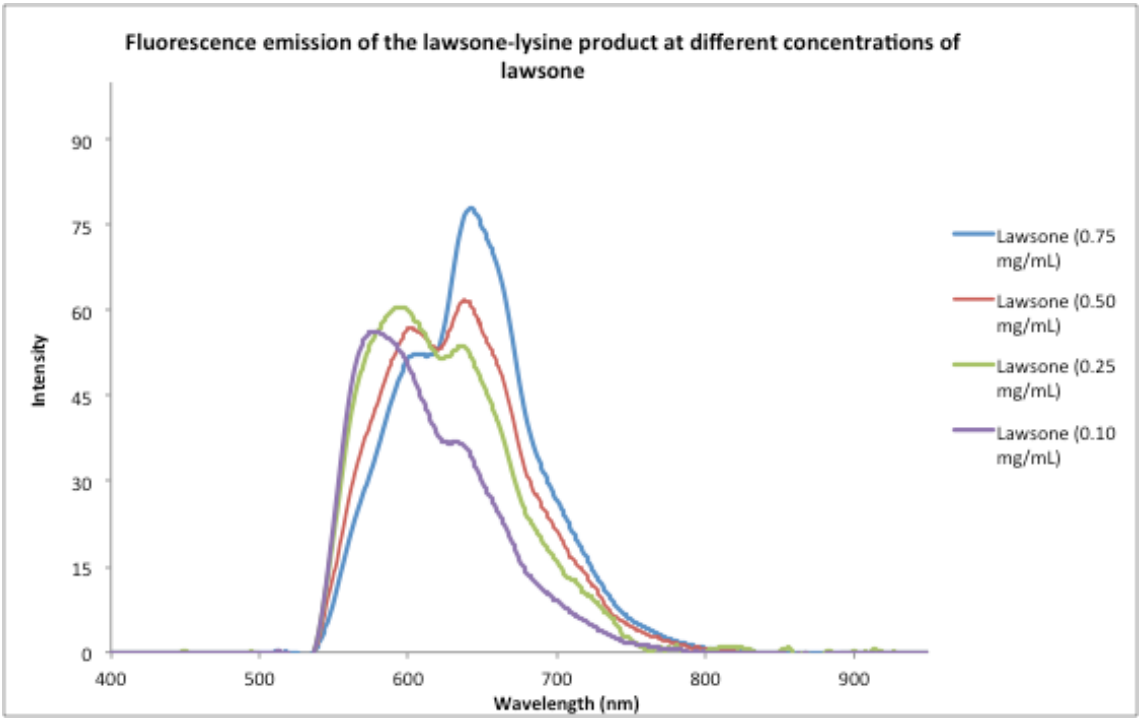


Figure 4-13 Emission spectra of the lawsone-lysine product at four different concentrations of lawsone.

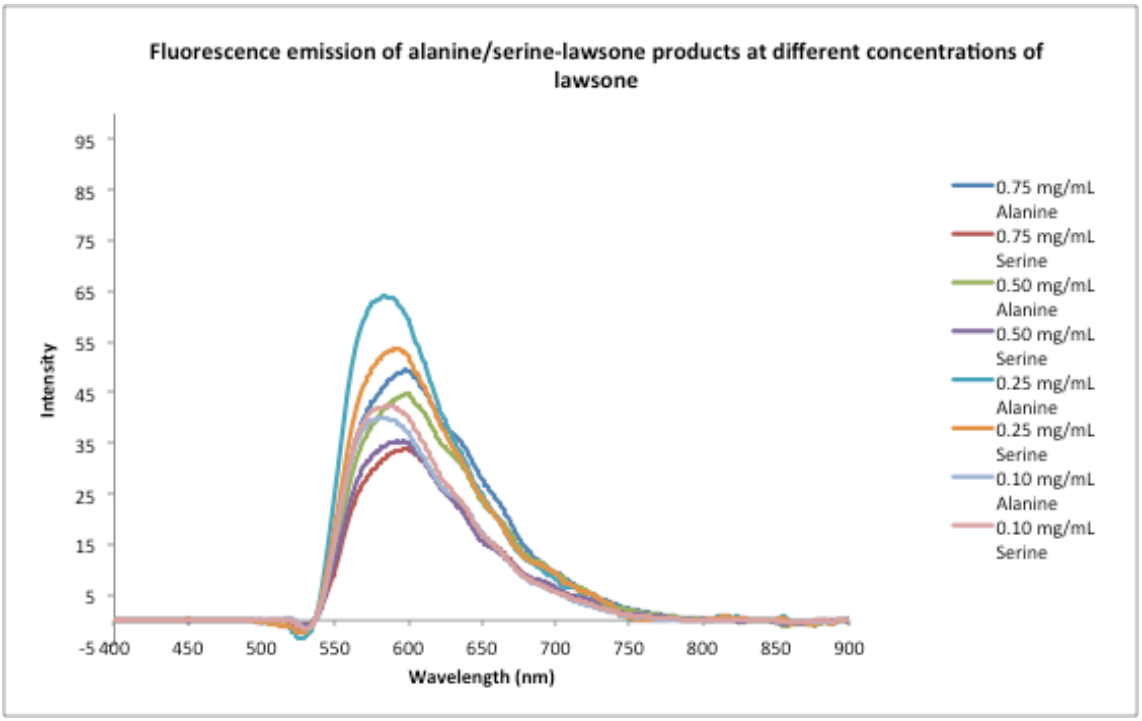


Figure 4-14 Fluorescence emission spectra of the products from the reactions resulting from alanine and serine with at different concentrations of lawsone.

Altering the pH of the working solution from pH 4 – 7 did not improve the fluorescence of the amino acid test strips or fingermark ridge details. As such, experimental studies on the optimisation of metal salts were conducted where the working solution was maintained at pH 5, which is the pH of lawsone in solution. Unlike the results obtained for NQS and those found by Jelly *et al.*, it was determined that there were slight improvements in the fluorescence that is obtained when small amounts of zinc chloride were added to the working solution. However, there was little difference between the three ratios of zinc chloride to lawsone that were examined.

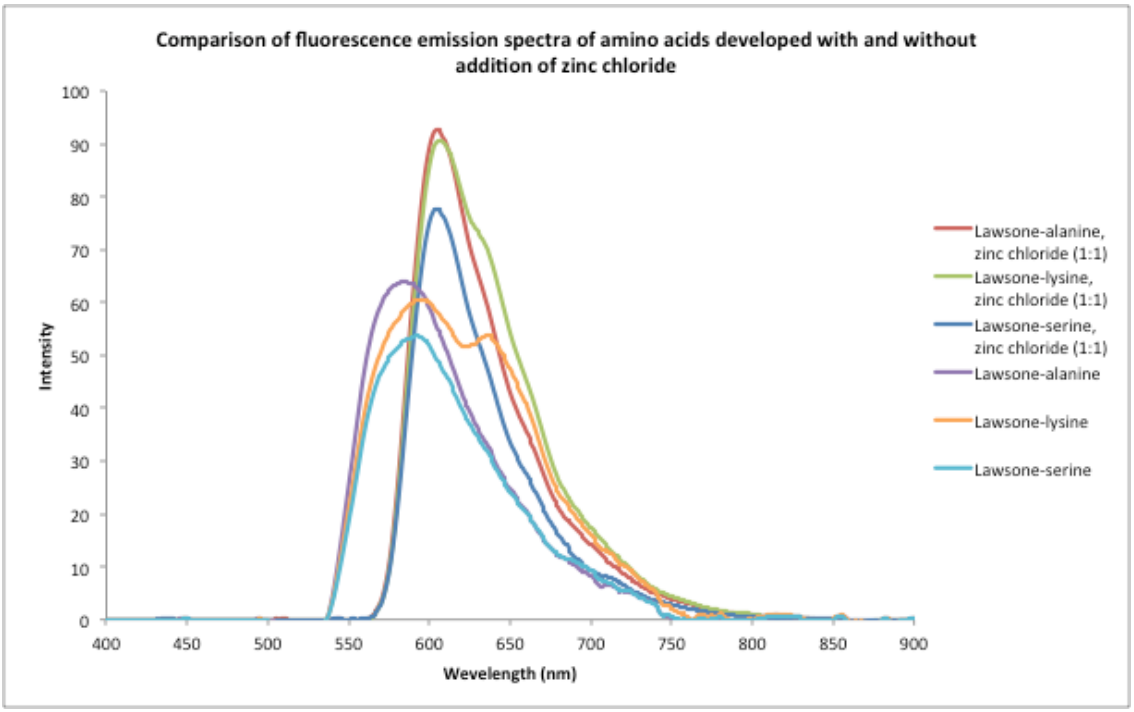


Figure 4-15 Comparison of the emission spectra obtained from the reaction of amino acids developed with and without the addition of zinc chloride.

Figure 4-15 shows a slight blue shift (approximately 10 nm) and an improvement in fluorescence emission intensity when zinc chloride was added into the lawsone working solution. The change in emission spectrum could indicate the formation of a complex, and/or a catalytic reaction. Any improvements that were observed with amino acids did not translate well to fingermarks on paper, where no real difference in fluorescence was observed. When the test substrates were treated with the zinc chloride solution, either as a pre-stain treatment or as a post treatment, the emission intensity was observed to have decreased significantly. This is likely to be caused by the ethanolic solution of zinc chloride 'washing' away the amino acids and fingermark ridges. Figure 4-16 shows that at the bottom of the fingermark, the ridges appear more diffuse and sections were washed away.

Figure 4-16 Ethanolic solution of zinc chloride 'washing' away some fingermark ridges (bottom of the fingermark).

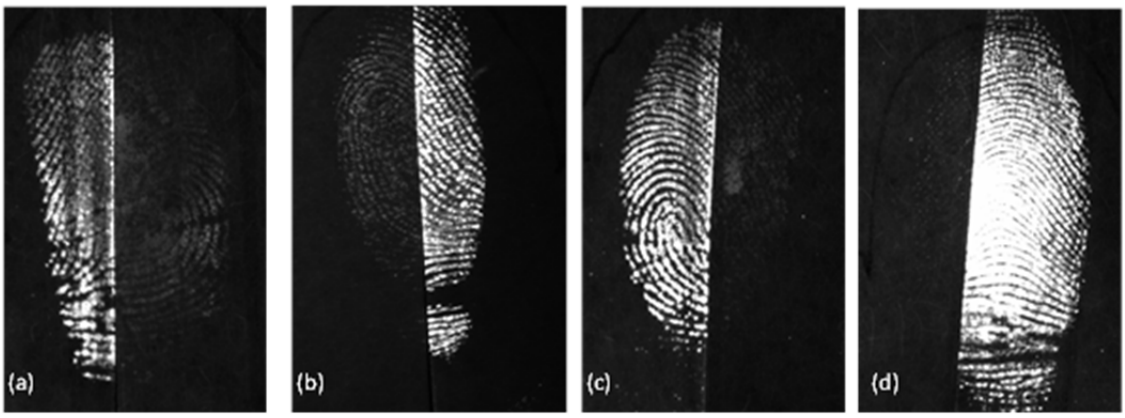


Figure 4-17 (a) Fingermark on lined paper, left: optimised lawsone, right: Jelly *et al.*'s lawsone (exposure 8s), (b) fingermark developed on white Reflex® paper, left: Jelly *et al.*'s lawsone, right: optimised lawsone (exposure 8s), (c) fingermark on lined paper, left: IND-Zn, right: optimised lawsone, (d) fingermark developed on white Reflex® paper, left: optimised lawsone, right: IND-Zn (Lawsone: photographed under excitation 555 nm, 610 nm filter, F5.6, exposure 2s. IND-Zn: Photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

The optimised formulation found during this study provided better results than those found by Jelly *et al*. (133). Unfortunately, the lawsone treated fingermarks were mostly not visible under white light and only resulted in faintly fluorescent fingermarks for

freshly deposited charged fingermarks. As was expected, when compared with IND-Zn treated fingermarks, any improvements were insignificant. This is demonstrated Figure 4-17 where representative photos of the most fluorescent fingermarks are shown. Figure 4-17 (a) and (b) are fingermarks developed on lined paper and white copy paper, respectively. The figures compare fingermarks developed by the original lawsone formula and the optimised formulation from this study, the more fluorescent half of the fingermark is developed by the lawsone formulation from this study. The decrease in fluorescence of the fingermarks in the original lawsone formulation is likely to be caused by over-saturation of lawsone in the formulation. The more fluorescent halves shown in Figure 4-17 (a) and (b) showed strong development. Note that long exposure times required for photography is not ideal in a policing environment due to case work and the timely provision of results. These development results are promising, however, Figure 4-17 (c) and (d) show split prints comparing lawsone and IND-Zn developed fingermarks. This illustrates that at 2 second exposure, the lawsone treated fingermarks are only faintly fluorescent, while the IND-Zn treated fingermark is almost over exposed (Figure 4-17 (d)).

Figure 4-17 (a) and (b) show that the optimised lawsone solution performs better overall across different porous surfaces than the working solution proposed by Jelly *et al*. When compared with IND-Zn, the fluorescence and details that could be captured are inferior. The split prints that were developed by IND-Zn, have high levels of clarity at short exposure times while the same level of detail could not be captured for the lawsone developed prints even with increased length of exposure. Attempts were also made to developed 'aged' fingermarks using the lawsone working solution, but only fresh fingermarks were developed with faint ridge continuity and clarity. Fingermarks older than 7 days generally developed with either very faint fluorescent or no viable ridges were observed.

4.4.3 Anthraquinones

Optimisation of the reagent concentration was tested concurrently with solvent systems and developmental conditions. The solutions were further optimised by altering the pH followed by the addition of metal salts. During the course of this study, observation of the developed amino acid strips and fingermarks by various anthraquinones was performed under the same conditions as the naphthoquinones. The optimised working solutions were compared with the current AFP IND-Zn formulation. Due to the structural similarity of the anthraquinones, the absorption maxima of all compounds tested occurred in a range between 515 – 540 nm. The increase in conjugation and substitution led to absorption at slightly longer wavelengths.

4.4.3.1 1,4-Dihydroxy-9,10-anthraquinone (quinizarin)

Initial tests conducted on amino acid test strips developed by quinizarin (1 mg/mL to 0.1 mg/mL) indicated that it is highly fluorescent. However, the reaction is not specific to amino acids, staining the controlled blank on the test strips. Therefore, all further research on quinizarin was abandoned.

4.4.3.2 9-Chloro-10-hydroxy-1,4-anthraquinone, 2

The results from **2** were shown to be highly fluorescent. Initial testing with amino acid strips showed the reagent reacted most strongly with the 0.1 M amino acid spot and less observed fluorescence at lower concentrations. However, the solution stained the controlled blank (filter paper spotted with deionised water) and the background. This shows that **2** was not selective towards amino acids. As a result, further research on using **2** was abandoned.

4.4.3.3 2,9-Dichloro-10-hydroxy-1,4-anthraquinone, 3

A working solution of **3** (1 mg/mL) resulted in the precipitation of the reagent onto the surface of the substrates indicating that the solution was oversaturated. Attempts were made to reduce the concentration of the reagent. The concentrations examined included 0.5, 0.25, 0.1, 0.05 and 0.025 mg/mL. At higher concentrations, 0.5, 0.25 and 0.1 mg/mL, precipitation of the reagent also occurred on the substrates. A comparison of the fluorescence emission spectra produced by the three amino acids at 1.0, 0.5 and 0.1 mg/mL is shown in Figure 4-18. The spectra show that at higher concentrations of **3** in the working solution there is an inverse relationship with the fluorescence intensity that can be attributed to over-saturation.

At lower concentrations 0.05 and 0.025 mg/mL, over-saturation of the reagent was no longer an issue. Figure 4-19 shows that the fluorescence emissions of the amino acids improved at lower concentrations and that 0.025 mg/mL was in fact the optimum concentration. Lower concentrations of the reagent, 0.02 and 0.01 mg/mL, were also evaluated. The fluorescence produced by the amino acid spots treated with these solutions was significantly diminished. It was found that out of all of the combination of solvents tested, the use of a small amount of methanol (10 % v/v) in conjunction with HFE7100 (90 % v/v) and heat treatment using oven at 150 °C for 1 hour provided the optimum fluorescence.

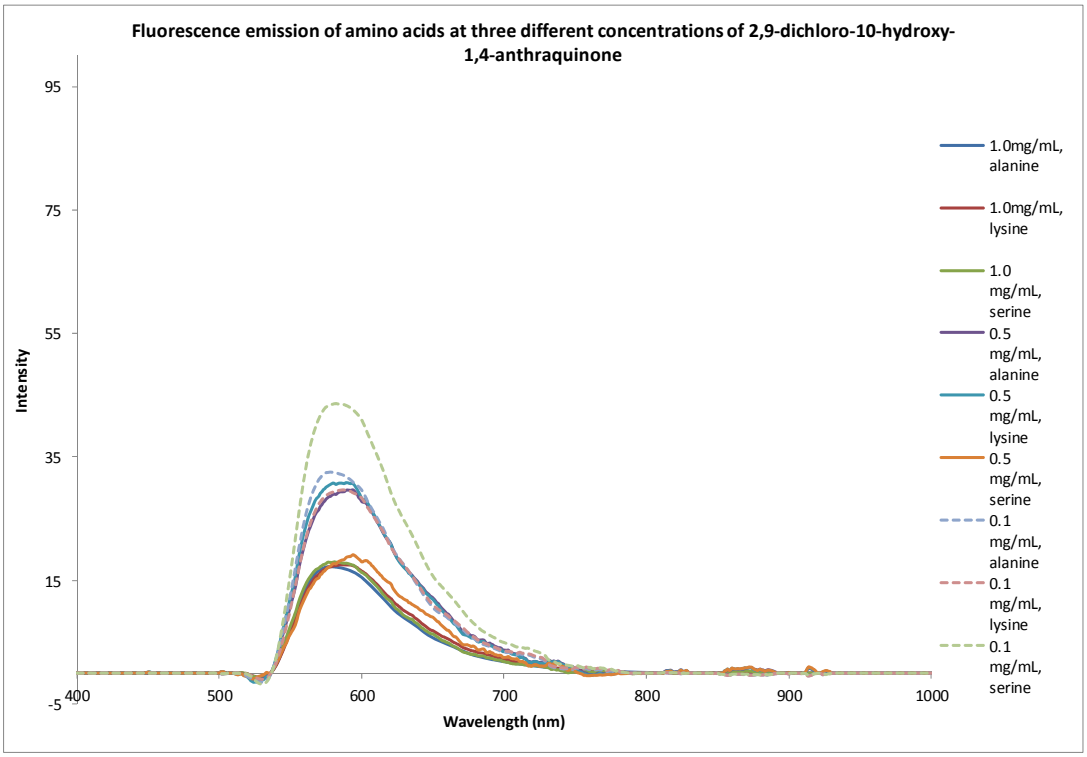


Figure 4-18 Fluorescence emission spectra of three amino acids treated with 1.0, 0.5 and 0.1 mg/mL 2,9-dichloro-10-hydroxy-1,4-anthraquinone (excitation at 485 – 535 nm).

The pH of the solutions was also altered in order to determine if any improvements could be made to the intensity of the fluorescence observed. A range of pH, from pH 4 to 7, was examined and pH ranges from 4 to 7 showed little to no difference in the fluorescence intensity. Note that at higher pH, the reagent will be ionised in the solution and insoluble in HFE7100. As the other synthesised anthraquinones also bear a hydroxy substituent, they would also become ionised in basic solutions. Therefore, these anthraquinones would not be examined under basic conditions.

Metal salts were also added into the optimised solutions of **3**. No differences in fluorescence emission in the amino acid test strips or fingermarks were observed across all concentrations and developing conditions that were analysed. The use of zinc chloride solution resulted in a darkening of the background and a decrease in fluorescence of the fingermark. Therefore, further research with metal salts was abandoned.

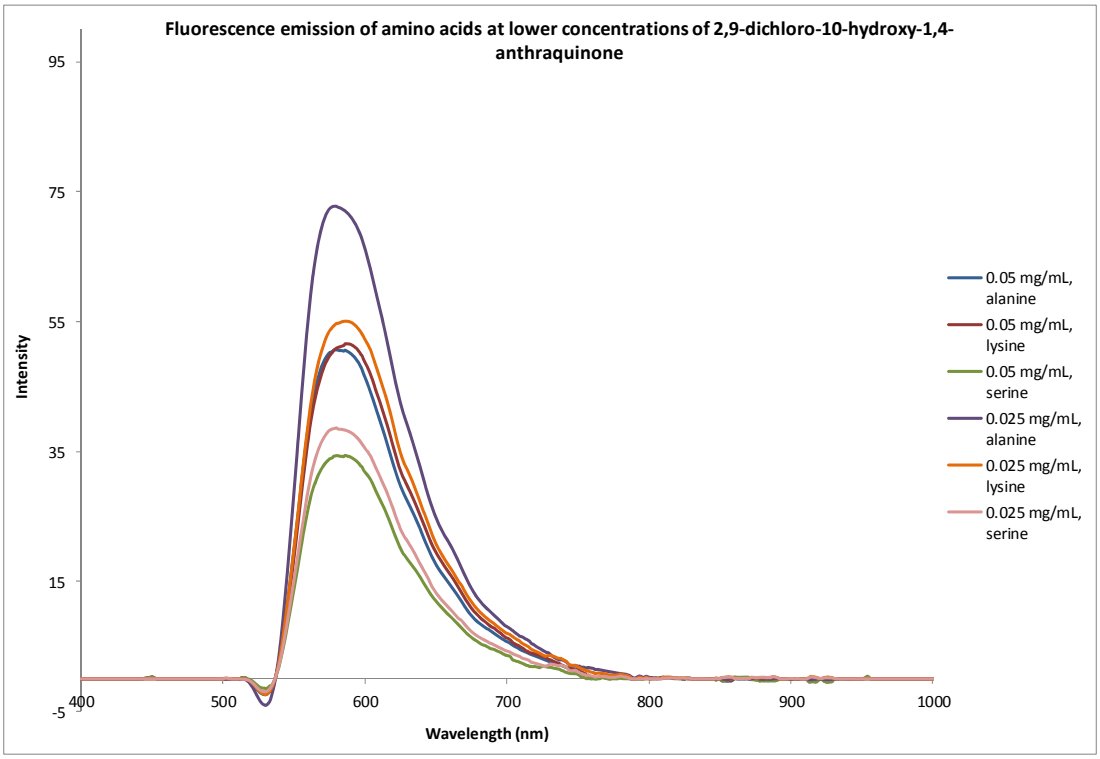


Figure 4-19 Fluorescence emission spectra of three amino acids treated with 0.05 and 0.025 mg/mL 2,9-dichloro-10-hydroxy-1,4-anthraquinone (excitation at 485 – 535 nm).

The optimised solution of **3** was used to develop fingermarks across a number of different porous surfaces. The results showed that across all the surfaces analysed, it was only able to develop fingermarks that are less than 7 days old. These results indicated that limited ridges details were developed and only faint characteristics were observed. Figure 4-20 shows the split fingermarks that have been developed with the optimised solution of **3** and IND-Zn. Fingermarks developed with IND-Zn solutions were clearer due to greater contrast between the fluorescent ridges and the background. Furthermore, defined ridge details and other characteristics were also observed at faster shutter speeds. Across all fingermarks developed **3**, only charged prints were developed with up to five to six minutiae points. Examination of the fingermarks on two different types of lined paper showed similar results (Figure 4-20 (a) and (b)). Fingermarks that were developed on white copy paper were more fluorescent and as a result have greater contrast between the background than those on coloured copy paper.

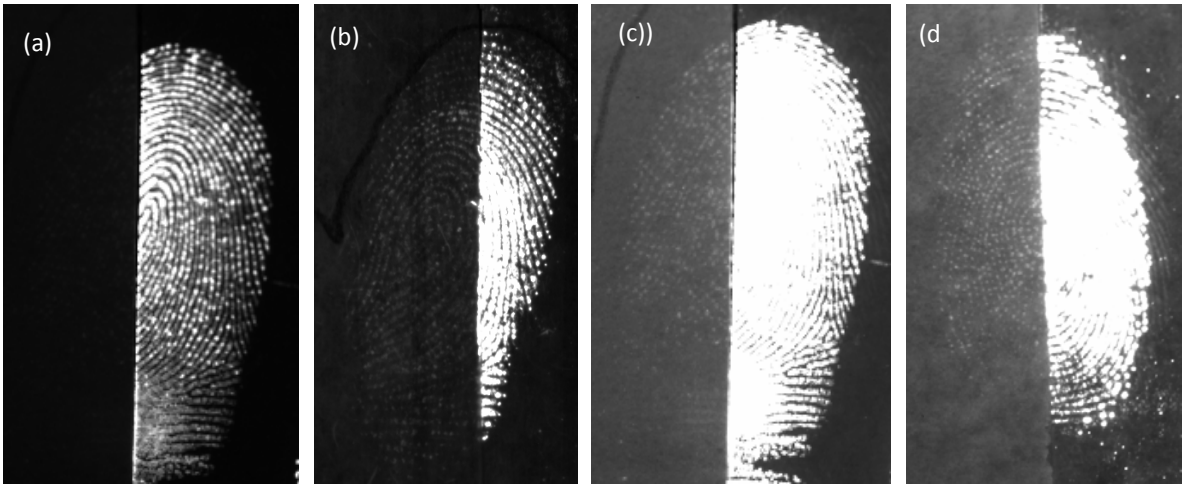


Figure 4-20 (a) Fingermark on lined paper, left: 2,9-dichloro-10-hydroxy-1,4-anthraquinone, right: IND-Zn, (b) fingermark on recycled lined paper, left: 2,9-dichloro-10-hydroxy-1,4-anthraquinone, right: IND-Zn, (c) fingermark on white Reflex® paper, left: 2,9 -dichloro-10-hydroxy-1,4-anthraquinone, right: IND-Zn, (d) fingermark developed on pink Reflex® paper, left: 2,9-dichloro-10-hydroxy-1,4-anthraquinone, right: IND-Zn (2,9-dichloro-10-methoxy-1,4-anthraquinone: photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s. IND-Zn: Photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

Although very faintly fluorescent fingermark ridges with **3** could be observed, Figure 4-20 (a) – (d) show that IND-Zn is a more fluorogenic fingermark reagent. The reaction products between amino acids and anthraquinones were expected to be more fluorescent than those formed from naphthoquinone reactions as they have greater π conjugation. Furthermore, the compounds that were synthesised have greater electron withdrawing groups in its moiety that also have a positive effect on fluorescence.

4.4.3.4 9-Chloro-10-hydroxy-2-methoxy-1,4-anthraquinone, 4

Initial experimental work conducted on **4** tested concentrations from 1.0 to 0.1 mg/mL. Congruent to the results found for the other anthraquinone, over-saturation occurred across these concentrations. Therefore, lower concentrations of the reagent were examined; ranging from 0.05, 0.025, 0.01, and 0.005 mg/mL. The concentrations of interest were 0.025 and 0.01 mg/mL, as the fluorescence intensity measured at 0.05 mg/mL was similar to that at 0.025 mg/mL. At low concentrations such as 0.005

mg/mL, the volume of reagent to be added was difficult to prepare, especially if used in routine analysis. Furthermore the fluorescence emission intensity provided was dramatically less than that of 0.01 mg/mL. When fingermarks were tested using the 0.005 mg/mL solution, there was little contrast between the fingermark and the background. The fluorescence intensity at 0.005 mg/mL compared poorly with those developed by the 0.01 mg/mL solution.

Figure 4-21 to 4-23 show the fluorescence emission spectra of the three amino acids developed by three different concentrations (0.025, 0.01 and 0.005 mg/mL) of **4**. Overall, the figures show that a solution of 0.01 mg/mL provided the greatest fluorescence. In addition, results indicate that a combination of ethyl acetate/HFE7100 and ethanol/HFE7100 provided greater fluorescence than the other mixed solvent systems. Although these two combinations provided similar results, it is more prudent to use a combination of ethyl acetate (20 % v/v) and HFE7100 (80 % v/v) because polar solvents can cause the diffusion of inks, thus destroying evidence.

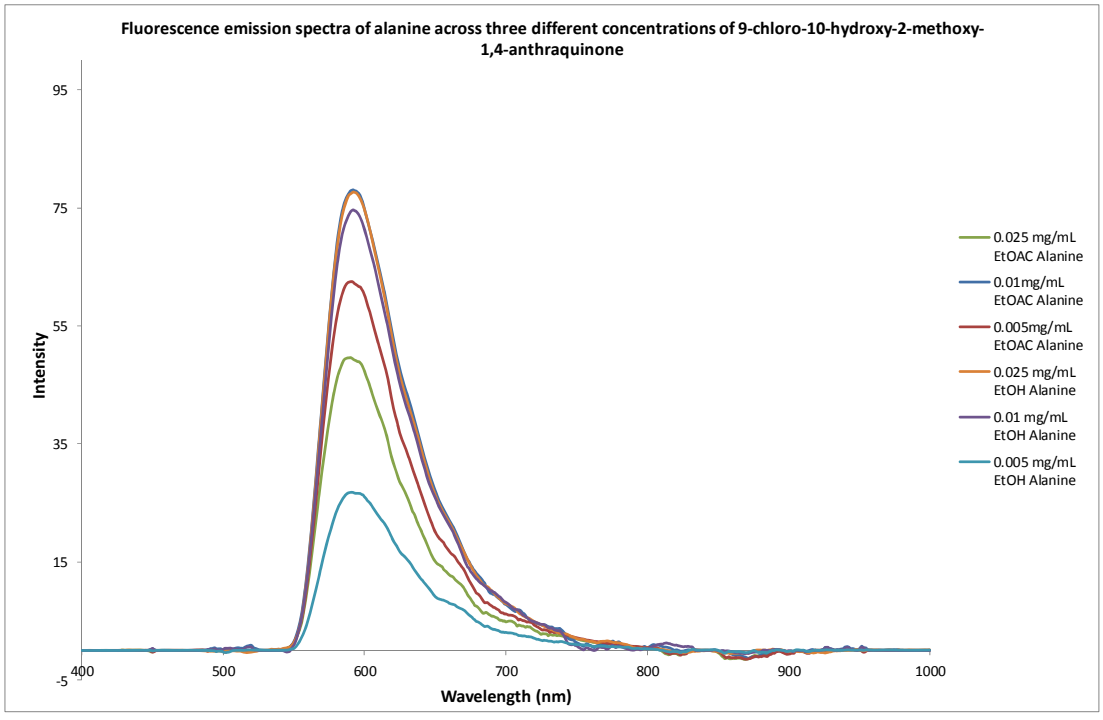


Figure 4-21 Fluorescence emission spectra of alanine across three different concentrations of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone (excitation at 485 – 535 nm).

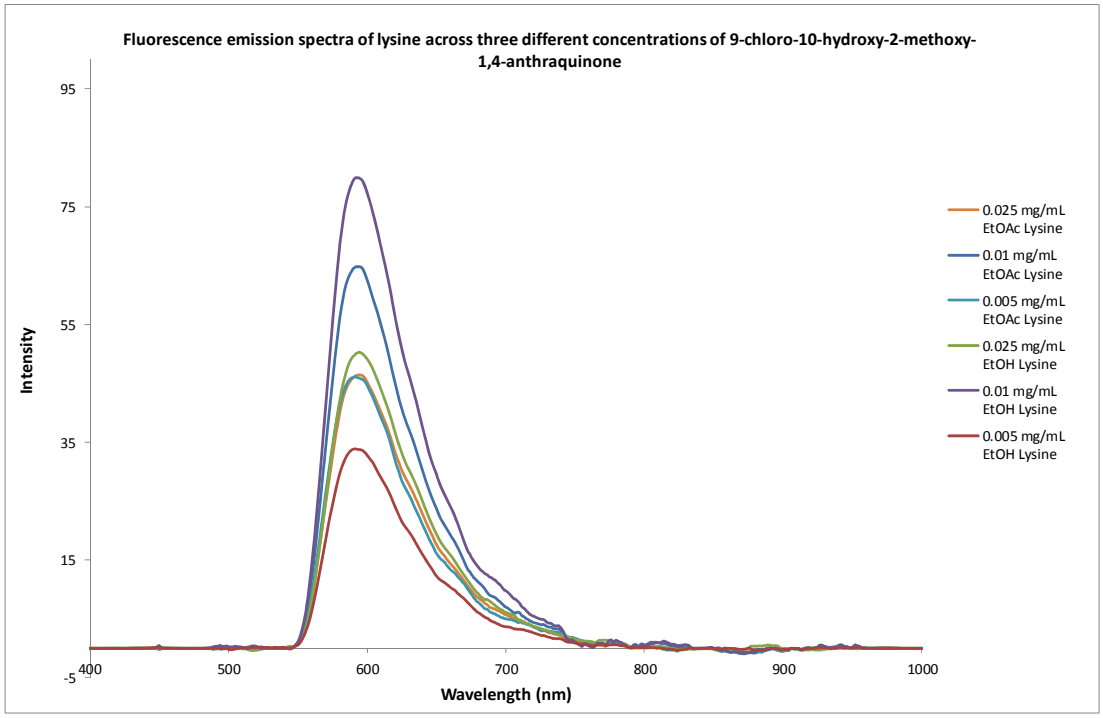


Figure 4-22 Fluorescence emission spectra of lysine across three different concentrations of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone (excitation at 485 – 535 nm).

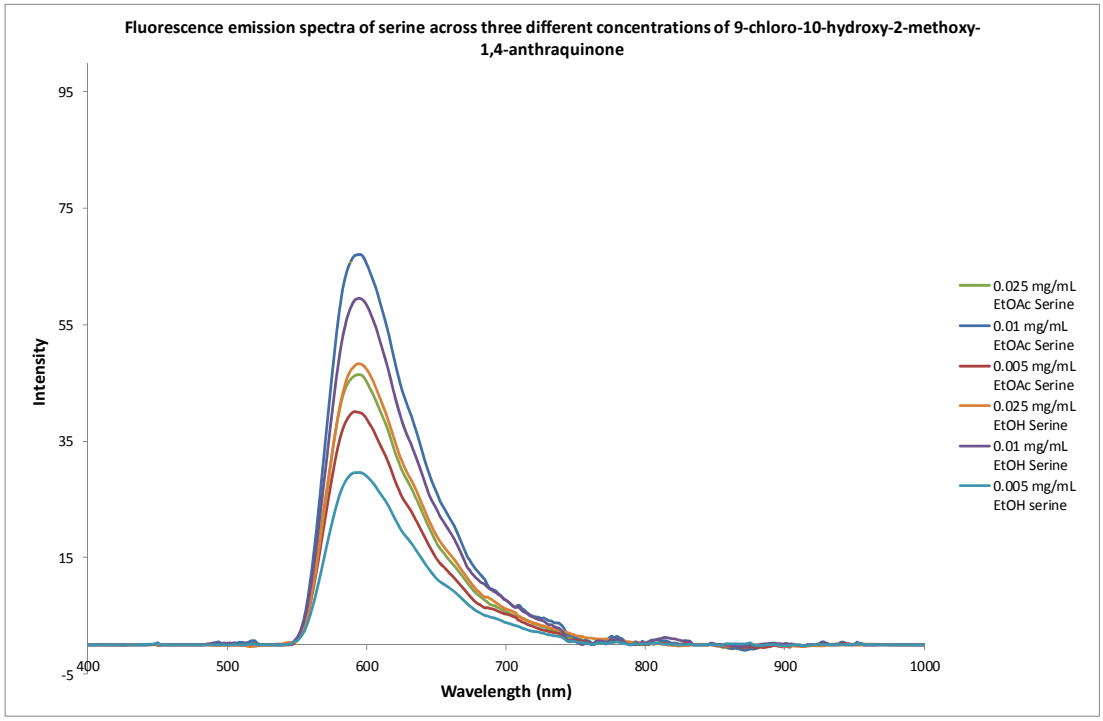


Figure 4-23 Fluorescence emission spectra of serine across three different concentrations of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone (excitation at 485 – 535 nm).

A number of development conditions were tested and it was found that oven development at 150 °C for approximately one hour provided optimum development

conditions. This was similar to the results found for the quinones that have already been examined.

Furthermore, the results for pH and metal salts optimisation did not provide improvements. Adjustments to the pH of the optimised solution showed that pH 5 provided the best fluorescence results. All other results from the pH studies resulted in a decrease of fluorescence emission across the three amino acids that were tested. The addition of zinc salts also produced no improvements in fluorescence emission. Figure 4-24 depicts the fluorescence emission spectra of three amino acids developed by solutions of **4** with and without added zinc chloride. It shows a slight blue shift (approximately 10 nm) across the three different amino acids following the addition of zinc chloride. This was accompanied by a decrease in fluorescence intensity and this effect was observed with higher concentrations of zinc chloride. In reality, the slightly longer wavelength is too small to be practical as there is no improvement in observed fluorescence. In addition, the emission wavelength of the quinone-amino acid product without zinc chloride overlaps that of the zinc complex. As expected, when this process was used to treat fingermarks, no real improvements were observed and a slight darkening of the background was noted. Similar results were obtained with zinc nitrate.

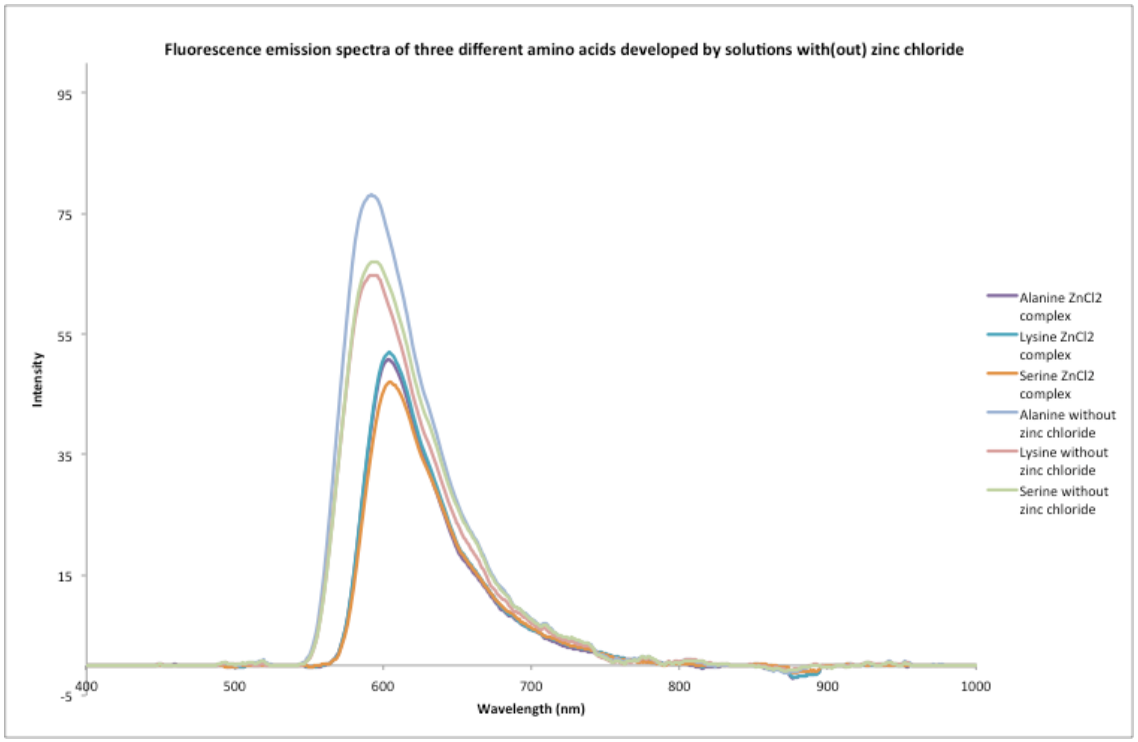


Figure 4-24 Comparison of the fluorescence emission spectra of three different amino acids developed by solutions of 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone with and without the addition of zinc chloride (excitation 485 – 535 nm).

Post-treatment and staining of the fingermark with a metal salt solution prior to treatment with **4** also failed to produce either a change in colour or any positive changes to fluorescence.

The four sets of split fingermarks in Figure 4-25 compare **4** and IND-Zn treated fingermarks. It showed that despite the shorter exposure times, IND-Zn produced far more fluorescent fingermarks with clear and defined minutiae. The optimised solution of **4** was also best used on fingermarks less than 7 days old. When it was used to treat fingermarks that were older than 7 days, either no fingermarks were observed or intermittent fluorescent ridges were developed. An example of this characteristic is shown in Figure 4-26. Figure 4-25 (a) to (c), faint ridges were observed with the halves developed by **4** on the two different types of lined paper and white Reflex® paper. Figure 4-25 (d) shows a split fingermark developed by IND-Zn and **4** on pink Reflex® paper. It can be noted that no ridges were developed by **4**.

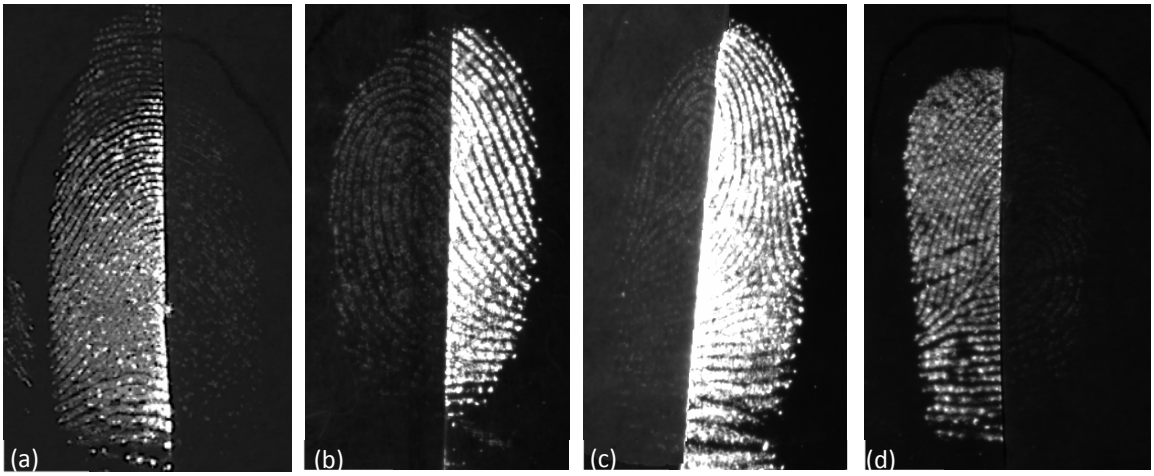


Figure 4-25 (a) Fingermark on line paper, left: IND-Zn , right: 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone, (b) fingermark on recycled lined paper, left: 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone, right: IND-Zn, (c) fingermark on white Reflex® paper, left: 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone, right: IND-Zn, (d) fingermark developed on pink Reflex® paper, left: IND-Zn, right: 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone (9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone: photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s. IND-Zn: Photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

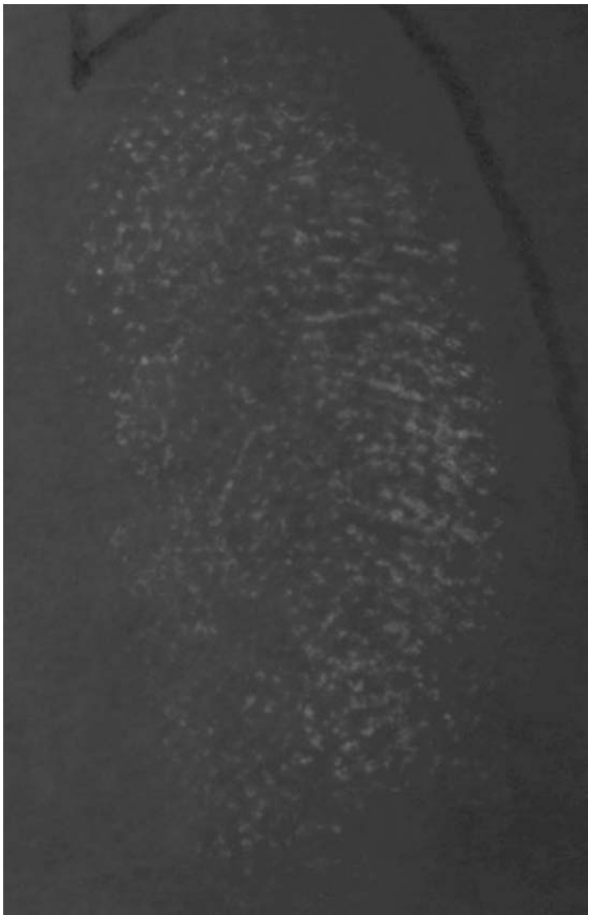


Figure 4-26 Charged fingermark, aged 1 month, fingermark on white Reflex® paper, developed by 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquionone (photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s).

4.4.3.5 9-Chloro-2,10-dihydroxy-1,4-anthraquinone, 5

Optimisation of the concentration for **5** was conducted over a range of 1.0 to 0.01 mg/mL. Results from these sets of experiments showed over-saturation and background darkening at higher concentrations. These results were also congruent with the results obtained with other substituted anthraquinones that were described in the previous sections. Therefore, it was determined that a concentration of 0.02 mg/mL was the optimum concentration using a combination of ethyl acetate (2 % v/v), ethanol (18 % v/v) and HFE 7100 (80 % v/v). Oven development at 150 °C for one hour provided optimum development condition.

Using this concentration, pH testing was conducted from pH 4 to 7. The fluorescence spectra of the three amino acids spotted on the filter paper strips showed that there was little difference between the results over between pH 5 - 7. However, slight improvements were noted in the results from pH 4. These changes were not apparent in the results conducted on paper where the clarity and fluorescence of fingermark ridges were faint. The developed ridges only showed very faint brown colouration, which were difficult to photograph under white light.

Metal salts were used in an attempt to improve the colour and fluorescence intensity of the fingermark ridges. Similar to ninhydrin, DFO and the current AFP formulation of IND-Zn, the addition of zinc chloride to a solution of **5** appeared to improve the fluorescence emission of the developed amino acids and fingermarks. At a concentration of 1:2 molar ratio of zinc chloride to **5,** there were no noticeable effects. Figure 4-27 shows improvements in results for the amino acid strips developed with a solution of quinone with added zinc chloride when compared with a solution without zinc chloride. No improvements in fluorescence were observed when zinc chloride was added either as a pre-staining or as a post-staining solution regardless of the concentration. These results indicate that zinc (II) ions act as a Lewis acid catalyst that provides stabilisation to intermediates that may be formed during the hydrolysis step of the reaction.

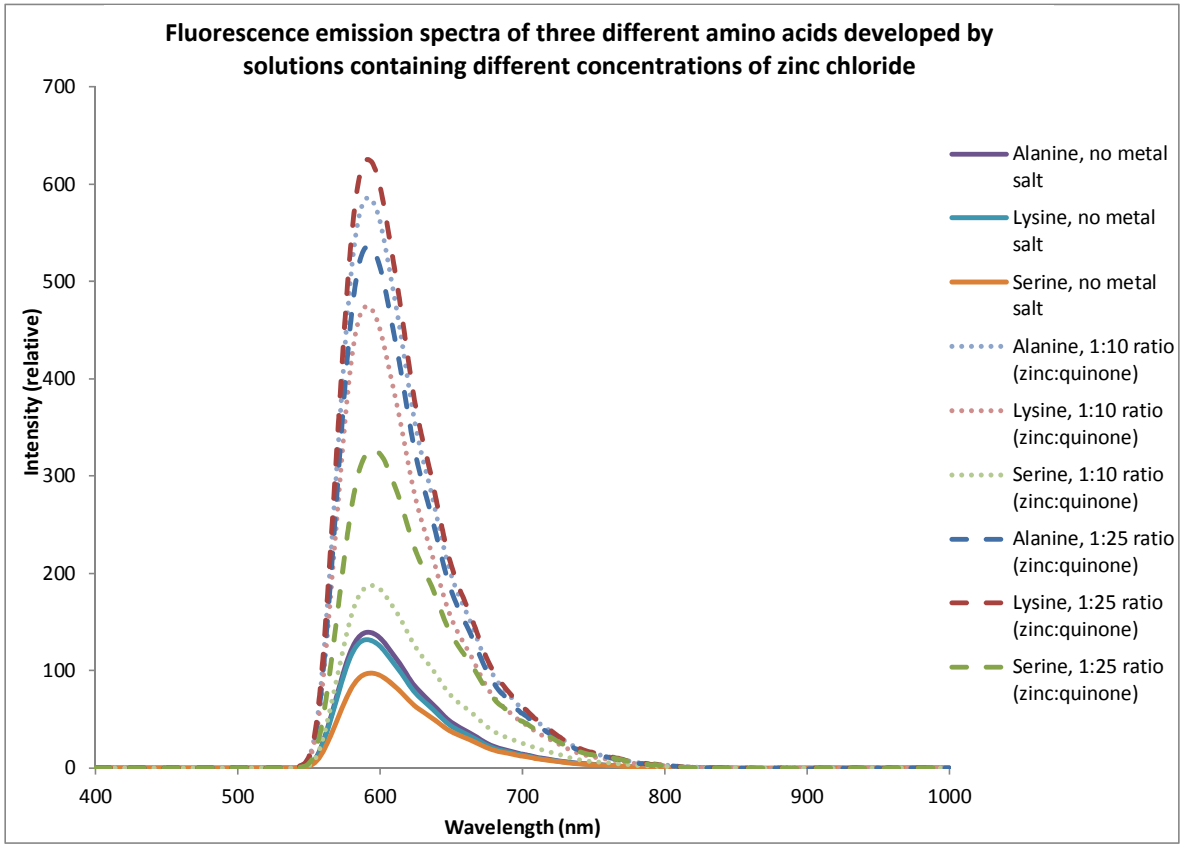


Figure 4-27 Comparison of fluorescence intensity of three different amino acids developed by solutions of 9-chloro-2,10-dihydroxy-1,4-anthraquinone with different ratios of zinc chloride (excitation at 485 – 535 nm).

When the optimised solution of **5** was used to develop fingermarks, very faint brown coloured ridges were developed for charged fingermarks only. Other developed fingermarks were not observed under white light or luminescence mode. Consequently, charged fingermarks were also more fluorescent with minutiae details evident and sufficient for photographs to be captured for analysis by fingerprint experts. Figure 4-28 shows four set of split prints developed by a solution containing **5** and IND-Zn. The fingermarks developed with **5** were not as fluorescent as the IND-Zn developed fingermarks on the substrates that were tested. There was also greater contrast between the ridges and the background with the IND-Zn developed fingermarks. Although these ridges were not as fluorescent, there were sufficient details present that, if encountered in real casework, would warrant further investigation by fingerprint experts.

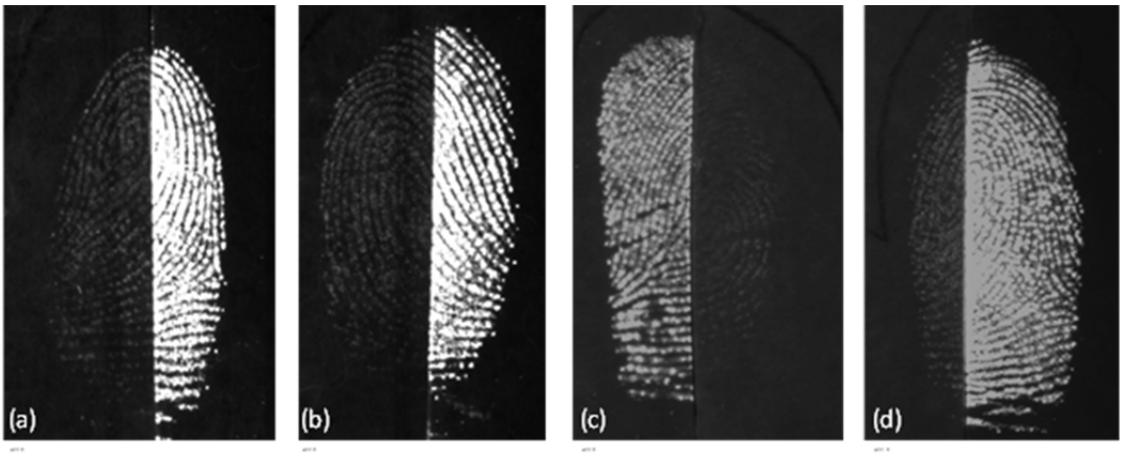


Figure 4-28 (a) Fingermark on line paper, left: 9-chloro-2,10-dihydroxy-1,4-anthraquinone, right: IND-Zn, (b) fingermark on recycled lined paper, left: 9-chloro-2,10-dihydroxy-1,4-anthraquinone, right: IND-Zn, (c) fingermark on white Reflex® paper, left: IND-Zn, right: 9-chloro-2,10-dihydroxy-1,4-anthraquinone, (d) fingermark developed on pink Reflex® paper, left: 9-chloro-2,10-dihydroxy-1,4-anthraquinone, right: IND-Zn (9-chloro-2,10-dihydroxy-1,4-anthraquinone: photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s. IND-Zn: Photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

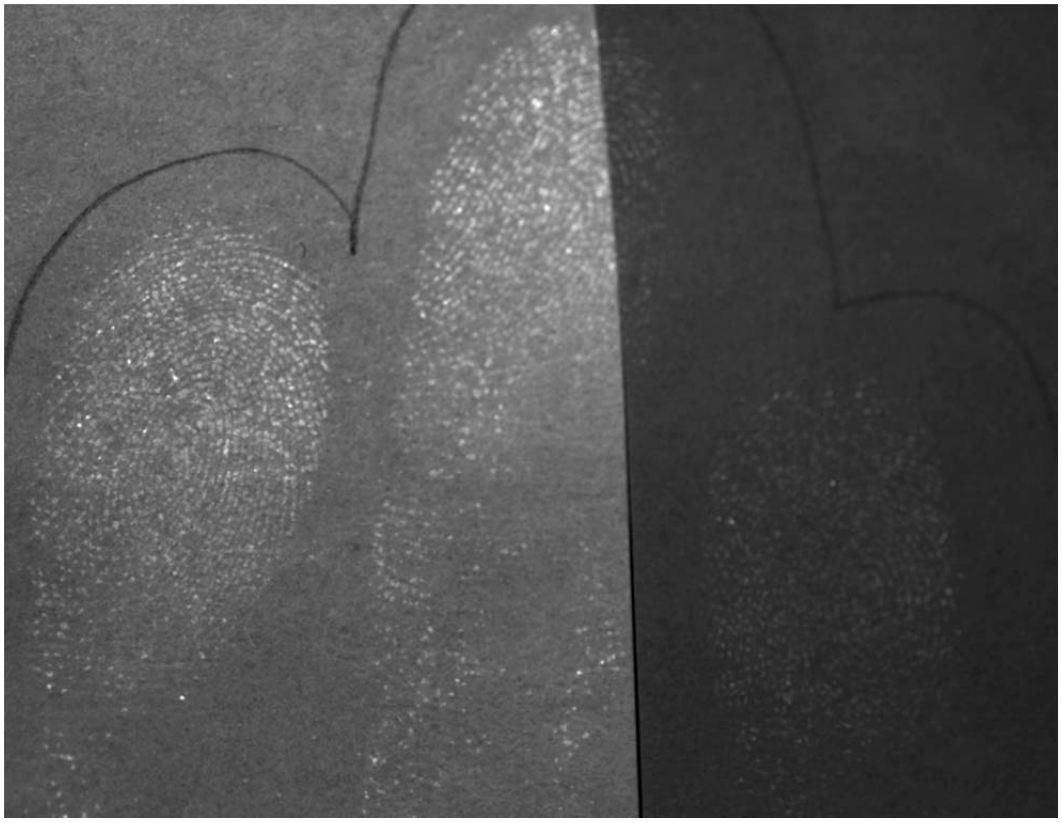


Figure 4-29 Split fingermark on white Reflex® paper developed by left: 9-chloro-2,10-dihydroxy-1,4-anthraquinone, right: lawsone (photographed under excitation 530 nm, 610 nm filter, F.5.6, exposure 2s).

Comparisons were made between the working solutions of **5** and lawsone. Figure 4-29 shows an example a split fingermarks that was developed. fingermarks developed by lawsone were not as clear or fluorescent as the ridges developed by **5** despite the lighter background. These improvements were likely due to greater conjugation and the number of electron withdrawing groups in the anthraquinone compound.

4.4.3.6 1,2-Anthraquinone-4-sulfonic acid, ammonium salt (AQS)

Optimisation of the concentration for AQS was conducted over a range of 1.0 to 0.01 mg/mL. Similar to the results obtained from the experiments conducted for naphthoquinones and anthraquinones, over-saturation and background darkening occurred at higher concentrations. These issues were not problematic at lower concentrations ranging from 0.05 to 0.01 mg/mL. However, there was a distinct decrease in fluorescence emission at concentrations below 0.05 mg/mL. A number of solvent systems were also assessed, with the aim of maximising the volume of HFE 7100 in the working solution. It was determined that a concentration of 0.05 mg/mL was the optimum concentration using a combination of ethyl acetate (5 % v/v), butanol (15 % v/v) and HFE7100 (80 % v/v). Optimum development condition was achieved by heating the substrates (amino acids and fingermarks) in the oven at 150 °C for one hour. Since oven development delivered the greatest fluorescent intensity for all the quinones tested in this thesis, this technique will be used to develop the substrates treated by **6 - 8**.

The pH of the working solution was also modified in order to determine its effects. The results indicate that there was little to no difference in the fluorescence or colour produced at pH 4 – 7. At pH 7 reduced contrast due to background staining was observed. These results were consistent with those obtained for NQS. This was to be expected as the two compounds are structurally similar, differing by an aromatic ring.

The addition of metal salts, either zinc chloride or nitrate, into the solution of AQS did not have any effect on the developed amino acids or fingermarks. When the metal salt

solution was used on the substrate prior to and after development with the AQS solution, any fluorescent fingermark ridges appeared to be diffused. This was also the case with the amino acid spots.

The optimised solution of AQS and IND-Zn were used to develop fingermarks on porous surfaces. All results indicated that IND-Zn is a far superior fingermark development reagent in terms of producing a coloured and fluorescent fingermark. In nearly all instances, unless it was a heavily secreted fingerprint, little to no viable ridge details were obtained using the AQS solution. Figure 4-30 (a) and (b) show charged fingermarks deposited on lined paper. In these two instances, only very faint ridges were developed by AQS and they were clearly not as fluorescent as the IND-Zn developed split prints. On lined paper, the AQS solution was not able to develop fingermark ridges over the printed lines, whereas the IND-Zn split prints did not have the same issues. Figure 4-30 (c) and (d) show split fingermarks developed on white and pink Reflex® copy paper, respectively. AQS was unsuccessful in developing fingermarks on the coloured paper that was examined while limited success was achieved on white paper. When comparisons were made between AQS and NQS, very little difference in fluorescence was observed. Some examples are shown in Figure 4-31 (a) and (b). Note that these fingerprints were charged, freshly deposited fingermarks on white Reflex® copy paper. While slight improvements in fluorescence were expected between the AQS and NQS developed fingermarks, the increase in conjugation may not have been possible to overcome steric hindrance that can affect its interaction with fingermark secretions.

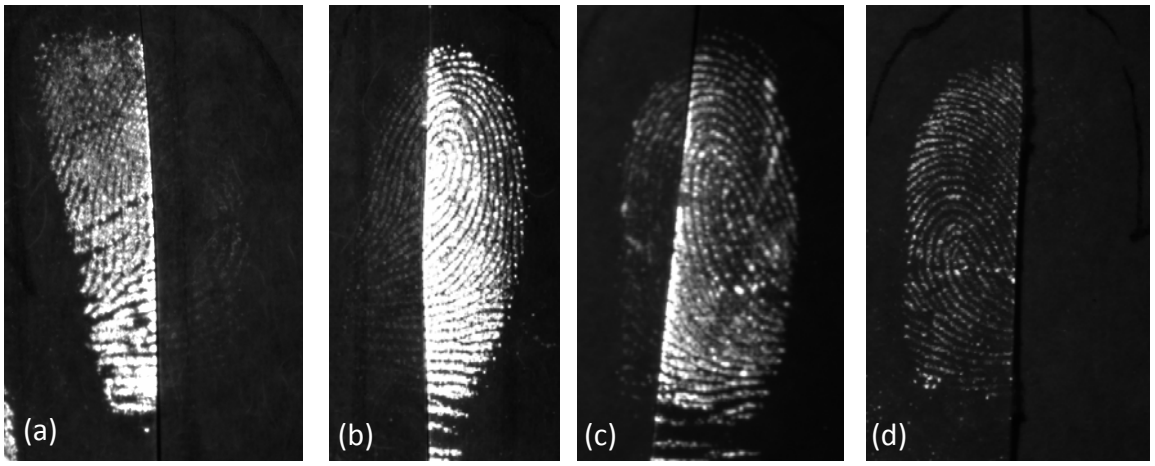


Figure 4-30 (a) Fingermark on lined paper, left: IND-Zn, right: AQS, (b) fingermark on recycled lined paper, left: AQS right: IND-Zn, (c) fingermark on white Reflex® paper, left: AQS, right: IND-Zn, (d) fingermark on pink Reflex® paper, left: IND-Zn, right: AQS (AQS: photographed under excitation 530 nm, 590 nm filter, F5.6, exposure 2s. IND-Zn: Photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

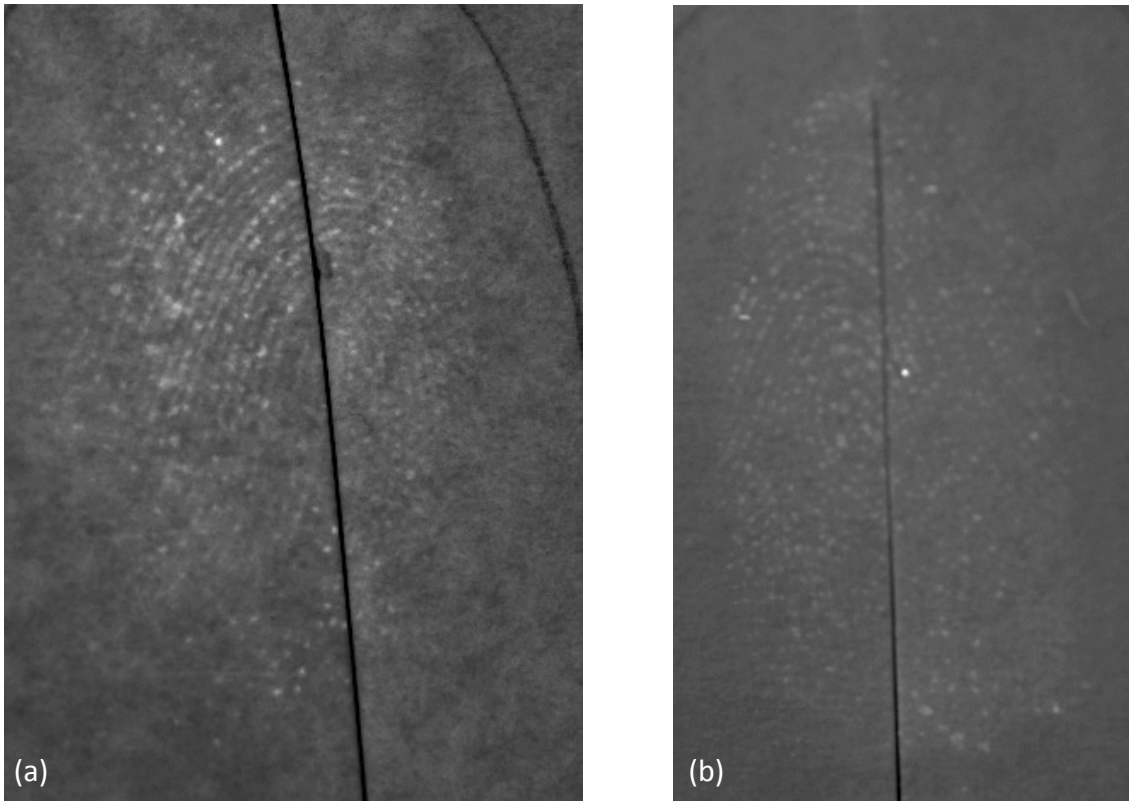


Figure 4-31 (a) and (b) Fingermarks deposited on white Reflex® paper, left: AQS, right NQS (photographed under excitation 555 nm, 610 nm filter, F5.6, exposure 2s)

4.4.3.7 1,4-Anthraquinone, 6

Similar to the results found for quinizarin, the amino acid test strips developed with solutions of **6** were indiscriminately stained. Since **6** is non-selective towards amino acids, further research on optimising **6** as a potential fingermark reagent was abandoned.

4.4.3.8 2-Methoxy-1,4-anthraquinone, 7

Initial experimental work conducted on **7** tested concentrations from 1.0 to 0.01 mg/mL. At high concentrations, low fluorescence emission and lack of background contrast were noted. These characteristics were not prominent at lower concentrations. Adjustment to the solvent system and concentration of the reagent were made. It was determined that **7** at a concentration of 0.05 mg/mL in a combination of ethyl acetate (5 % v/v), butanol (15 % v/v) and HFE 7100 (80 % v/v) provided the most fluorescent and clarity in fingermarks developed. This solvent system was then used for further testing.

Various pH and metal salts were adjusted for the optimisation of the working solution. Analysis of the fluorescence emission from the developed amino acid test strips by the working solution showed that there was no difference between the measured intensity over a pH range of 4 to 7. Therefore, the pH of the working solution was maintained at the pH of the solution (pH 5). The addition of metal salts also produced no improvements in fluorescence emission. Congruent with other results in this chapter, pre- and post-staining with a zinc chloride solution caused diffusion of amino acid spots and fingermark ridges. As expected, when these solutions were used to develop fingermarks on porous surfaces, no improvements were noted. Similar results were obtained with zinc nitrate.

The optimised solution of **7** and IND-Zn was used to develop fingermarks on porous surfaces. All results indicated that IND-Zn is a far superior fingermark development reagent in terms of producing coloured and highly fluorescent fingermarks. Unless it

was a heavily secreted fingerprint, little to no viable fingermarks were obtained using **7** in nearly all instances. Some of these fingermarks s are depicted in Figure 4-32. The background contrast of the half fingermark developed by **7** in Figure 4-32 (d) has been digitally enhanced to reveal some fingermark ridges. As mentioned previously, no enhancement of ridges was observed using **7**.

When **4** was compared with **7**, the results were noticeably better. Some of these examples are shown in Figure 4-33, where (a) was a charged freshly deposited fingermark and (b) was the second print deposited in the series. The improvements are likely the result of the increase in the number of electron withdrawing groups that are present on the highly substituted anthraquinone moiety as compared with **7**.

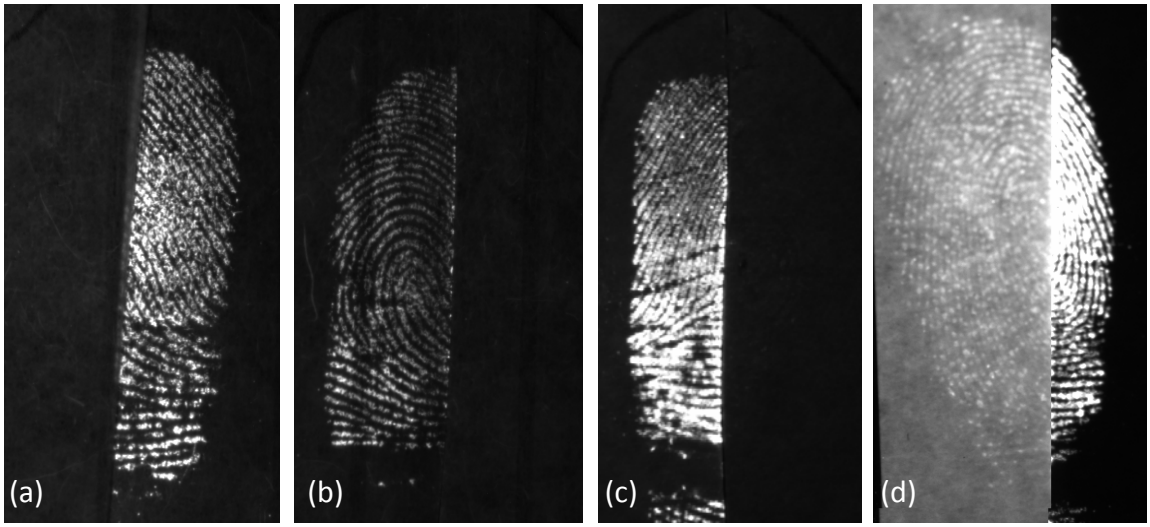


Figure 4-32 (a) Fingermark on lined paper, left: MAQ, right: IND-Zn, (b) fingermark on recycled lined paper, left: IND-Zn, right: MAQ, (c) fingermark on white Reflex® paper, left: IND-Zn, right: MAQ (d) fingermark on pink reflex® paper, left: MAQ, right: IND-Zn (MAQ: photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s. IND-Zn: Photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

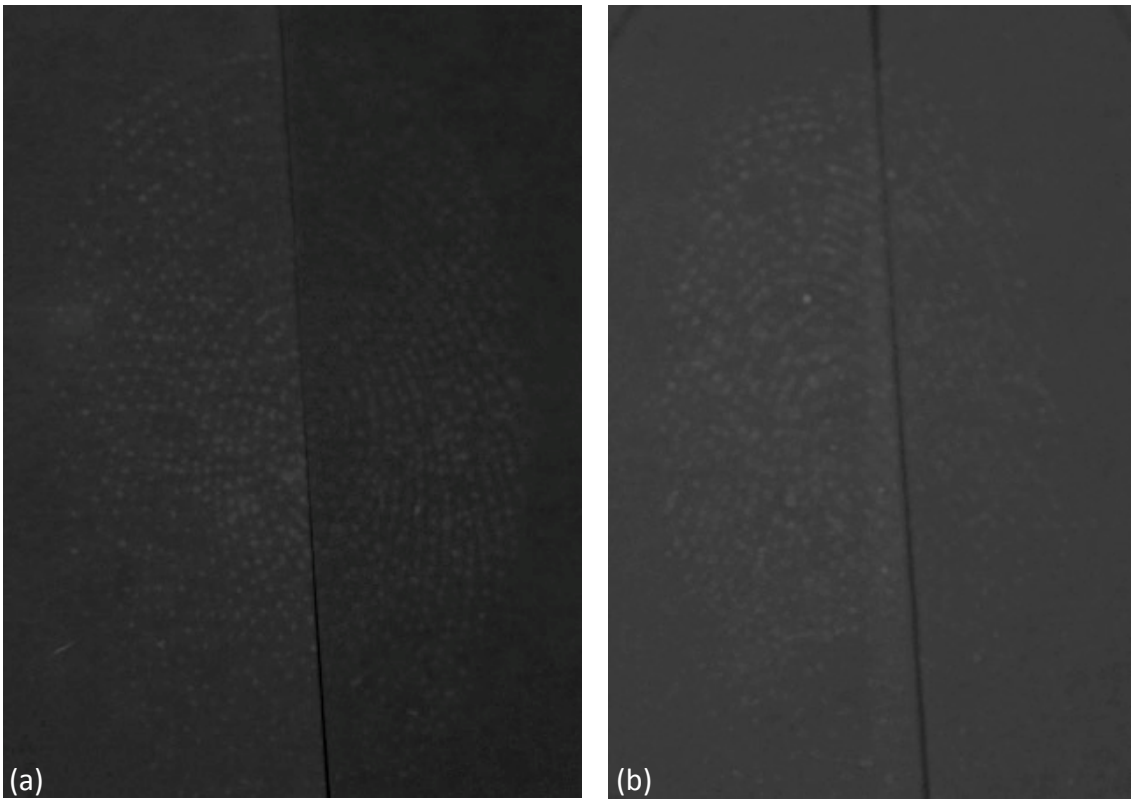


Figure 4-33 (a) and (b) Split fingermark deposited on white Reflex® paper developed by: left: 9-chloro-10-hydroxy-2-methoxy-1,4-anthraquinone, right: MAQ (photographed under excitation 530 nm, 610 nm filter, F5.6, exposure 2s).

4.4.3.9 2-Hydroxy-1,4-anthraquinone, 8

Initial experimental work conducted using **8** evaluated concentrations from 1.0 to 0.01 mg/mL. At high concentrations, the background of the substrate was stained a dark yellow. In luminescence mode, little to no luminescence was obtained with fresh fingermarks. By altering the concentration of solvents in the working solution and the reagent, and it was found that **8** (0.02 mg/mL) in a combination of propanol (5 % v/v), butanol (15 % v/v) and HFE7100 (80 % v/v) appeared to have provided better results.

The pH and metal salts were adjusted for the optimisation of the working solution. Analysis of the fluorescence emission from the developed amino acid test strips by the working solution showed that there was no difference between the measured intensity over a pH range of 5 - 7. Following the addition of a small volume of acetic acid to reach pH 4, the fluorescent intensity of the amino acid spots increased

dramatically relative to the other solutions. These results also translated well when tested on fingermarks deposited on porous surfaces. However, fingermarks only developed when the donor was a heavy eccrine secretor and only on fresh fingermarks. The addition of metal salts produced no improvements in fluorescence emission. Pre-and post-staining with a zinc chloride solution resulted in diffused amino acid spots and fingermark ridges. As expected, when these solutions were used to develop fingermarks on porous surfaces, no improvements were noted. These results were in line with all the other results that were obtained for other anthraquinones and naphthoquinones that were examined in this thesis. Similar results were obtained with zinc nitrate.

The optimised solution of **8** and IND-Zn were used to develop fingermarks on porous surfaces. All results obtained showed that IND-Zn is a far superior fingermark development reagent. Unless it was a heavily eccrine secreted fingerprint, little to no viable fingermarks were obtained using the solution containing **8**. Figure 4-34 shows examples of some split fingermarks comparing **8** and IND-Zn. From the experimental work conducted, **8** can only develop freshly deposited charged fingermarks and failed to develop fingermarks older than 7 days old.

When lawsone was compared with **8**, there was very little difference in the quality of the developed fingermarks. Across most surfaces, both these reagents produced faint, non-continuous fingermark ridges that were mostly 'spotty'. There was also lack of contrast between the developed fingermark and the background. These characteristics made the identification of fingermark ridges very difficult due to its poor quality. In theory, it was expected that **8** would be superior to lawsone because of increased π conjugation, however, in reality there was little variation between the results. This is likely due to steric hindrance of **8** (in comparison with lawsone) preventing successful reaction with the various amino acids that are present in eccrine secretions. A split fingermark deposited on white copy paper comparing the differences between **8** and IND-Zn is shown in Figure 4-35.

Comparisons were made using split fingermarks with **5** and **8**. Some of these examples are shown in Figure 4-36 (a) and (b), with little to no difference observed between the two split fingermarks.

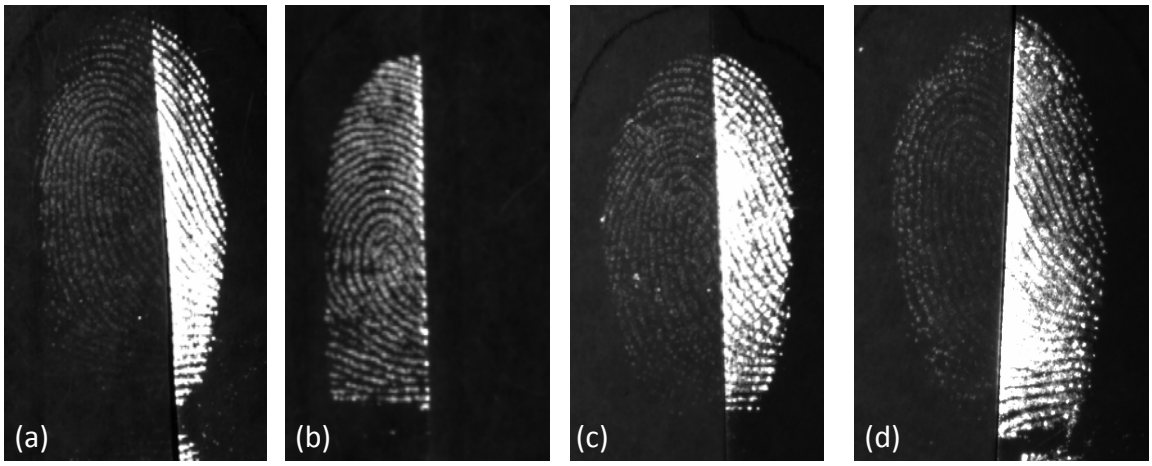


Figure 4-34 (a) Fingermark on lined paper, left: HAQ, right: IND-Zn, (b) 'aged' (7 day old) fingermark on lined paper, left: IND-Zn, right: HAQ, (c) fingermark on white Reflex® paper, left: HAQ, right: IND-Zn (d) fingermark on pink reflex® paper, left: HAQ, right: IND-Zn (HAQ: photographed under excitation 530 nm, 590 nm filter, F5.6, exposure 2s. IND-Zn: photographed under excitation 505 nm, 555 nm filter, F5.6, exposure 1s).

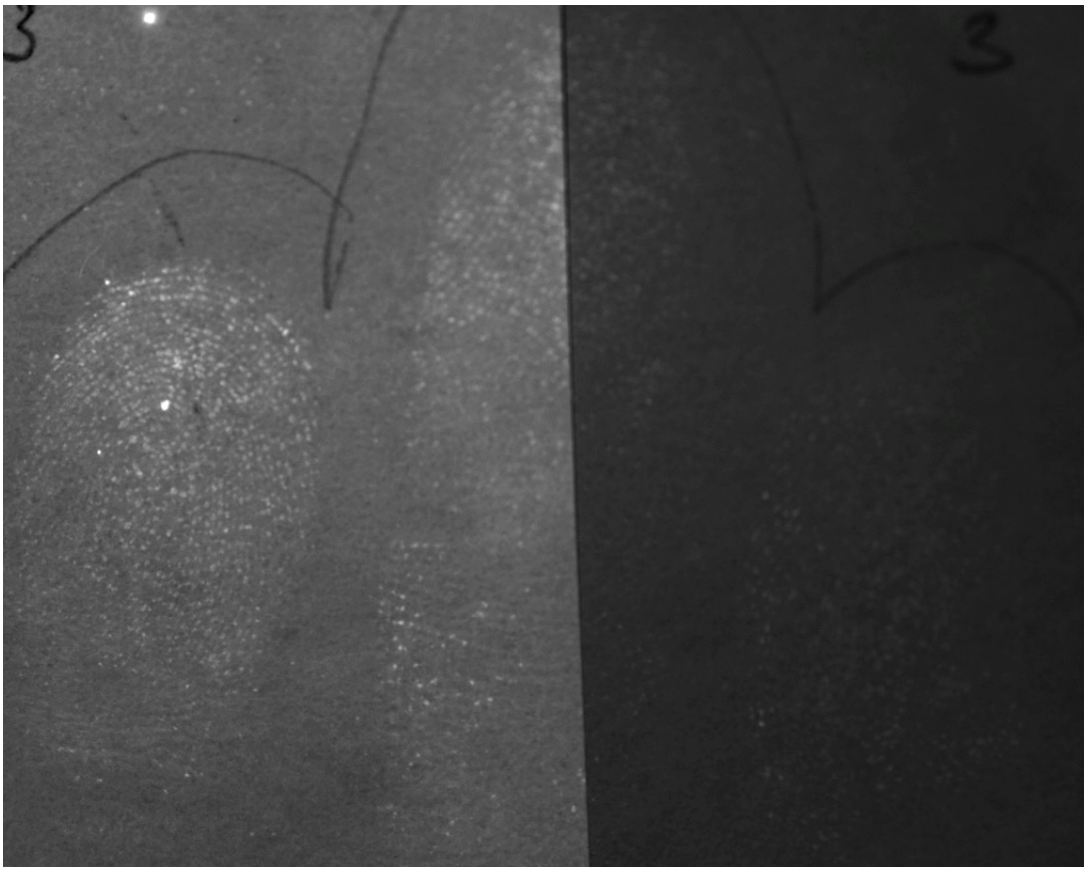


Figure 4-35 Fingermark deposited on white Reflex® paper, developed by left: HAQ, right: lawsone (photographed under excitation 530 nm, 590 nm filter, F5.6, exposure 2s)

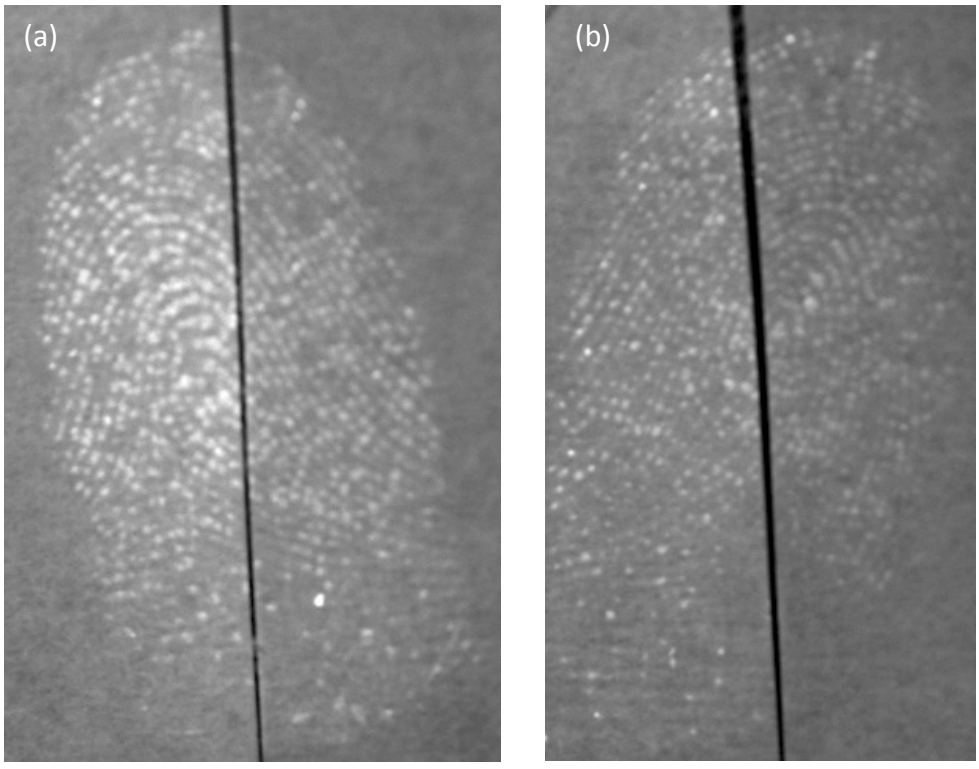


Figure 4-36 Fingermark deposited on white Reflex® paper developed by (a) 9-chloro-2,10-dihydroxy-1,4-anthraquinone, (b) HAQ (photographed under excitation 530 nm, 590 nm filter, F5.6, exposure 3s)

4.4 Conclusions

It was hypothesised that the synthesised anthraquinones would be more reactive to the amino acid components of a fingermark than its benzoquinones and naphthoquinones equivalent due to greater conjugation. This theory was investigated in this pilot study in which quinones with varying substituents and conjugation were used to develop fingermarks on porous surfaces. In this study, a limited age range of fingermarks was used, ranging from fresh (less than 24 hours) to 1 month and a small pool of fingermark donors was used. Evaluation of older test substrates was not conducted due to the poor results obtained with fresh fingermarks. Furthermore, all the quinones that were tested showed little to no fingermark development when used on 1 month old aged fingermarks.

1 was unstable and therefore not suitable for testing. Some of the anthraquinones (quinizarin, **2**, and **6**) were non-selective toward fingermark components and were deemed unsuitable for the project. The increase in conjugation between naphthoquinones and anthraquinones can lead to steric hindrance that is likely to be inhibiting its reaction with the eccrine components in the fingermark. As a result, only slight improvements in fluorescence were observed with the anthraquinone reagents.

During this study, a preliminary comparison with IND-Zn using split fingermarks against the quinones was conducted. The experimental work showed that IND-Zn produced far greater fluorescence in all the fingermarks that were examined. The fingermarks treated with IND-Zn could be developed quickly with the heat press as opposed to thermal development in the oven. Due to greater sensitivity of IND-Zn to amino acids and thus the eccrine secretions within fingermarks, photographs of fingermarks can be acquired rapidly. While naphthoquinones and anthraquinones were able to develop fingermarks, all the fingermarks were comparably less fluorescent than the ones developed by IND-Zn. As such, photographs of the fingermarks required longer exposure times. Furthermore, solutions of IND-Zn can be made with ease and are stable for long periods of time. These properties make IND-Zn an attractive working solution for the development of latent fingermarks for routine casework. As the timely provision of results for investigations is an important component of forensic

laboratories due to the dynamic nature of crime and the backlog of casework, the use of these novel quinones will require further research and development. Although each of the compounds examined did not perform better than IND-Zn for the development of latent fingermarks when viewed in isolation, they may have added value when examined in a sequence with other fingermark reagents. However, this was not investigated due to the preliminary nature of the study and may be an avenue of exploration for later research.

The use of anthraquinones for the development of latent fingermarks on porous surfaces is an interesting concept as these compounds are generally used in industry as dyes or are incorporated as part of medical treatments. The experimental work conducted in Chapter 4 shows that these compounds can be used for fingermark development and their effects are dependent on conjugation and substituents on the molecular structure of the compound. If research based on these anthraquinones is to extend beyond this project, various factors will need to be considered, these include the cost-effectiveness (in terms of time required for the synthesis of these compounds as well as the cost of the starting materials), the work health and safety aspect of these compounds and also the ease of use of these compounds.

Chapter 5: Conclusions

5.1 Conclusions and recommendations

Fingermark evidence is an important component of forensic case work. It is a fundamental tool in linking individuals to a crime scene and/or a piece of evidence. Fingermarks deposited on porous items are more robust than those deposited on non-porous items as eccrine secretions tend to be absorbed into the porous matrix. Therefore, there has been continual research into new fingermark development reagents for porous surfaces.

Jelly *et al.,* have shown that naphthoquinones, can be used to develop fingermarks on porous surfaces (97; 127; 133; 135). In published works by the same authors, structural elucidation of the reaction product(s) between lawsone and various amino acids were pursued with unconfirmed results. In order to understand the reaction between 1,4-quinones and fingermarks, the nature of the reaction was explored. While the ninhydrin, and 1,2-indanedione reactions with amino acids have been investigated, the lawsone reaction products have not been elucidated to an extensive degree. Chapter 3 documents a number of reaction products that were detected when lawsone was reacted with three amino acids and a common product was found to be present. The proposed structure of this product was characterised using FTIR spectroscopy, NMR spectroscopy and LC-MS. A reaction mechanism for the formation of this common product has been proposed. The use of LC-MS has allowed for minor reaction products to be detected and tentative structures were proposed.

In this current study, quinones were synthesised on the premise that greater conjugation and more reactive functional groups would translate to greater fluorescence upon the compounds' reaction with latent fingermarks. The synthesised quinones were characterised using FTIR spectroscopy, NMR spectroscopy and LC-MS.

A part of this thesis was a preliminary study in which the synthesised anthraquinones and benzoquinones were investigated for their ability to develop fingermarks on porous surfaces. In addition, the effects of increasing conjugation and electron withdrawing groups on a reagent were examined. This was achieved by comparing the amino acid standard results and the fingermarks that were developed by each reagent.

A small number of donors and substrates were selected for the study due to the availability of donors and the preliminary nature of the project. The age of the fingermarks tested ranged from fresh, 1 day, 1 week to 1 month. This thesis also compared the reactivity of the synthesised compounds with the naphthoquinones that were examined by Jelly *et al*. (135). Attempts at making the naphthoquinone working solutions using the published methods were met with unsuccessful results. Therefore, all formulations involving these naphthoquinones were revisited due to various issues such as solubility.

When solutions of all the compounds were optimised, comparisons between the developed fingermarks were conducted. Through experimental data, it was determined that slight differences could be observed, with anthraquinones being more reactive than the corresponding naphthoquinones. Although these differences were insignificant in practical terms, these observations indicate that increasing conjugation and reactive functional groups on a molecule led to greater quantum yield.

Another aspect of this study was to use these reagents and perform preliminary comparisons with a routinely used technique. The latent fingermarks from the naphthoquinones and anthraquinones examined produced poor results. It is unsurprising that fingermarks developed by IND-Zn were more fluorescent and had greater clarity than those developed by the naphthoquinones and anthraquinones. The superior fluorescence results are likely due to the ability of IND-Zn to consume amino acids to a greater extent than the novel reagents. The ability of IND-Zn to react with amino acids is well documented with comparisons made with ninhydrin and DFO. Consequently, these amino acid reagents are placed in a sequence, in order to react with all the amino acids that may be present in a fingermark. In reality, this ability translates to greater sensitivity, less time required for development and ease of use.

When viewed in isolation, the quinones that were synthesised did not surpass the results produced by IND-Zn. However, it may be worthwhile in future research, to investigate their potential when used in a sequence with other reagents. Future research can also extend to a greater variety of donors as well as substrates that exhibit background interference.

In this study, a series of quinones were successfully synthesised and characterised using a number of analytical techniques. The reaction products from the lawsone-amino acid reactions were investigated and structures of these were proposed. Due to the structural similarities of these 1,4-quinone compounds, it was hypothesised that the reaction product (quinone-amino acid product) would be comparable to the benzoquinone and anthraquinone series. The experimental work in this thesis showed the potential of these quinones for the detection of latent fingermarks on porous surfaces. However, we were unable to discover a fingermark reagent that could potentially surpass the reactivity of IND-Zn based on the preliminary results from the study.

During the course of this study, numerous sets of experimental data arising from the fluorescence studies were collected. A potential pathway for future researchers would be to consider the use and development of an artificial neural network at the outset of the study. Using such a network would likely reduce the number of experiments conducted and thus improve the efficiency of the work.

There are further avenues for potential research that arose from the current study. These include the characterisation and elucidation of the reaction products arising from the reactions of amino acids with the synthesised anthraquinone as the lawsone reaction serves only as a model. It was noted that when a metal salt, such as zinc chloride, was added to a solution of lawsone, the fluorescence emission spectra changed dramatically. This could indicate the formation of a complex as well as a catalytic reaction. The isolation of this product was not attempted in this thesis. However, the isolation of the product could be attempted in further research. The current research used two different zinc salts, other metal salts such as cadmium, divalent copper and mercury could also be considered for future research.

Although the synthesised anthraquinones were found to be inadequate as fingermark reagents, their fluorescent nature may allow them to be used in biochemistry as fluorescent dyes for the staining of molecules such as DNA, or as DNA tags. During the literature review conducted for this thesis, it was found that medicinal chemistry

incorporates the use of quinones within the moiety of therapeutic preparations. The quinones prepared in this thesis may be used in such a manner.

Chapter 6: References

6.1 References

1. Ashbaugh DR. 1999. Quantitative-qualitative friction ridge analysis: an introduction to basic and advanced ridgeology. Boca Raton, Florida: CRC Press
2. Maltoni D, Maio D, Jain AK, Prabhaker S. 2009. Handbook of fingerprint recognition. London: Springer-Verlag
3. Kücken M. 2007. Models for fingerprint pattern formation. *Forensic Science International* 171:85-96
4. Prabhaker S, Jain AK, Sharath P. 2003. Learning fingerprint minutiae location and type. *Pattern Recognition* 36:1847 - 57
5. Knowles AM. 1978. Aspects of physicochemical methods for the detection of latent fingerprints. *Journal of Physics E: Scientific Instruments* 11:713-21
6. Champod C, Lennard C, Margot P, Stoilovic M. 2004. Fingerprints and other ridge skin impressions. Boca Raton, Florida: CRC Press
7. Berry J, Stoney DA. 2001. History and Development of Fingerprinting. In *Advances in Fingerprint Technology*, ed. HC Lee, RE Gaensslen. Boca Raton, Florida: CRC Press
8. Cummins H. 1942. Ancient finger prints in clay. *Journal of Criminal Law and Criminology* 32:468 - 81
9. Hoover JE. 1972. The role of identification in law enforcement: an historical adventure. *St. John's Law Review* 46:613 - 31
10. Caplan RM. 1990. How fingerprints came into use for personal identification. *Journal of the American Academy of Dermatology* 23:109-14
11. Saferstein R. 2004. *Criminalistics: an introduction to forensic science*. Upper Saddle River, New Jersey: Pearson Education International
12. Kaye DH. 2003. Questioning a courtroom proof of the uniqueness of fingerprints. *International Statistical Review / Revue Internationale de Statistique* 71:521-33
13. Sombat JM. 2002. Latent justice: *Daubert's* impact on the evaluation of fingerprint identification testimony. *Fordham L.aw Review* 70:2819 - 68
14. Langenburg G. 2011. Chapter 14: Scientific research supporting the foundations of friction ridge examinations. In *The Fingerprint Sourcebook*, ed. A McRoberts, pp. 14-1-31. Washington, DC: National Institute of Justice
15. Cole SA. 2003. Fingerprint: the first junk science? *Oklahoma City University Law Review* 28:73 - 92

16. Haber L, Haber RN. 2008. Scientific validation of fingerprint evidence under Daubert. *Law, Probability and Risk* 7:87 - 110
17. Langenburg G, Champod C, Wertheim P. 2009. Testing for potential contextual bias effects during the verification stage of the ACE-V methodplogy when conducting fingerprint comparisons. *Journal of Forensic Sciences* 54:571 - 82
18. Dror IE, Champod C, Langenburg G, Charlton D, Hunt H, Rosenthal R. 2011. Cognitive issues in fingerprint analysis: inter- and intra-expert consistency and the effect of a 'target' comparison. *Forensic Science International* 208:10 - 7
19. Champod C. 2013. Friction ridge skin impression evidence – standards of proof. In *Encyclopedia of Forensic Sciences*, ed. JA Siegel, PJ Saukko, MM Houck. Waltham: Elsevier Science & Technology
20. Rivaldería N, Gutiérrez-Redomero E, Alonso-Rodríguez C, Dipierri JE, Martín LM. 2017. Study of fingerprints in Argentina population for application in personal identification. *Science and Justice* 57:199-208
21. Stoney D, Thornton J. 1986. A critical analysis of quantitative fingerprint individuality models. *Journal of Forensic Sciences* 31:1187-216
22. Neumann C, Evett IW, Skerrett JE, Mateos-Garcia I. 2011. Quantitative assessment of evidential weight for a fingerprint comparison I. Generalisation to the comparison of a mark with set of ten prints from a suspect. *Forensic Science International* 207:101-5
23. Neumann C, Evett IW, Skerrett JE, Mateos-Garcia I. 2012. Quantitative assessment of evidential weight for a fingerprint comparison. Part II: a generalisation to take account of the general pattern. *Forensic Science International* 214:195-9
24. Neumann C, Mateos-Garcia I, Langenburg G, Kostroski J, Skerrett JE, Koolen M. 2011. Operational benefits and challenges of the use of fingerprint statistical models: a field study. *Forensic Science International* 212:32-46
25. Egli NM, Champod C, Margot P. 2007. Evidence evaluation in fingerprint comparison and automated fingerprint identification systems - modelling within finger variability. *Forensic Science International* 167:189-95
26. Abraham J, Champod C, Lennard C, Roux C. 2013. Modern statistical models for forensic fingerprint examinations: a critical review. *Forensic Science International* 232:131-50

27. Gutiérrez-Redomero E, Rivaldería N, Alonso-Rodríguez C, Martín LM, Dipierri JE, et al. 2012. Are there population differences in minutiae frequencies? A comparative study of two Argentinian population samples and one Spanish sample. *Forensic Science International* 222:266-76
28. Scheil HG, Schmidt HD, Baltova S, Djordjevic D, Vulpe C, et al. 2005. Dermatoglyphic studies in eastern and south-eastern Europe. *Anthropologischer Anzeiger* 63:393-9
29. Ramotowski RS. 2001. Composition of latent prints. In *Advances in Fingerprint Technology*, ed. HC Lee, RE Gaensslen. Boca Raton, Florida: CRC Press
30. Girod A, Ramotowski R, Weyermann C. 2012. Composition of fingermark residue: a qualitative and quantitative review. *Forensic Science International* 223:10 - 24
31. Van Slyke DD, Dillion RT, MacFadyen DA, Hamilton P. 1941. Gasometric determination of carboxyl groups in free amino acids. *Journal of Biological Chemistry* 141:627 - 69
32. Zuo L, Yao S, Wang W, Duan W. 2008. An efficient method for demethylation of aryl methyl ethers. *Tetrahedron Letters* 49:4054-6
33. Hardorn B, Hanimann F, Anders P, Curtius HC, Halverson R. 1967. Free amino-acids in human sweat from different parts of the body. *Nature* 215:416 - 7
34. Oro J, Skewes HB. Nature. Free amino-acids on human fingers: the question of contamination in microanalysis. *Nature* 207:1042 - 5
35. Almog J, Azoury M, Elmaliah Y, Berenstein L, Zaban A. 2004. Fingerprints' third dimension: the depth and shape of fingerprints penetration into paper - cross section examination by fluorescence microscopy. *Journal of Forensic Sciences* 49:981 - 5
36. Ferreira LFV, Freixo MR, Garcia AR. 1992. Photochemistry on surfaces: fluorescence emission quantum yield evaluation of dyes adsorbed on microcrystalline cellulose. *Journal of the Chemical Society, Faraday Transaction* 88:15 - 22
37. Margot P, Lennard C. 1994. *Fingerprint detection techniques*. Lausanne: Institut de police scientifique et de criminologie
38. Lennard C. 2007. Fingerprint detection: current capabilities. *Australian Journal of Forensic Sciences* 39:55 - 71
39. Lee HC, Gaensslen RE. 2012. Fingerprint detection techniques. In *Advances in fingerprint technology*, ed. RS Ramotowski. Boca Raton, Florida: CRC Press

40. Jones N, Stoilovic M, Lennard C, Roux C. 2001. Vacuum metal deposition: factors affecting normal and reverse development of latent fingerprints on polyethylene substrates. *Forensic Science International* 115:73-88
41. Jones N, Mansour D, Stoilovic M, Lennard C, Roux C. 2001. The influence of polymer type, print donor and age on the quality of fingerprints developed on plastic substrates using vacuum metal deposition. *Forensic Science International* 124:167-77
42. Williams G, ap Llwyd Dafydd H, Watts A, McMurray N. 2011. Latent fingermark visualisation using reduced-pressure sublimation of copper phthalocyanine. *Forensic Science International* 204:e28-e31
43. Haque F, Westland AD, Milligan J, Kerr FM. 1989. A small particle (iron oxide) suspension for detection of latent fingerprints on smooth surfaces. *Forensic Science International* 41:73-82
44. Jasuja OP, Singh G, Almog J. 2011. Development of latent fingermarks by aqueous electrolytes. *Forensic Science International* 207:215-22
45. Haque F, Westland A, Kerr FM. 1983. An improved non-destructive method for detection of latent fingerprints on documents with iodine-7,8-benzoflavone. *Forensic Science International* 21:79-83
46. Jasuja OP, Kaur A, Kumar P. 2012. Fixing latent fingermarks developed by iodine fuming: a new method. *Forensic Science International* 223:e47-e52
47. Kelly PF, King RSP, Bleay SM, Daniel TO. 2012. The recovery of latent text from thermal paper using a simple iodine treatment procedure. *Forensic Science International* 217:e27-e30
48. Wang YF, Yang RQ, Wang YJ, Shi ZX, Liu JJ. 2009. Application of CdSe nanoparticle suspension for developing latent fingermkars on the sticky side of adhesives. *Forensic Science International* 185:96 - 9
49. Stoilovic M, Lennard C. 2010. *Fingerprint detection and enhancement*. Canberra: Australian Federal Police
50. Navarro E, Castelló A, López-Alfaro JA, Verdú F. 2007. More about the developing of invisible lipstick-contaminated lipmarks on human skin: the usefulness of fluorescent dyes. *Journal of Forensic and Legal Medicine* 14:340-2
51. Castello A, Alvarez M, Verdu F. 2002. A new chemical aid for criminal investigation: dyes and latent prints. *Coloration Technology* 118:316-8

52. Au C, Jackson-Smith H, Quinones I, Jones BJ, Daniel B. 2011. Wet powder suspensions as an additional technique for the enhancement of bloodied marks. *Forensic Science International* 204:13-8
53. Bossers LCAM, Roux C, Bell M, McDonagh AM. 2011. Methods for the enhancement of fingermarks in blood. *Forensic Science International* 210:1-11
54. Fieldhouse SJ. 2011. An investigation into the use of a portable cyanoacrylate fuming system (SUPERfume®) and aluminum powder for the development of latent fingermarks*. *Journal of Forensic Sciences* 56:1514-20
55. Wargacki SP, Lewis LA, Dadmun MD. 2007. Understanding the chemistry of the development of latent fingerprints by superglue fuming. *Journal of Forensic Sciences* 52:1057-62
56. Czekanski P, Fasola M, Allison J. 2006. A Mechanistic Model for the Superglue Fuming of Latent Fingerprints*. *Journal of Forensic Sciences* 51:1323-8
57. Payne G, Reedy B, Lennard C, Comber B, Exline D, Roux C. 2005. A further study to investigate the detection and enhancement of latent fingerprints using visible absorption and luminescence chemical imaging. *Forensic Science International* 150:33-51
58. Morimoto S-i, Kaminogo A, Hirano T. 1998. A new method to enhance visualization of latent fingermarks by sublimating dyes, and its practical use with a combination of cyanoacrylate fuming. *Forensic Science International* 97:101-8
59. Algaier D, Baskaran D, Dadmun M. 2011. The influence of temperature on the polymerization of ethyl cyanoacrylate from the vapor phase. *Reactive & Functional Polymers* 71:809-19
60. Paine M, Band HL, Bleay SM, Wilson H. 2011. The effect of relative humidity on the effectiveness of the cyanoacrylate fuming process for fingermark development and on the microstructure of the developed marks. *Forensic Science International* 212:130-42
61. Fung TC, Grimwood K, Shimmon R, Spindler X, Maynard P, et al. 2011. Investigation of hydrogen cyanide generation from the cyanoacrylate fuming process used for latent fingermark detection. *Forensic Science International* 212:143-9
62. Fairley C, Bleay SM, Sears VG, NicDaeid N. 2012. A comparison of multi-metal deposition processes utilising gold nanoparticles and an evaluation of their aplication to 'low yield' surfaces for finger mark development. *Forensic Science International* 217:5-18

63. Burow D, Seifert D, Cantu AA. 2003. Modifications to the silver physical process. *Journal of Forensic Sciences* 48:1 - 7
64. Wilson JD, Cantu AA, Antonopoulos G, Surrency MJ. 2007. Examination of the steps leading up to the physical developer process for developing fingerprints. *Journal of Forensic Sciences* 52:320 - 9
65. De la Hunty M, Moret S, Chadwick S, Lennard C, Spindler X, Roux C. 2015. Understanding physical developer (PD): Part I - Is PD targeting lipids? *Forensic Science International* 257:481 - 7
66. De la Hunty M, Moret S, Chadwick S, Lennard C, Spindler X, Roux C. 2015. Understanding physical developer (PD): Part II - Is PD targeting eccrine constituents? *Forensic Science International* 257:488 - 95
67. Wood MM, James T. 2009. ORO. The physical developer replacement? *Science and Justice* 49:272-6
68. Braasch K, de la Hunty M, Deppe J, Spindler X, Cantu AA, et al. 2013. Nile red: alternative to physical developer for the detection of latent fingermarks on wet porous surfaces? *Forensic Science International* 230:74-80
69. De la Hunty M, Spindler X, Chadwick S, Lennard C, Roux C. 2014. Synthesis and application of an aqueous nile red microemulsion for the development of fingermarks on porous surfaces. *Forensic Science International* 244:e48 - e55
70. Becue A, Champod C, Margot P. 2007. Use of gold nanoparticles as molecular intermediates for the detection of fingermarks. *Forensic Science International* 168:169-76
71. Becue A, Scoundrianos A, Champod C, Margot P. 2008. Fingermark detection based on the in situ growth of luminescent nanoparticles - towards a new generation of multimetal deposition. *Forensic Science International* 179:39-43
72. Stauffer E, Becue A, Singh KV, Thampi KR, Champod C, Margot P. 2007. Single-metal deposition (SMD) as a latent fingermark enhancement technique: An alternative to multimetal deposition (MMD). *Forensic Science International* 168:e5 - e9
73. Newland TG, Moret S, Becue A, Lewis SW. 2016. Further investigations into the single metal deposition (SMD II) technique for the detection of latent fingermarks. *Forensic Science International* 268:62 - 72
74. Hansen DB, Joullie MM. 2005. The development of novel ninhydrin analogues. *Chemical Society Reviews* 34:408-17

75. Petrovskaia OG. 1999. *Design and synethsis of chromogenic and fluorogenic reagents for amino acid detection*. Ann Arbor: University of Pennsylvania
76. Friedman M, David Williams L. 1974. Stoichiometry of formation of Ruhemann's purple in the ninhydrin reaction. *Bioorganic Chemistry* 3:267-80
77. Friedman M. 2004. Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. *Journal of Agricultural and Food Chemistry* 52:385-406
78. Oden S, Von Hofsten B. 1954. Detection of fingerprints by the ninhydrin reaction. *Nature* 173:449 - 50
79. Morris JR. 1974. NFN - An improved ninhydrin reagent for detection of latent fingerprints. *Police Research Bulletin* 24:45 - 53
80. Pounds CA. 1988. Development in fingerprint visualisation. *Forensic Science Progress* 3:93 - 119
81. Wilkinson D. 2000. Study of the reaction mechanism of 1,8-diazafluoren-9-one with the amino acid, L-alanine. *Forensic Science International* 109:87-103
82. Hewlett DF, Sears VG, Suzuki S. 1997. Replacements for CFC113 in the ninhydrin process: Part 2. *Journal of Forensic Identification* 47:300 - 6
83. Wilkinson D. 2000. Spectroscopic study of 1,2-indandione. *Forensic Science International* 114:123-32
84. Herod DW, Menzel ER. 1982. Laser detection of latent fingerprints: ninhydrin. *Journal of Forensic Sciences* 27:200 - 4
85. Kobus HJ, Stoilovic M, Warrener RN. 1983. A simple luminescent post-ninhydrin treatment for the improved visualisation of fingerprints on documents in cases where ninhydrin alone gives poor results. *Forensic Science International* 22:161-70
86. Liberti A, Calabrò G, Chiarotti M. 1995. Storage effects on ninhydrin-developed fingerprints enhanced by zinc complexation. *Forensic Science International* 72:161-9
87. Pounds CA, Phil M, Grigg R, Mongkolaussavaratana T. 1990. The use of 1,8-diazafluoren-9-one (DFO) for the fluorescent detection of latent fingerprints on paper. A preliminary evaluation. *Journal of Forensic Sciences* 35:169 - 75
88. Conn C, Ramsay G, Roux C, Lennard C. 2001. The effect of metal salt treatment on the photoluminescence of DFO-treated fingerprints. *Forensic Science International* 116:117-23

89. Porpiglia N, Bleay S, Fitzgerald L, Barron L. 2012. An assessment of the effectiveness of 5-methylthioninhydrin within dual action reagents for latent fingerprint development on paper substrates. *Science & Justice* 52:42-8
90. Almog J, Klein A, Davidi I, Cohen Y, Azoury M, Levin-Elad M. 2008. Dual fingerprint reagents with enhanced sensitivity: 5-methoxy- and 5-methylthioninhydrin. *Journal of Forensic Sciences* 53:364-8
91. Joullie MM, Thompson TR. 1991. Ninhydrin and ninhydrin analogs. Syntheses and applications. *Tetrahedron Letters* 47:8791 - 830
92. Chan J, Shimmon R, Spindler X, Maynard P, Lennard C, et al. 2010. An investigation of isatin as a potential reagent for latent fingermark detection on porous surfaces. *Journal of Forensic Identification* 60:320 - 36
93. Hauze DB, Petrovskaia OG, Taylor BM, Joullie MM, Ramotowski RS, Cantu AA. 1998. 1,2-Indanediones: new reagents for visualizing the amino acid components of latent prints. *Journal of Forensic Sciences* 43:744 - 7
94. Roux C, Jones N, Lennard C, Stoilovic M. 2000. Evaluation of 1,2-indanedione and 5,6-dimethoxy-1,2-indanedione for the detection of latent fingerprints on porous surfaces. *Journal of Forensic Sciences* 45:761 - 9
95. Gardner SJ, Hewlett DF. 2003. Optimization and initial evaluation of 1,2-indanedione as a reagent for fingerprint detection. *Journal of Forensic Sciences* 48:1288 - 92
96. Fish FT, Miller LS, Braswell MC, Wallace EW. 2013. *Crime scene investigation*. Oxford, United Kingdom: Elsevier
97. Jelly R, Patton ELT, Lennard C, Lewis SW, Lim KF. 2009. The detection of latent fingermarks on porous surfaces using amino acid sensitive reagents: a review. *Analytica Chimica Acta* 652:128-42
98. Wiesner S, Springer E, Sasson Y, Almog J. 2001. Chemical development of latent fingerprints: 1,2-indanedione has come of age. *Journal of Forensic Sciences* 46:1082 - 4
99. Wallace-Kunkel C, Lennard C, Stoilovic M, Roux C. 2007. Optimisation and evaluation of 1,2-indanedione for use as a fingermark reagent and its application to real samples. *Forensic Science International* 168:14-26
100. Almog J, Levinton-Shamuilov G, Cohen Y, Azoury M. 2007. Fingerprint reagents with dual action: color and fluorescence. *Journal of Forensic Sciences* 52:330-4

101. Bicknell DE, Ramotowski RS. 2008. Use of an optimized 1,2-indanedione process for the development of latent prints*. *Journal of Forensic Sciences* 53:1108-16
102. Ramotowski RS, Cantu AA, Joullie MM, Petrovskaia OG. 1997. 1,2-Indanediones: a preliminary evaluation of a new class of amino acid visualizing compounds. *Fingerprint Whorld* 23:131 - 40
103. Almog J, Glasner H. 2010. Ninhydrin thiohemiketals: basic research towards improved fingermark detection techniques employing nano-technology*. *Journal of Forensic Sciences* 55:215-20
104. Alaoui IM, Troxler T, Joullie MM. 2012. Fingerprint visualization and spectroscopic properties of 1,2-indanedione-alanine followed by zinc chloride or europium chloride. *Journal of Forensic Identification* 62:1 - 13
105. Petrovskaia O, Taylor BM, Hauze DB, Carroll PJ, Joullié MM. 2001. Investigations of the reaction mechanisms of 1,2-indanediones with amino acids. *Journal of Organic Chemistry* 66:7666-75
106. Spindler X, Shimmon R, Roux C, Lennard C. 2011. The effect of zinc chloride, humidity and substrate on the reaction of 1,2-indanedione-zinc with amino acids in latent fingermark secretions. *Forensic Science International* 212:150 - 7
107. Alaoui IM, Menzel ER, Farag M, Cheng KH, Murdock RH. 2005. Mass spectra and time-resolved fluorescence spectroscopy of the reaction product of glycine with 1,2-indanedione with methanol. *Forensic Science International* 152:215 -9
108. Ramotowski RS. 2012. Amino acid reagents. In *Lee and Gaensslen's Advances in Fingerprint Technology, Third Edition*, ed. RS Ramotowski. Boca Raton, Florida: CRC Press
109. Chan J, Spindler X, Brust A, Shimmon R, Maynard P, et al. 2010. Evaluation of DFO and 1,2-indanedione formulations under two different Australian conditions. *Science & Justice* 50:38
110. Sears V, Batham R, Bleay S. 2009. The effectiveness of 1,2-indandione-zinc formulations and comparison with HFE-based 1,8-diazafluoren-9-one for fingerprint development. *Journal of Forensic Identification* 59:654-78
111. Joseph A. 2012. Ninhydrin and Ninhydrin Analogues. In *Lee and Gaensslen's Advances in Fingerprint Technology, Third Edition*, ed. R Ramotowski. Boca Raton, Florida: CRC Press
112. Alaoui IM, Menzel ER, Farag M, Cheng KH, Murdock RH. 2005. Mass spectra and time-resolved fluorescence spectroscopy of the reaction product of glycine with 1,2-indanedione in methanol. *Forensic Science International* 152:215-9

113. Almog J, Cohen Y, Azoury M, Hahn TR. 2004. Genipin - a novel fingerprint reagent with colorimetric and fluorogenic activity. *Journal of Forensic Sciences* 49:255 - 7
114. Ramos-de-la-Peña AM, Renard CMGC, Wicker L, Montañez JC, García-Cerda LA, Contreras-Esquivel JC. 2014. Environmental friendly cold-mechanical/sonic enzymatic assisted extraction of genipin from genipap (Genipa americana). *Ultrasonics Sonochemistry* 21:43-9
115. Lee S-W, Lim J-M, Bhoo S-H, Paik Y-S, Hahn T-R. 2003. Colorimetric determination of amino acids using genipin from Gardenia jasminoides. *Analytica Chimica Acta* 480:267-74
116. Winotapun W, Opanasopit P, Ngawhirunpat T, Rojanarata T. 2013. One-enzyme catalyzed simultaneous plant cell disruption and conversion of released glycoside to aglycone combined with in situ product separation as green one-pot production of genipin from gardenia fruit. *Enzyme and Microbial Technology* 53:92-6
117. Muzzarelli RAA. 2009. Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids. *Carbohydrate Polymers* 77:1-9
118. Touyama R, Inoue K, Takeda Y, Yatsuzuka M, Ikumoto T, et al. 1994. Studies on the blue pigments produced from genipin and methylamine. II. On the formation mechanisms of brownish-red intermediates leading to the blue pigment formation. *Chemical & Pharmaceutical Bulletin* 42:1571-8
119. Fujikawa S, Fukui Y, Koga K. 1987. Structure of genipocyanin G1, a spontaneous reaction product between genipin and glycine. *Tetrahedron Letters* 28:4699 - 700
120. Fujikawa S, Nakamura S, Koga K. 1988. Genipin, a new type of protein crosslinking reagent from gardenia fruits. *Agricultural Biology and Chemistry* 52:869 - 70
121. Kirkland D, Marzin D. 2003. An assessment of the genotoxicity of 2-hydroxy-1,4-naphthoquinone, the natural dye ingredient of Henna. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 537:183-99
122. Gallo FR, Multari G, Giambenedetti M, Federici E. 2008. Chemical fingerprinting of *Lawsonia inermis* L. using HPLC, HPTLC and densitometry. *Phytochemical Analysis* 19:550-9
123. Singh DK, Luqman S, Mathur AK. 2015. *Lawsonia inermis* L. - A commerically important primaeval dying and medicinal plant with diverse pharmacological activity: a review. *Industrial Crops and Products* 65:269-86

124. Rahmoun NM, Boucherit-Atmani Z, Benabdallah M, Boucherit K, Villemin D, Choukchou-Braham N. 2013. Antimicrobial activites of the henna extract and some synthetic naphthoquinones derivatives. *American Journal of Medical and Biological Reserach* 1:16-22
125. Cofrancesco AJ. 2009. Dyes, Natural. In *Kirk-Othmer Encyclopedia of Chemical Technology*, pp. 1 - 30: John Wiley & Sons, Inc.
126. Kraeling MEK, Bronaugh RL, Jung CT. 2007. Absorption of lawsone through human skin. *Cutaneous and Ocular Toxicolgy* 26
127. Jelly R, Lewis SW, Lennard C, Kieran FL, Almog J. 2008. Lawsone: a novel reagent for the detection of latent fingermarks on paper surfaces. *Chemical Communications* 30:3513-5
128. Hernando D, Saurina J, Hernández-Cassou S. 1999. Liquid chromatographic determination of aniline in table-top sweeteners based on pre-column derivatization with 1,2-naphthoquinone-4-sulfonate. *Journal of Chromatography A* 859:227-33
129. Hlabangana L, Hernández-Cassou S, Saurina J. 2006. Determination of biogenic amines in wines by ion-pair liquid chromatography and post-column derivatization with 1,2-naphthoquinone-4-sulphonate. *Journal of Chromatography A* 1130:130-6
130. Latorre RM, Hernandez-Casso S, Saurina J. 2001. Strategies for in-capillary derivatization of amino acids in capillary electrophoresis using 1,2-naphthoquinone-4-sulfonate as labeling reagent. *Journal of Chromatography A* 934:105 - 12
131. Saurina J, Hernández-Cassou S. 1994. Determination of amino acids by ion-pair liquid chromatography with post-column derivatization using 1,2-naphthoquinone-4-sulfonate. *Journal of Chromatography A* 676:311-9
132. Saurina J, Hernández-Cassou S. 1996. Chromatographic determination of amino acids by pre-column derivatization using 1,2-naphthoquinone-4-sulfonate as reagent. *Journal of Chromatography A* 740:21-30
133. Jelly R, Lewis SW, Lennard C, Lim KF, Almog J. 2010. Substituted naphthoquinones as novel amino acid sensitive reagents for the detection of latent fingermarks on paper surfaces. *Talanta* 82:1717 - 24
134. Thomas P, Farrugia K. 2013. An investigation into the enhancement of fingermarks in blood on paper with genipin and lawsone. *Science & Justice* 53:315-20

135. Jelly R. 2011. *Natural products as novel reagents for the detection of latent fingermarks on porous surfaces*. Curtin University of Technology
136. Xu F, Zou L, Ong CN. 2010. Experiment-originated variations, and multi-peak and multi-origination phenomena in derivatization-based GC-MS metabolomics. *Trends in Analytical Chemistry* 29:269-80
137. Kopka J. 2006. Current challenges and developments in GC-MS based metabolite profiling technology. *Journal of Biotechnology* 124:312-22
138. Ashnagar A, Shiri A. 2011. Isolation and characterization of 2-hydroxy-1,4-naphthoquinone (lawsone) from the powdered leaves of henna plant marketed in Ahwaz city of Iran. *International Journal of Chem Tech Research* 3:1941-4
139. Petraco NDK, Proni G, Jackiw JJ, Sapse A-M. 2006. Amino acid alanine reactivity with the fingerprint reagent ninhydrin. A detailed ab initio computational study. *Journal of Forensic Sciences* 51:1267-75
140. Kavitha Rani PR, Fernandez A, George A, Remadevi VK, Sudarsanakumar MR, et al. 2015. Synthesis, spectral characterization, molecular structure and pharmacological studies of N'-(1,4-naphtho-quinone-2yl)isonicotinohydrazide. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 135:1156-61
141. Monks TJ, Hanzlik RP, Cohen G, Ross D, Graham DG. 1992. Quinone chemistry and toxicology. *Toxicological and Applied Pharmacology* 111:2-16
142. Frechard A, Fabre N, Pean C, Montaut S, Fauvel M, et al. 2001. Novel indole-type glucosinolates from woad (*Isatis tinctoria* L.). *Tetrahedron Letters* 42:9015 - 7
143. Clark RJH, Cooksey CJ, Daniels MAM, Withnall R. 1993. Indigo, woad, and Tyrian Purple important vat dyes from antiquity to the present. *Endeavour* 17:191 - 9
144. Baranyovits FLC. 1978. Cochineal carmine: an ancient dye with a modern role. *Endeavour* 2:85 - 92
145. Fieser LF. 1930. The discovery of synthetic alizarin. *Journal of Chemical Education* 7:2609 - 33
146. Loyson P. 2010 Chemistry in the time of the pharaohs. *Journal of Chemical Education* 88:146 - 50
147. Abraham I, Joshi R, Pardasani P, Pardasanim RT. 2011. Recent advances in 1,4-benzoquinone chemistry. *Journal of Brazilian Chemical Society* 22:385-421

148. Koskinen AMP. 2012. *Asymmetric synthesis of natural products*. West Sussex, United Kindom: John Wiley & Sons, Ltd
149. Phillips M. 1929. The chemistry of anthraquinone. *Chemical Reviews* 6:157-74
150. Klipstein KH. 1926. The development of synthetic anthraquinone. *Industrial & Engineering Chemistry* 18:1327-9
151. Brightman R. 1956. Perkin and the dyestuffs industry. *Nature* 177:815-21
152. Holme I. 2006. Sir William Henry Perkin: a review of his life, work and legacy. *Coloration Technology* 122:235-51
153. Caro Y, Anamale L, Fouillaud M, Laurent P, Petit T, Dufosse L. 2012. Natural hydroxyanthraquinoid pigments as potent food grade colorants: an overview. *Natural Products and Bioprospecting* 2:174 - 93
154. Shahid M, Shahid ul I, Mohammad F. 2013. Recent advancements in natural dye applications: A review. *Journal of Cleaner Production* 53:310-31
155. Ran N, Knop DR, Draths KM, Frost JW. 2001. Benzene-free synthesis of hydroquinone. *Journal of the American Chemical Society* 123:10927-34
156. Champod C, Lennard C, Margot P, Stoilovic M. 2004. The friction ridge identification process. In *Fingerprints and other ridge skin impressions*. Boca Raton, Florida: CRC Press
157. Biamonte MA, Wanner J, Le Roch KG. 2013. Recent advances in malaria drug discovery. *Bioorganic & Medicinal Chemistry Letters* 23:2829-43
158. Gupta AK, Gover MD, Nouri K, Taylor S. 2006. The treatment of melasma: a review of clinical trials. *Journal of the American Academy of Dermatology* 55:1048-65
159. Rendon M, Berneburg M, Arellano I, Picardo M. 2006. Treatment of melasma. *Journal of the American Academy of Dermatology* 54:S272-S81
160. Prati F, Bartolini M, Simoni E, De Simone A, Pinto AC, et al. 2013. Quinones bearing non-steroidal anti-inflammatory fragments as multitarget ligands for Alzheimer's disease. *Bioorganic & Medicinal Chemistry Letters* 23:6254-8
161. Ravichandiran P, Kannan R, Ramasubbu A, Mauthusubramanian S, Samuel VK. 2012. Green synthesis of 1,4-quinone derivatives and evaluation of their fluorescent and electrochemical properties. *Journal of Saudi Chemical Society* 12:S93 - S9

162. Uchimiya M, Stone AT. 2009. Reversible redox chemistry of quinones: Impact on the biogeochemical cycles. *Chemosphere* 77:451 - 8
163. Kim RS, Park W, Hong H, Chung TD, Kim S. 2014. Quinone electrochemistry altered by local hydrophobic environment and hydrogen bonding interactions. *Electrochemistry Communications* 41:39 - 43
164. Ryan DE, Meyerhof TP, Fairhurst MT. 1976. Quinone-cyanide reactions: their use for the determination of quinones and cyanide. *Analytical Chimica Acta* 86:195 - 201
165. Wang HU, Xu LX, Xiao Y, Han J. 2004. Spectrophotometric determination of dapsone in pharaceutical products using sodium 1,2-naphthoquinone-4-sulfonic as the chromatographic reagent. *Spectrochimica Acta Part A* 60:2933 - 9
166. Bolzan AD, Bianchi MS. 2001. Gentoxicity of streptonigrin: a review. *Mutation Research* 488:25 - 37
167. Bringmann G, Reichert Y, Kane VV. 2004. The total synthesis of streptonigrin and related antitumor antibiotic natural products. *Tetrahedron* 60:3539 - 74
168. Agudelo D, Bourassa P, Berube G, Tajmir-Riahi HA. 2016. Review on the binding of anticancer drug doxorubicin with DNA and tRNA: Structural models and antitumor activity. *Journal of Photochemistry & Photobiology, B: Biology* 158:274 - 9
169. Mitry MA, Edwards JG. 2016. Doxorubicin induced heart failure: phenotype and molecular mechanisms. *IJC Heart & Vasculature* 10:17 - 24
170. Golunski G, Borowik A, Derewonko N, Kawiak A, Rychlowski M, et al. 2016. Pentoxifylline as a modulator of anticancer drug doxorubicin. Part II: reduction of doxorubicin DNA binding and alleviation of its biological effects. *Biochimie* 123:95 - 102
171. Wang J, Ozsoz M, Cai X, Rivas G, Shiraishi H, et al. 1998. Interactions of antitumor drug daunomycin with DNA in solution and at the surface. *Bioelectrochemistry and Bioenergetics* 45:33 - 40
172. Rabbani A, Davoodi J. 1994. Effects of anthracycline antibiotic, daunomycin on thymus chromatin: the role of chromosomal proteins. *General Pharmacology: The Vascular System* 25:787 - 93
173. Ford L, Rayner CM, Blackburn RS. 2015. Isolation and extraction of ruberythric acid from *Rubia tinctorum* L. and crystal structure elucidation. *Phytochemistry* 117:168 - 73
174. Dufosse L. 2014. Anthraquinones, the Dr Jekyll and Mr Hyde of the food pigment family. *Food Research International* 65:132 - 6

175. Nekouei F, Nekouei S, Tyagi I, Gapta VK. 2015. Kinetic thermodynamic and isotherm studies for acid blue 129 removal from liquids using copper oxie nanoparticle-modified activated carbon as a novel adsorbent. *Journal of Molecular Liquids* 201:124 - 33
176. Feng G, Quia HF, Bai G, Liu YC, Hu LL. 2016. Synthesis, characterization, and application of diester/diurethane tethered azo disperse dyes: a new strategy to improve dye's fastness properties. *Dyes and Pigments* 129:54 - 9
177. Chakraborty JN. 2014. Fundamentals and practices in colouration of textiles. New Delhi: CRC Press
178. Borges ME, Tejera RL, Diaz L, Esparza P, Ibanez E. 2012. Natural dye extraction from cochineal (*Dactylopius coccus*). New extraction methods. *Food Chemistry* 132:1855 - 60
179. Chieli A, Sanyova J, Doherty B, Brunetti BG, Miliani C. 2016. Chromatographic and spectroscopic identification and recognition of *ammoniacal cochineal* dyes and pigments. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 162:86 - 92
180. Hussain H, Krohn K, Ahmad VU, Miana GA, Green IV. 2007. Lapachol: an overivew. *Arkivoc* 2:145 - 71
181. Boga C, Depivo C, Ballarin B, Morigi M, Galli S, et al. 2013. Investigation on the dyeing power of some organic natural compounds for a green approach to hair dyeing. *Dyes and Pigments* 97:9 -18
182. Mallavadhani UV, Prasad CV, Shrivastava S. 2014. Synthesis and anticancer of some novel 5,6-fused hybrids of juglone based 1,4-naphthoquinones. *European Journal of Medicinal Chemistry* 83:84 - 91
183. Seshadri P, Rajaram A, Rajaram R. 2011. Plumbagin and juglone induce caspase-3-dependent apoptosis involving the mitochondria through ROS generation in human peripheral blood lymphocytes. *Free Radical Biology and Medicine* 51:2090 - 107
184. Kurien KC, Robins PA. 1970. Photolysis of aqueous solutions of *p*-benzoquinone: a spectrophotometric investigation. *Journal of Chemical Society B: Physical Organic*:855-9
185. Brunmark A, Cadenas E. 1988. Reductive addition of glutathione to *p*-benzoquinone, 2-hydroxy-*p*-benzoquinone, and *p*-benzoquinone epoxides. Effect of the hydroxy- and glutathionyl substitents on *p*-benzohydroquinone autoxidation. *Chemico-Biological Interactions* 68:273 - 98

186. Schuchmann MN, Bothe E, von Sonntag J, von Sonntag C. 1998. Reaction of OH radicals with benzoquinone in aqueous solutions. A pulse radiolysis study. *Perkin Transactions 2*:791-6
187. Armstrong S, Patel TR, Whalen M. 1993. Detoxification mechanisms for 1,2,4-benzenetriol employed by a *Rhodococcus* sp. BPG-8. *Archives of Microbiology* 159:136-40
188. Jackson H. 1939. CLXXVII. The oxidation of catechol and 1,2,4-trihydroxybenzene by polyphenol oxidase. *Biochemical Journal* 33:1452-9
189. Corbett JF. 1967. The chemistry of hydroxyquinones. Part II. The autoxidation of 3,6-dimethylbenzene-1,2,4-triol. *Journal of the Chemical Society C*:611 - 20
190. Fieser JF, Ardao MI. 1956. Investigation of the chemical nature of gonyleptidine. *Journal of the American Chemical Society* 78:774 - 81
191. Chibber SS, Dutt SL. 1982. Oxidative decarbonylation of phenolic acids by singlet oxygen. *Indian Journal of Chemistry, Section B.* 21B:352 - 3
192. Haines A. 2012. Methods for oxidation of organic compounds V2: alcohol, alcohol derivatives, alky halides, nitroalkanes, alkyl azides, carbonyl compounds hydroxyarenes and aminoarenes. Elsevier
193. Tojo G, Fernandez M. 2006. Fetizon's reagent: silver carbonate on celite. In *Oxidation of Alcohols to Aldehydes and Ketones: A Guide to Current Common Practice*, ed. G Tojo, pp. 281 - 8 New York: Springer
194. Kakis FJ, Fetizon M, Douchkine N, Golfier M, Mourgues P, Prange T. 1974. Mechanistic studies regarding the oxidation of alcohols by silver carbonate on celite. *Journal of Organic Chemistry* 39:523 - 33
195. Smith MB. 2016. *Organic synthesis*. London, United Kingdom: Academic Press, Elsevier Inc.
196. Sutter P, Weis CD. 1985. The chlorination of 1,4-dihydroxyanthraquinone with thionyl chloride. *Dyes and Pigments* 6:435-43
197. Green A. 1926. CLXXXVI.—The action of thionyl chloride on hydroxyanthraquinones. Part II. Quinizarin. *Journal of the American Chemical Society (Resumed)*:1428 - 37
198. Hua DH, Tamura M, Huang X, Stephany HA, Helfrich BA, et al. 2002. Syntheses and bioactivities of substituted 9,10-dihydro-9,10-[1,2]benzenoanthracene-1,4,5,8-tetrones. Unusual

reactivities with amines. *Journal of Organic Chemistry* 67:2907-12
199. Wang Z. 2010. *Thiele-Winter acetoxylation*. John Wiley & Sons, Inc.
200. Mundy BP, Ellerd MG, Favaloro Jr F, .G. 2005. Name reactions and reagents in organic synthesis. John Wiley & Sons Inc.
201. McOmie JFW, Blatchly JM. 2011. The Thiele-Winter acetoxylation of quinones. *Organic Reactions* 19:199 - 277
202. Spyroudis S, Xanthopoulou N. 2002. Triptycene quinones in synthesis: preparation of triptycene cyclopentenedione and its reactivity as a dienophile. *The Journal of Organic Chemistry* 67:4612-4
203. Villemin D, Bar N, Hammadi M. 1997. Triflic acid an efficient catalyst for the Thiele-Winter reaction. *Tetrahedron Letters* 38:4777-8
204. Yadav JS, Reddy BVS, Swamy T, Rao KR. 2004. Bi(OTf)3-catalyzed acylation of p-quinones: a facile synthesis of acylated hydroquinones. *Tetrahedron Letters* 45:6037-9
205. Villemin D, Bar N, Hammadi M, Hachemi M. 2000. Liquid and solid acids as catalysts for the Thiele-Winter reaction of menadione. *Journal of Chemical Research (S)*:356 - 85
206. Vliet EB. 1941. Hydroxyhydroquinone triacetate. *Organic Syntheses* 1:317 - 8
207. Ahluwalia VK, Aggarwal R. 2000. *Preparations: hydroxyhydroquinone triacetate*. New Delhi: University Press (India) Private Limited
208. Fieser LF. 1948. Naphthoquinone antimalarials. III. Diene synthesis of 1,4-naphthoquinones. *Journal of Organic Chemistry* 70:3165 - 74
209. Fieser LF. 1926. The alkylation of hydroxynaphthoquinone I. Ortho-ethers. *Journal of the American Chemical Society* 48:2922-37
210. Fieser LF. 1928. The tautomerism of hydroxy quinones. *Journal of the American Chemical Society* 50:439-65
211. Fieser LF. 1928. 2-Hydroxy-1,4-anthraquinone. *Journal of the American Chemical Society* 50:465-74
212. Fieser LF, Fieser M. 1935. A new diene synthesis of anthraquinones. *Journal of the American Chemical Society* 57:1679-81
213. Fieser LF, Sachs DH. 1968. Structure of the diquinone resulting from oxidation of .beta.-naphthoquinone. *Journal of the American Chemical Society* 90:4129-33

214. Fieser LF, Martin EL. 1955. 2-Hydroxy-1,4-naphthoquinone. *Organic Syntheses* 21:465 - 7
215. Fellows TJ, Hughes G. 1972. The radiation-induced oxidation of aqueous solutions of hydroquinone. *Journal of the Chemical Society, Perkins Transaction 2*:1182 - 5
216. Philipp B, Schink B. 1998. Evidence of two oxidative reaction steps initiating anaerobic degradation of resorcinal (1,3-dihydroxybenzene) by the denitrifying bacterium azoarcus anaerobius. *Journal of Bacteriology* 180:3644 - 9
217. Wheeler AS, Scott JW. 1919. The halogenation of juglone: a new type of naphthalene dyes. *Journal of the American Chemical Society* 41:833 - 41
218. Avendano C, Balci M, Böker N, Couladouros EA, Echavarren AM. 2014. Science of synthesis: Houben-Weyl methods of molecular transformations. Volume 28: quinones and heteroatom analogues. ed. E Schaumann. Stuttgart: Georg Thieme Verlag KG
219. Lou K. 2007. *Syntheses of novel antitumor 1,4-anthracenediones and functionized cyclododeciptycene based molecular gears*. Kansas State University
220. Nor SM, Sukari MA, Azziz SS, Fah WC, Alimon H, Juhan SF. 2013. Synthesis of new cytotoxic aminoanthraquinone derivatives via nucleophilic substitution reactions. *Molecules* 18:8046 - 62
221. Fieser LF, Martin EL. 1944. 2-Hydroxy-1,4-naphthoquinone. *Organic Syntheses* 3:465 - 58
222. Singh MW, Karmakar A, Barooah N, Baruah JB. 2007. Variations in product in reactions of naphthoquinone with primary amines. *Beilstein Journal of Organic Chemistry* 3
223. Schmidt EG. 1939. Reaction between amines and sodium 1,2-naphthoquinone-4-sulfonate. *Industrial & Engineering Chemistry Analytical Edition* 11:99 - 100
224. Cranwell PA, Haworth RD. 1971. Humic Acid - IV. The reaction of alpha-amino acid esters with quinones. *Tetrahedron* 27:1831 - 7
225. Jali BE, Masud K, Baruah JB. 2013. Selectivity in changes of fluorescence emission of 1,4-naphthoquinone derivatives by manganese and cadmium ions. *Polyhedron* 51:75 - 81
226. Li Q, Li J, Yang Z. 2007. Study of the sensitization of tetradecyl benzyl dimethyl ammonium chloride for the spectrophotometric determination of dopamine hydrochloride using sodium 1,2-naphthoquinone-4-sulfonate as the chemical derivative chromogenic reagent. *Analytica Chimica Acta* 583:147 - 52
227. Li QM, Yang ZJ. 2007. Spectrophotometric determination of aminomethylbenzoic acid using sodium 1,2-naphthoquinone-4-

sulfonate as the chemical derivative chromogenic reagent. *Spectrochimica Acta Part A* 66:656 - 61
228. Li Q, Zhang T. 2007. Study of the sensitization of tetradecyl benzyl dimethyl ammonium chloride for the color development reaction between lysine and sodium 1,2-naphthoquinone-4-sulfonate and the determination of lysine in pharmaceutical and biological samples. *Spectrochimica Acta Part A* 7:360 - 7
229. Pierpoint WS. 1982. A class of blue quinone-protein coupling products: the allagochromes? *Phytochemistry* 21:91 - 5
230. Spindler X, Stoilovic M, Lennard C, Lennard A. 2009. Spectral variation for reaction products formed between different amino acids and latent fingermark detection reagents on a range of cellulose-based substrates. *Journal of Forensic Identification* 59:308 - 24
231. Hubbe MA, Gill RA. 2016. Fillers for papermaking: a review of their properties, usage practices, and their mechanistic role. *Bioresources* 11:2886 - 963
232. Modica M, Aprea GM, Chiuri A, Zampa F, Lago G. 2014. NIR luminescence for the inspection of thermal paper: a novel tool for fingermarks detection. *Forensic Science International* 244:50 - 6
233. Yu P-H, Wallace MM. 2007. Effect of 1,2-indanedione on PCR-STR typing of fingerprints deposited on thermal and carbonless paper. *Forensic Science International* 168:112-8
234. Stoilovic M. 1993. Improved method for DFO development of latent fingerprints. *Forensic Science International* 60:141-53
235. Merrick S, Gardner SJ, Sears VG, Hewlett DF. 2002. An operational trial of ozone friendly DFO and 1,2-indanedione formulations for latent fingermark detection. *Journal of Forensic Identification* 52:595 - 605
236. Berdejo S, Rowe M, Bond JW. 2011. Latent fingermark development on a range of porous substrates using ninhydrin analogs - a comparison with ninhydrin and 1,8-diazofluoren. *Journal of Forensic Sciences* 57:509-14
237. (IFRG) IFRG. 2014. Guidelines for the assessment of fingermark detection techniques. *Journal of Forensic Identification* 64:174 - 200
238. Chadwick S, Maynard P, Kirkbride P, Lennard C, McDonagh A, et al. 2012. Styryl dye coated metal oxide powders for the detection of latent fingermarks on non-porous surfaces. *Forensic Science International* 219:208 - 14

239. Chadwick S, Maynard P, Kirkbride P, Lennard C, Spindler X, Roux C. 2011. Use of Styryl 11 and STaR 11 for the luminescence enhancement of cyanoacrylate-developed fingermarks in the visible and near-infrared regions. *Journal of Forensic Sciences* 56:1505 - 13