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Original article

REVISITING THE STRUCTURE OF STRYCHNINE AND RELATED COMPOUNDS: AN "INADEQUATE" APPROACH TO WOODWARD AND ROBINSON'S WORK.

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ABSTRACT

Background: The structural elucidation of strychnine and brucine by Woodward and Robinson is a landmark in natural products chemistry. At the time it was done by classic degradation techniques.
Aims: This research was taken place to confirm and advance the findings of original work by innovative scientists such as Woodward and Robinson via modern analysis. As well as investigating older structure elucidation techniques to determine which method has the largest impact on degrading complex alkaloids such as strychnine and brucine, we will apply these compounds to modern NMR experiments such as INADEQUATE.
Methods: a range of degradation techniques such as base hydrolysis, amination, dehydrogenation and oxidation reactions were reproduced and monitored with modern instruments such as EI-LC MS and NMR to deduce structural insights surrounding strychnine's degradant products. The modern equipment was also used to further current data surrounding strychnine via running elaborate 2D NMR procedures such as 13C-13C INADEQUATE.
Results: dehydrogenation via bromination and sulfonation had the greatest influence on degrading strychnine. NMR experiments revealed molecular relationships between the carbons to be able to piece together the skeletal backbone of the compound.
Conclusion: Overall, the development in modern analysis has been a key turning point in structure determination, further degradations would be running paired with more extensive 2D NMR processes and older methods are effective but are much more beneficial when hybridised with new technology.

Keywords: Alkaloids; Degradation reactions; Mass spectrometry; Nuclear Magnetic Resonance; History of Chemistry

1 INTRODUCTION

1.1 The story of structure elucidation

This chronicle of structure determination spans centuries to get to the current methods used in modern society. It begins with methods such as alchemy and element theory. Alchemy is the process of converting metals into gold, first performed by a range of native ancient native cultures such as the Greeks and Egyptians (Winter, 1932). Slightly later, element theory began to be employed by the Greek philosopher Democritus (460–370 BCE). Democritus suggested that matter is made up of small, unbreakable components known as atoms. Although this latter was deemed as true, his theory experienced a lack of support (G., 1932),

Next came along, the phlogiston theory, which wrongly suggested phlogiston was emitted during the burning of hydrocarbons. However, this proved to be a prevalent theory of combustion and rusting in the 17th and the beginning of the 18th century (Mamlok-Naaman, 2023).

As advancements began to emerge, the 19th century became the era of atomic theory. In this period, Lavoisier disproved the phlogiston hypothesis and created the law of conservation of mass (Hoffmann and Laszlo, 1991).

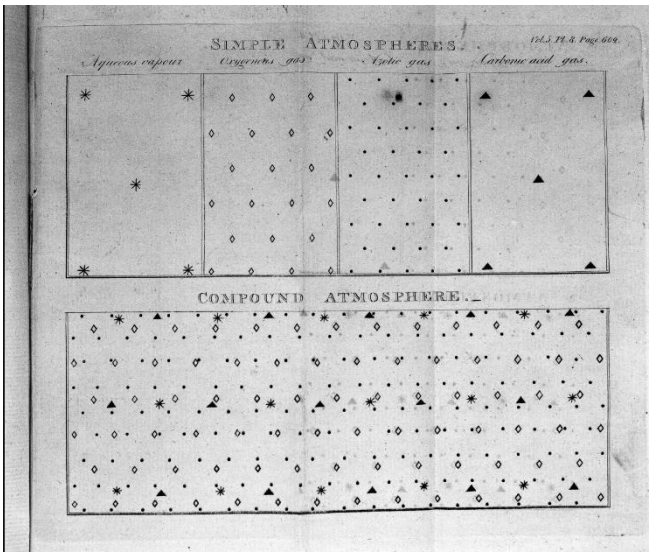


Figure 1. John Dalton's original diagrams for his proposed structure of atomic theory (Science and Industry Museum, 2019).

Shortly after, in 1803, Dalton established that atoms of a single, distinct kind make up each element, and that these atoms are rearranged during chemical processes. A conceptual basis for comprehending chemical composition and reaction was established by this theory (Lappert et al, 2003). Then, Avogadro's constant suggested that the number of molecules in equal quantities of gases at equal temperatures and pressures is equal. The determination of molecular formulae and related molecular masses depended heavily on this theory (Sarikaya, 2011).

Further into the 19th century, structural chemistry began to blossom, starting off with Kekulé proposing the theory of benzene in 1865 (Kekulé, 1865).

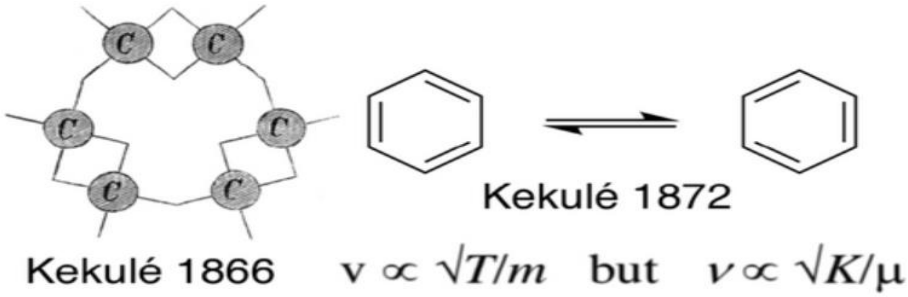


Figure 2. This shows the original structure of benzene, discovered by Kekulé (Wentrup, 2023).

Then, in 1874, the tetrahedral structure of carbon was hypothesised by Jacobus Henricus van der Hoff and Joseph Le Bel, explaining the spatial arrangement of atoms and paving the way for the creation of stereochemistry (Jorge Lindner, 1974).

In the 20th century, is when the switch into more in-depth discoveries on molecular structures began to prevail, with key discoveries made by Robert Woodward and Robert Robinson.

Woodward was one of the most influential chemists who changed the outlook with his vast contributions to structure determination, one of his key discoveries being the elucidation of strychnine in 1947 via degradation methods (Woodward et al, 1947). He also successfully determined the structure of several alkaloids such as quinine (Woodward et al, 1945), as well as steroids, including cholesterol and cortisone (Woodward et al, 1951) and (Woodward et al, 1951) Woodward as well had many contributions within total synthesis and produced the Woodward-Hoffman rules. These rules offer a theoretical foundation for comprehending the stereochemistry of pericyclic reactions. These regulations, which have a significant influence on synthetic organic chemistry and reaction processes, are founded on the ideas of orbital symmetry conservation (Seeman, 2023). Robinson contributed in many ways, his contribution to determine structures of alkaloids, Robinson's rules to understand natural products and his work on total synthesis (Robinson, 1917).

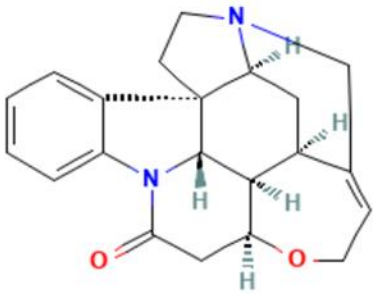


Figure 3. This shows the final accurate structure of strychnine, elucidated by Woodward.

As well as Woodwards, another outstanding chemists who significantly contributed to this field is Linus Pauling He had a vast understanding of quantum mechanics and X-ray crystallography which enabled him to anticipate and identify the structures of numerous complex compounds, such as the triple helix of collagen (Pauling and Corey, 1953) and the alpha helix of proteins (Pauling and Corey, 1951). Dorothy Hodgkin, a leading lady in structure determination, discovered the structures of penicillin, B12 and insulin. She was awarded the Nobel Peace Prize in 1964. Her discoveries had huge impacts on the industry (Hodgkin, 1965).

Biemann and Ernst are two analytical chemists whose work changed the way people deduce structures. Biemann helped to develop mass spectrometry as a critical tool for determining the molecular weights and structures of peptides, proteins, and other organic compounds (Klaus Biemann, 1986). Ernst enhanced two-dimensional NMR and the Fourier transform, significantly boosting the sensitivity and resolution in NMR. It made it possible to analyse complicated molecules' structures in great detail (Ernst, 1992).

In more recent years, Elias James Corey created a number of synthetic techniques and approaches that are now commonly used in organic synthesis. His research on the production of complex compounds such as prostaglandins has had a long-lasting influence on chemistry and pharmacology (Corey et al, 1970). John Bennett Fenn was the founder of ESI, electrospray ionisation. This discovery allowed the analysis of a range of molecules via the m/z (Muddiman, 2011).

This demonstrates how far structure determination has come and how influential this procedure is in the world of science.

1.2 Structure theory

Today, structure determination looks slightly different from decades prior, when these complicated molecules were originally eluded. There are a variety of staple techniques used to deduce the identity of an unknown compound.

1.2.1 NMR

The prominent method used for structure elucidation is nuclear magnetic resonance (NMR), which uses the principle of nuclei with or against a magnetic field. The strength of the magnetic field is dependent on the MHz of the instrument. Where the nuclei lie causes different energy levels. The nuclei are exposed to radiofrequency, resulting in different nuclei absorbing different amounts of energy. This energy is released and correlates as peaks within the spectrum (L. M. K. Vandersypen et al, 2005). Depending on the type of NMR method run, it varies on what can be inferred from the spectrum.

The techniques are split into 1D and 2D; all provide information that can be interpreted to show a sample's structure based on atom-to-atom bonds. 1D techniques, including proton NMR, ^{13}CNMR and DEPT Q, focus us on using a single axis to determine the presence of functional groups within the structure (Jacobsen, 2008). In comparison, 2D techniques such as HSQC, HMBC, COSY, etc., use two axes to evaluate cross-links between nuclei to piece together bonds between individual atoms (P Diehl et al, 1971).

A study carried out by (Molinski, 2010) discusses the importance of NMR, especially for elucidating the small structures of small molecules. Through these methodologies, many structures were successfully discovered.

In the current research, a range of varying NMR techniques was run based on several parameters. The 1D procedures were run due to the investigation by (Seeman et al, 2020). In their tests, they measured how efficient it would be to determine the structure of strychnine with today's technological advancements.

Other techniques, including 15N HMBC, were selected due to the presence of the indole ring, HMBC to investigate carbon-hydrogen bonds and INADEQUATE used to advance the information currently available on strychnine.

Certain groups, such as the methoxy group, require techniques like HMBC to locate the exact position in which they fall within a compound. The long-range coupling allows them to be accurately pieced into the structure. An investigation by (Furrer, 2010) demonstrates how effective the use of HMBC is. It demonstrates the positives when using this technique for complex molecules.

INADEQUATE NMR is an upcoming process, as it provides in-depth data on the carbon skeleton, which makes it particularly useful for complex compounds such as strychnine. Research by (Ismail et al, 2020) examined the success rate on eluding structures of small natural products via INADEQUATE NMR. Several were accurately assigned, therefore making it a well-rounded technique to be used within the current study.

1.2.2 EI LC-MS

Another vital method used is electrospray ionisation liquid chromatography mass spectrometry. EI LC-MS takes 3 separate analytical techniques and combines them to produce a spectrum. Electrospray ionisation is a soft technique that formats the ions from the already separated compounds (Konermann et al, 2012). The liquid chromatography section of the instrument differentiates parts of the sample based on the polarity or size of the fraction (Morley et al, 2021). The mass spectrometer is the section which detects the ions produced from electrospray ionisation (Buchberger et al, 2017).

The analytical technique provides information on the mass-to-charge ratio of the constituents within the sample. The base peak, representing the most abundant constituent within the sample (Palma et al, 2011). A range of parameters, including cone voltage and mode, can be changed to optimise the spectrum.

A study performed by (Cappiello et al, 2011) identifies how advantageous EI LC-MS is for analysing and confirming structural information about small molecules. Therefore, highlighting why this would be an effective method to demonstrate modern analysis in the current investigation.

Another set of experiments carried out by (Gabrielly et al, 2023) and (Seemann et al, 2015) showcase how EI LC-MS is an extremely beneficial method, particularly when within the assessment of molecules with a low molecular weight.

1.2.3 Structure misassignment

The biggest advocate for these advancements in modern analysis is to combat the issue of structure misassignment. Woodward discovered the correct structure of strychnine degradation methods in 1947. After a vigorous 30 years of trial and error of incorrect structures (Woodward et al, 1947)

Misassignment of structure is a frequent problem, especially in natural products, because of misinterpreted spectra or compound complexity. A study conducted by (Nicolaou, et al, 2005) concluded that the frequency of the problem in natural products and the origins of these misassignments. Consequently, it is crucial to use cutting-edge technologies to validate earlier findings.

1.3 Strychnine, a challenging molecule

Strychnine is a naturally occurring, highly intricate molecule which can be extracted from the seeds of the *Strychnos nux vomica* and *Strychnos ignatii* plants (Zlotos et al, 2022). There are several queries surrounding the "most complex molecule known for its size" (Robinson, 1952) as referred to by Robinson. One of the main concerns is the reputation surrounding the poisonous alkaloid.

Although in the current climate the use of strychnine is as a pesticide (Palmateer, 1990), it has, in several cases, been known to be used in a harmful manner. This is due to its severe effect on the human body (Patocka, 2015). In one of the original murder stories, Macbeth, strychnine was the murder weapon of choice to kill his sons (Shakespeare, 1623). In a real-life tale, Chemist Gail Bell explores the story of Macbeth. Then, further down the line, her grandpa, an Australian medicine vendor, is later suspected of poisoning his two boys with strychnine (Bell, 2017, p.276).

Other than being used as poison for humans, there are several other reservations surrounding the compound. In many countries, there are legal restrictions on the alkaloid. As ordered in the USA, there are limits on the quantity that can be used as a pesticide (Colvin et al, 1988). Also, in continents including Africa and Asia, there are strict laws regarding the alkaloid due to its high rates of misuse. In two cases in 1923 and 1926, a woman, Daisy Der Melker, poisoned her husband with strychnine to obtain both of their inheritance's (Grogan, 2016).

Strychnine also poses an environmental threat if not monitored correctly. If any spills occur and are not treated in the right manner leads to risks to wildlife and domestic animals in the area (Rattner et al, 2014) . Therefore, it is a necessity the compound is stored sufficiently and handled with caution.

Evidently, as strychnine is deemed a dangerous compound, many are hesitant to investigate the compound. However, the molecule can provide insight into structural advancements which can be applied when exploring other alkaloids; the molecule must be studied. Strychnine was originally extracted by the renowned French scientists Joseph Bienaimé Caventou and Pierre-Joseph Pelletier from the Saint-Ignatius

bean in 1818 (Pelletier PP, 1819). From these isolation experiments, it was determined that many of these plants contain fewer toxic derivatives of strychnine, such as brucine, that demonstrate similar complexity levels (Rathi et al, 2008).

Through degradations and older techniques carried out in a study by (Clemo, 1936) to examine strychnine atom by atom via NMR (Núria Marcó et al, 2017), LC-MS (Liao et al, 2008), UV-VIS (Show et al, 1978). The structural knowledge about strychnine and other alkaloids is continuously expanding. Currently, the ^{13}C-13C INADEQUATE data is yet to be published, therefore being tackled in the current research to add to Woodward's original work of the structure determination of strychnine (Woodward, 1947).

To advance the available structural information about molecules, different forms of modern analysis can be used. By gaining a deeper understanding of the structure and molecular relationships of compounds, it can help progress areas such as pharmaceuticals, the food industry and agriculture (Cleophas et al, 2017).

The study aims to further the molecular relationships of strychnine while conducting investigations into its structure and stereochemistry. This will be carried out by using 2D NMR techniques such as ^{13}C-13C INADEQUATE to advance the understanding of the molecular connections within the accurate structure of strychnine. Also, by comparing and contrasting older structure elucidation techniques against modern analysis. As well as retrieving structural data of strychnine degradant products via EI LC-MS and NMR.

2 EXPERIMENTAL

2.1 Materials

The materials and reagents used were from two main suppliers. The materials obtained from Sigma Aldrich are as follows: Strychnine – CAS 57-24-9; Brucine – CAS 357-57-3; Pilocarpine hydrochloride – CAS 54-71-7, and 2-methylquinoline – CAS 91-63-4. The reagents obtained from Sigma Aldrich were: Sodium hydroxide – CAS 1310-72-2; Barium hydroxide – CAS 12230-71-6; Diphenyl ether – CAS 101-84-8; Chromic acid – CAS 7738-94-5; Methylene blue – CAS 61-73-4; Potassium carbonate – CAS 584-08-7; Phosphoric tungstic acid – CAS 12501-23-4; Phenyl diamine (ortho) – CAS 95-54-5; Phenyl diamine (meta) – CAS 108-45-2 and Boric acid – CAS 10043-35-3. The reagents obtained from Fischer Scientific were: Deionised water – CAS 7732-18-5; Methanol – CAS 67-56-; Methanol LC-MS grade – CAS 67-56-1; Ethanol –CAS 64-17-5; Acetone – CAS 67-64-1; Dichloromethane – CAS 75-09-2; Sulfur flowers – CAS 7704-34-9; Acetonitrile – CAS 75-05-8; Potassium permanganate – CAS 7722-64-7; Hydrogen peroxide – CAS 7722-84-1; Morphaniline – CAS 110-91-8 and t-butyl amine – CAS 75-64-9.

2.1.1 Instrumental

Nuclear Magnetic Resonance – Bruker Ascend 600 MHz

Electrospray Ionisation Liquid chromatography Mass Spectrometry – LCT Premier – The Benchtop Exact Mass MS Solution.

2.2 Methodology

2.2.1 Degradation

2.2.1.1 Base hydrolysis of strychnine

Barium hydroxide

A 10 ml vial and mini stirrer bar were weighed and recorded; 100mg of strychnine was then added. To the vial, 1 molar eq (50 mg) of $Ba(OH)_2$ was added and stirred on a magnetic stirrer plate at 300 rpm for 1 week. Once stirring was complete, the sample was run on EI LC-MS for analysis.

Barium hydroxide + water

A 10 ml vial and mini stirrer bar were weighed and recorded, 100mg of strychnine and 5ml of deionised water were then added. To the vial, 1 molar eq (50 mg) of $Ba(OH)_2$ was added and stirred on a magnetic

stirrer plate at 300 rpm for 1 week. Once stirring was complete, the sample was run on EI LC-MS for analysis.

Barium hydroxide + water refluxed

A 50 ml round-bottom flask and mini stirrer bar were weighed and recorded, 100mg of strychnine and 25ml of deionised water were then added. To the flask, 1 molar eq (50 mg) of $Ba(OH)_2$ was added. The solution was refluxed at 100℃ and stirred on a magnetic stirrer plate at 300 rpm for 72 hours. Once stirring was complete, the sample was run on EI LC-MS for analysis.

Barium hydroxide + ethanol refluxed.

A 50 ml round-bottom flask and a mini stirrer bar were weighed and recorded. 100mg of strychnine and 25ml of ethanol were then added. To the flask, 1 molar eq (50 mg) of $Ba(OH)_2$ was added. The solution was refluxed at 100℃ and stirred on a magnetic stirrer plate at 300 rpm for 72 hours. Once stirring was complete, the sample was run on EI LC-MS for analysis.

Sodium hydroxide

A 10 ml vial was weighed and recorded, and a mini stirrer bar, 100mg of strychnine, was then added. To the vial, 2 molar eq 100 mg of NaOH was grinded then added and stirred on a magnetic stirrer plate at 300 rpm for 1 week. Once stirring was complete, the sample was run on EI LC-MS for analysis.

2.2.1.2 Base hydrolysis of brucine

Sodium hydroxide

A 10 m vial and a mini stirrer bar were weighed and recorded. 100mg of Brucine was then added. To the vial, 2 molar eq 100 mg of NaOH was grinded then added and stirred on a magnetic stirrer plate at 300 rpm for 1 week. Once stirring was complete, the sample was run on EI LC-MS for analysis.

All base hydrolysis was based on experiments studied by (Robinson-Fuentes et al, 1997) and (Patel et al., 2022)

2.2.1.3 Dehydrogenation with bromine

Reaction 1

A 10 ml vial and mini stirrer bar were weighed and recorded; equal moles of bromine (159 mg) and strychnine (334 mg) were added. 0.7 ml of $CDCl_3$ was taken up via a Pasteur pipette, then filtered through a glass pipette and paper. Once filtered, $CDCl_3$ was transferred into the vial. The reaction was then heated and stirred on a magnetic stirrer plate at 250 rpm until a colour change occurred from brown to colourless, which confirmed the presence of a double bond.

0.1 ml of this sample was transferred into a clean NMR tube, and a further 0.6 ml of filtered $CDCl_3$ was added dropwise to the NMR tube. The sample was run on a 600 MHz NMR instrument for analysis.

The rest of the sample was continuously stirred and heated for a further 24 hours to allow full bromination of strychnine to occur. Once stirring was complete, the sample was run on EI LC-MS for analysis.

Reaction 2

A 10 ml vial and mini stirrer bar were weighed and recorded; 668 mg of strychnine was added. The strychnine vial was placed in an ice bath. To the vial, equal moles of bromine (320 mg) were added dropwise via a dropping funnel (an average of 43 drops across 30 seconds) over 15 minutes. The reaction was then stirred on a magnetic stirrer plate at 250 rpm for 24 hours. Once stirring was complete, the sample was run on EI LC-MS.

This method followed similar processes that were performed by Berti and Marsili (1966).

2.2.1.4 Dehydrogenation with sulfur

A 10 ml vial and mini stirrer bar were weighed and recorded; 334 mg of strychnine was then added. To the vial, 1 molar equivalence of Sulfur flowers (16 mg) and 25 ml of diphenyl ether were added. The reaction

was placed in a sand bath and stirred on a magnetic stirrer at 300 rpm until a visible colour change occurred, from pale yellow to deep yellow/ brown. Once stirring was complete, the sample was run on EI LC-MS for analysis.

This reaction was carried out a further 4 times, each time the molar equivalence of Sulfur flowers was increased by 1 equivalence. Reaction 2 used 2 equivalents (32 mg), reaction 3 used 3 equivalents (48 mg), reaction 4 used 4 equivalents (64 mg) and reaction 5 used 5 equivalents (80 mg).

This method was based upon a procedure investigated by (Wang et al., 2020).

2.2.1.5 Chromic acid

334 mg of strychnine was added to a 250ml conical flask along with 100 ml of water. The reaction was heated and stirred until the reaction reached 60 degrees, which was measured with a 300℃ thermometer. 3 molar equivalence (900 mg) of potassium dichromate was then added to the preheated solution. The reaction was heated and stirred at 300 rpm until the temperature reached 100 ℃. The reaction was then taken off the heat and stirred for another hour.

After stirring for an hour, the solution was re-boiled and then left aside to cool until it returned to room temperature. Once at room temperature, the solid product was filtered via vacuum filtration and washed 3 times with 20 ml of deionised water. When the solid was dry, a small sample was run on EI LC-MS for analysis.

This method was based on a study performed by (Buchanan, et al 1964).

2.2.1.6 Singlet oxygen

In a fume cupboard, 334 mg of strychnine was added to a 100 ml test tube, along with 100 mg of methylene blue and 100 ml of water. The test tube was then clamped and fitted with a glass adapter. A PVC tube was attached to the glass adapter and bubbled oxygen through to the test tube. 10 cm away from each side of the test tube, 2 40-watt UV lamps. It was ensured that there was no other light getting to the reaction by covering the entire fume cupboard shutter with tin foil. The reaction was carried out for 48 hours, and then the test tube was removed from the setup. A sample of the solid was then run on for EI LC-MS analysis.

2.2.1.7 Permanganate reactions

Classic strychnine degradation via potassium permanganate

A 250 ml conical flask and stirrer bar were weighed and recorded. Into the flask, 158 mg of potassium permanganate and 218 mg of potassium carbonate were added. A solution of 10% sodium hydroxide was made by mixing 10 ml of sodium hydroxide and 90ml of deionised water. 0.13 ml of the 10% solution was added to the flask. A further 10 ml of deionised water was transferred into the solution, which was then stirred on a magnetic stirrer at 250 rpm until any solid was fully dissolved.

Into a 100 ml round-bottom flask, 334 mg of strychnine was added, then placed in an ice bath. The fully dissolved solution was added dropwise over 20 minutes, while being stirred on a magnetic stirrer plate at 250 rpm. The reaction was continuously stirred for a further 24 hours. Once stirring was complete, the sample was run on EI LC-MS for analysis.

Degradation with potassium permanganate and acetonitrile

A 100 ml round-bottom flask and stirrer bar were weighed and recorded. To the flask 334 (1 mmol) mg of strychnine and 25 ml of acetonitrile were added. The reaction was placed in an ice bath and stirred on a magnetic stirrer plate at 300 rpm.

While the strychnine solution was stirred, 996 mg (6.3 mmol) of potassium permanganate was ground in a mortar and pestle until it became a fine powder. This was added in small quantities into strychnine solution over 15 minutes. After all of the potassium permanganate was added, the reaction was stirred for a further 5 minutes. The reaction was then removed from the ice bath and stirred for an extra 50 minutes.

Once stirring was complete, the solution was filtered via vacuum filtration, with the addition of celite in the Hirsch funnel to ensure no solid was lost. The round-bottom flask was washed 3 times with 10 ml of

acetonitrile and filtered. To the final filtrate, 4 drops of hydrazine were added to reduce any remaining oxidant. The solid was then run on EI LC-MS for analysis.

These methods were based on research produced by (Shaabani, et al, 2005) and (Emerson, 1938).

2.2.1.8 Hydrogen peroxide reaction

A 250 ml round-bottom flask and stirrer bar were weighed and recorded. To the flask, 334 mg of strychnine, 2.8802 g of phosphoric tungstic acid, 25 ml of dichloromethane and 1 drop of Fairy Liquid were added. The flask was then reweighed to the final total weight, minus the weight of round bottom flask and stirrer bar. The total weight was 16 g; therefore, 16 ml of hydroxide peroxide was added, 1 ml for every g.

The reaction was then refluxed and heated to the boiling point of dichloromethane (40 ℃). After one hour of the reaction being refluxed, a sample was taken and run on Electrospray mass spectrometry, to compare against a sample of the full reflux reaction. The reaction was further refluxed for 72 hours. Once the reaction was complete, a sample was run on EI LC-MS for analysis.

This method was based on an investigation performed by (Pai et al, 2005).

2.2.1.9 Amination reactions

Phenyl diamine reactions

A 10 ml vial and mini stirrer bar were weighed and recorded. To the vial, 334 mg of strychnine was added, and 0.5 molar equivalence of the phenyl diamine (54 mg). 300mg of boric acid was then added to the vial. The reaction was placed in a sand bath and stirred on a magnetic stirrer plate at 300 rpm. Once stirring was complete, a sample was run on EI LC-MS.

This reaction was carried out for both ortho and meta isotopes of phenyl diamine.

Other amine reactions

A 10 ml vial and mini stirrer bar were weighed and recorded. To the vial, 334 mg of strychnine was added, and 1 molar equivalent of the amine. 300 mg of boric acid was then added to the vial. The reaction was placed in a sand bath and stirred on a magnetic stirrer plate at 300 rpm. Once stirring was complete, a sample was run on EI LC-MS.

The reaction was carried out for both morphaniline, where 87mg was used and t-butyl amine, where 158 mg was used.

These amination reactions were inspired by an experiment produced by (Nguyen et al, 2012).

2.2.2 Instrumental analysis

2.2.2.1 EI LC-MS

Preparation of the sample

5 mg of sample is transferred into a 1.5 ml vial and topped up with LC-MS grade methanol, then 2 more 1.5 more vials are filled with only methanol. A spatula was cleaned and dried, then dipped into a vial containing the 5mg of sample. The spatula was stirred around in the vial and into the first fully methanol vial; this was repeated twice. The spatula was then stirred in the first methanol vial and dipped into the second fully methanol vial; this was repeated twice. This process created an effective dilution of the sample to be run on EI LC-MS.

Data collection

The instrument was set up by turning on the argon gas and altering the dessolvation temperature from the base temperature to 300℃. The flow rate was then checked to ensure it was at a flow of 20µl / minute. Once the instrument parameters were correct, a blank of LC-MS grade methanol was run to flush out anything previously run, and blanks were run at a cone voltage of 30V.

After a blank was run, the most dilute sample was injected into the instrument to check that the sample was at

a correct dilution: the cone voltage was not altered for the first 30 seconds. If the sample is at an effective dilution, the ion counts falls between 200 and 1000 counts; if the counts were too low, the sample was too dilute, or if the counts were too high, the sample was too concentrated. Then any adjustments to the sample were made if the dilution was too strong/ weak.

When the sample was at an optimal concentration, the cone voltage was adjusted to determine the optimum voltage and to examine the sample at varying intensities. The cone voltage was lowered to 0V and increased by increments of 5 or 10 volts until the ion count on the spectrum reached 1 or revealed an insufficient spectrum.

2.2.2.2 Nuclear Magnetic Resonance

Preparation of the sample

In a clean NMR tube, between 100 and 300 mg of the sample was added into the tube. 0.7 ml of solvent ($CDCl_3$ or $DMSO_6$) was taken up via a Pasteur pipette, then filtered through a glass pipette and paper. Once filtered, the solvent was added to the NMR tube. The cap was placed onto the tube and then labelled.

Data collection

The prepared sample was wiped down once and inserted 3 quarters into a tube spinner. The sample was then placed into an available slot, and the number of the slot was noted. From the interface, the types of 1D and 2D NMR methods needed to be run were selected, based on the amount of sample, the hertz of the machine or the type of technique; the scans were altered. The lower hertz machine required more scans for an effective spectrum to be produced. Also, samples that contained smaller quantities (100-200mg) required a larger number of scans to produce an effective spectrum.

3 RESULTS

This section displays formatted data and key spectra due to the vast majority of analytical data produced in this research.

3.1 EI LC-MS

3.1.1 Strychnine

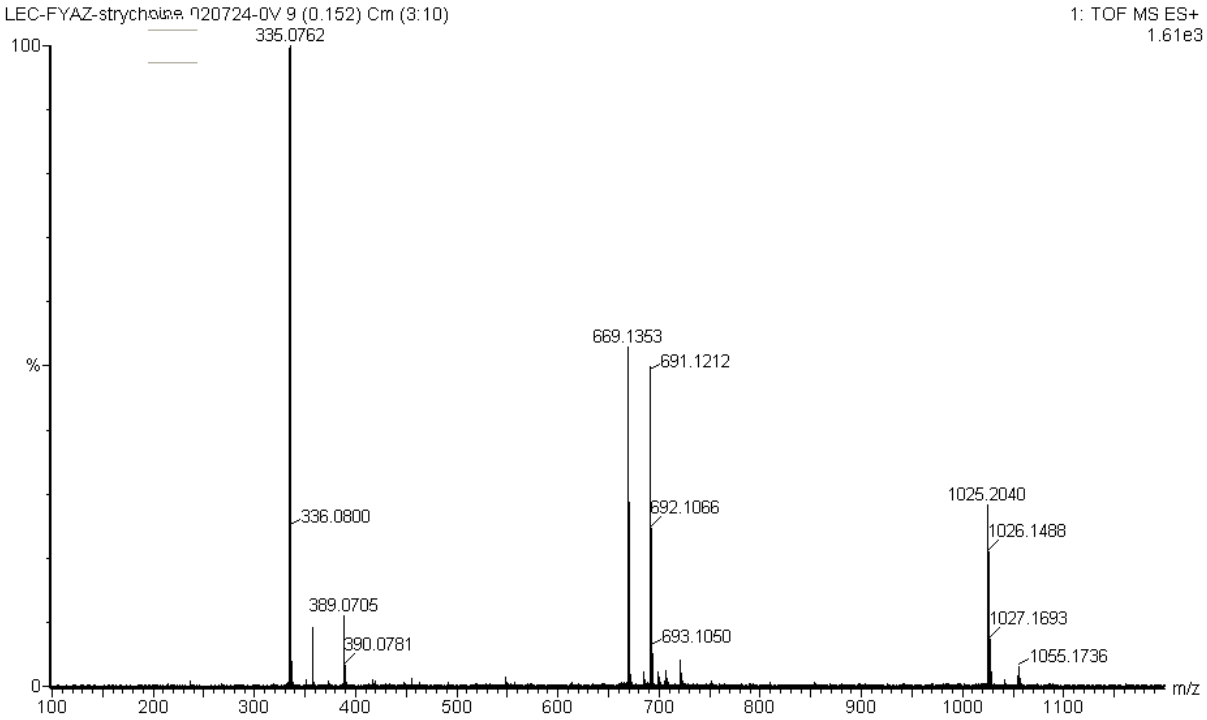


Figure 4. EI LC-MS of strychnine, ran in positive ion mode – at 0 volts.

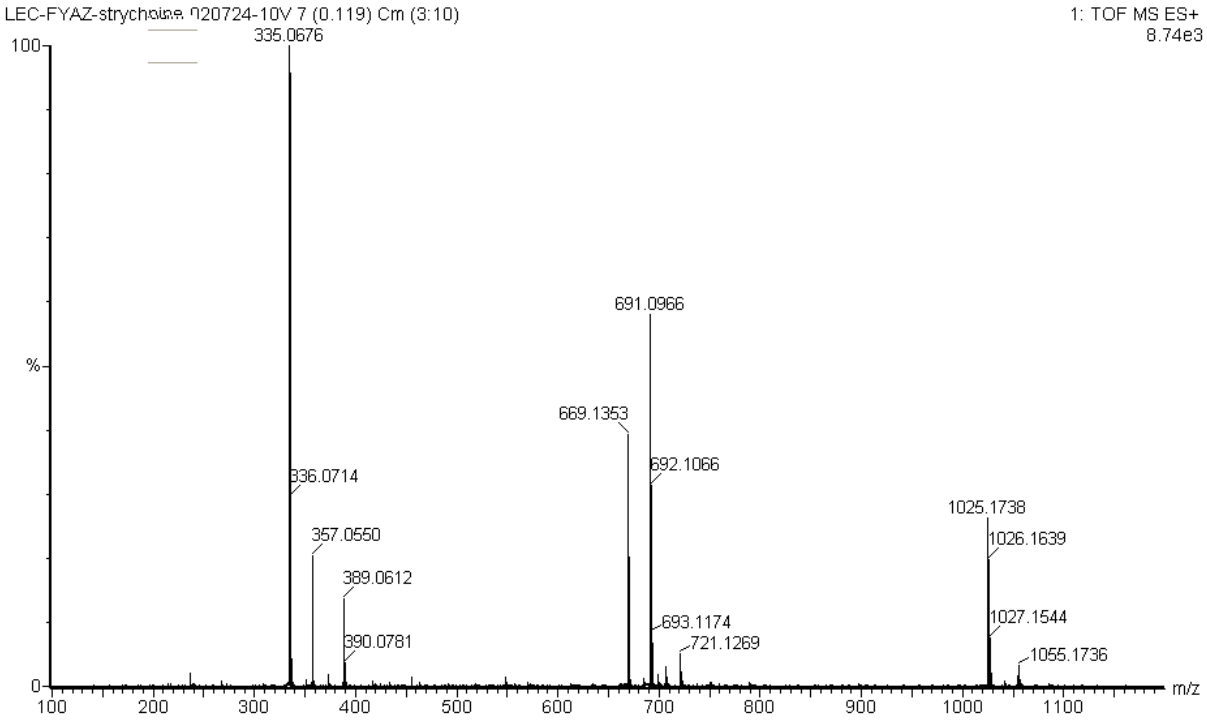


Figure 5. EI LC-MS of strychnine, run in positive ion mode – at 10 volts.

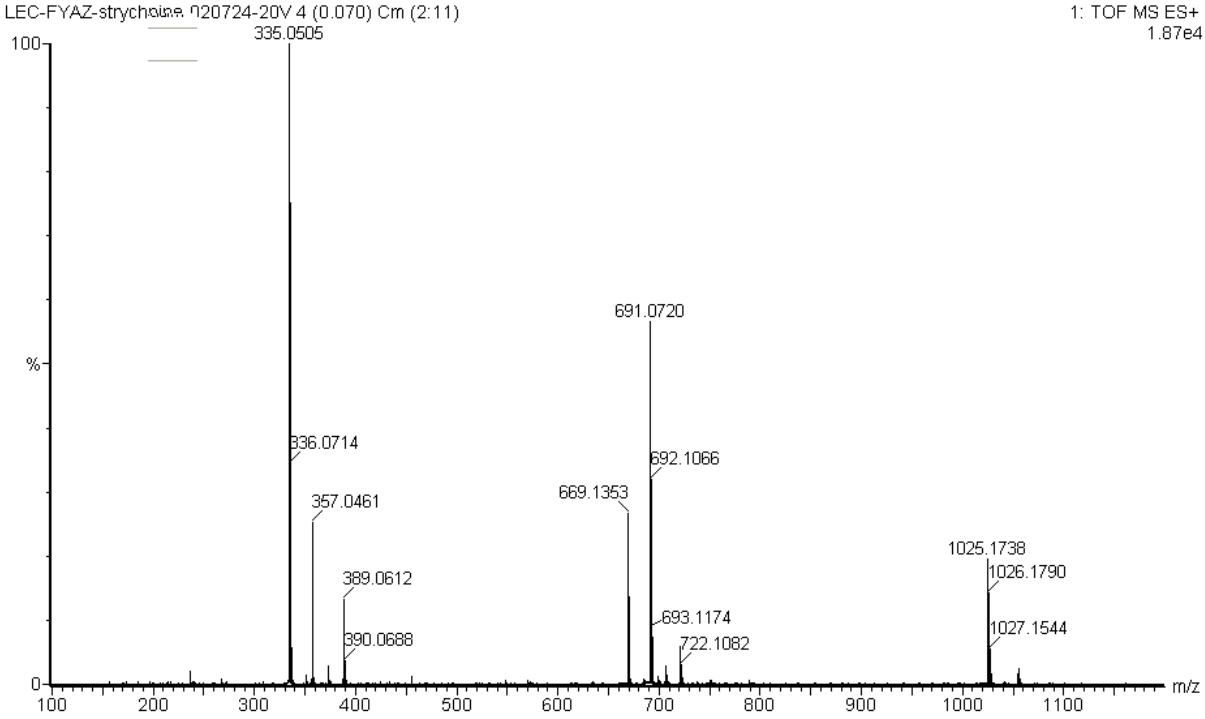


Figure 6. EI LC-MS of strychnine, run in positive ion mode – at 20 volts.

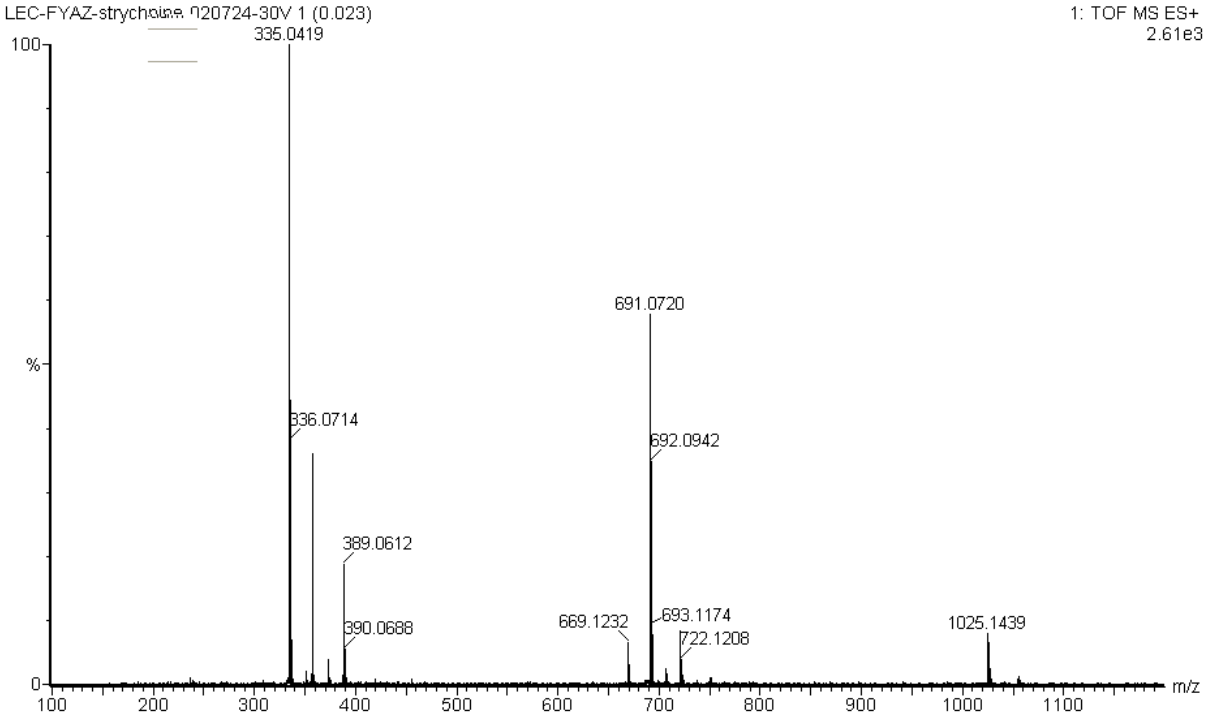


Figure 7. EI LC-MS of strychnine, run in positive ion mode at 30 volts.

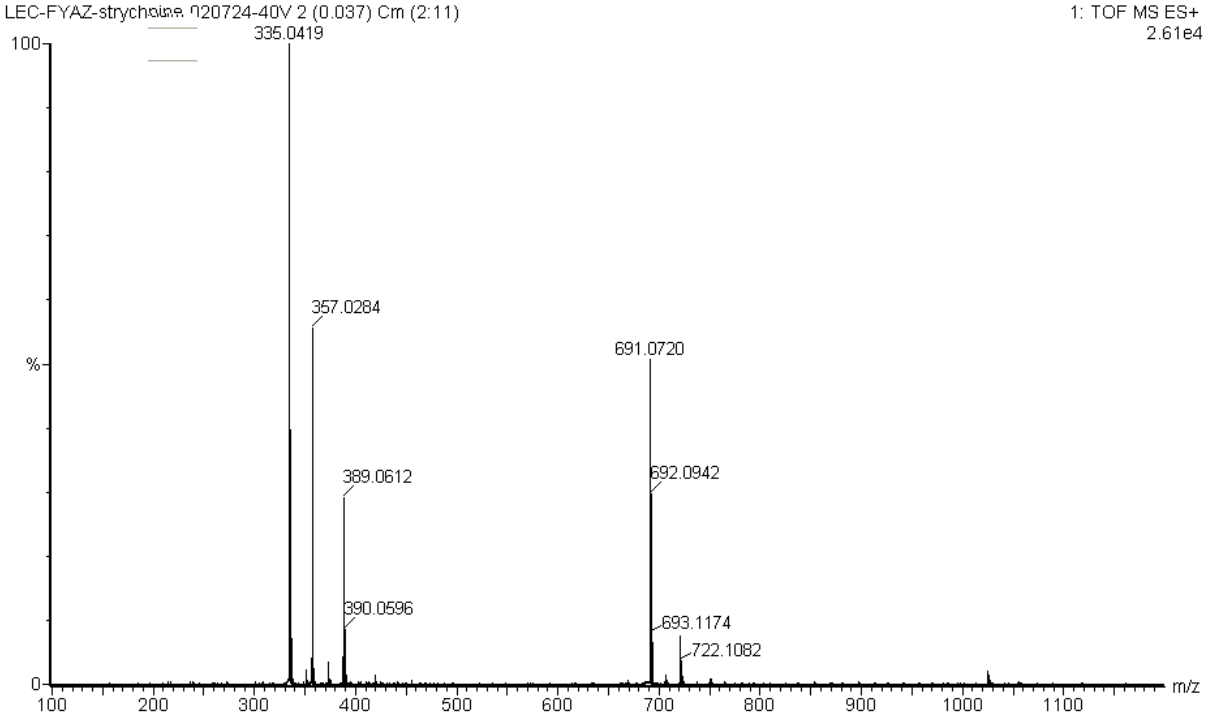


Figure 8. EI LC-MS of strychnine, run in positive ion mode – at 40 volts.

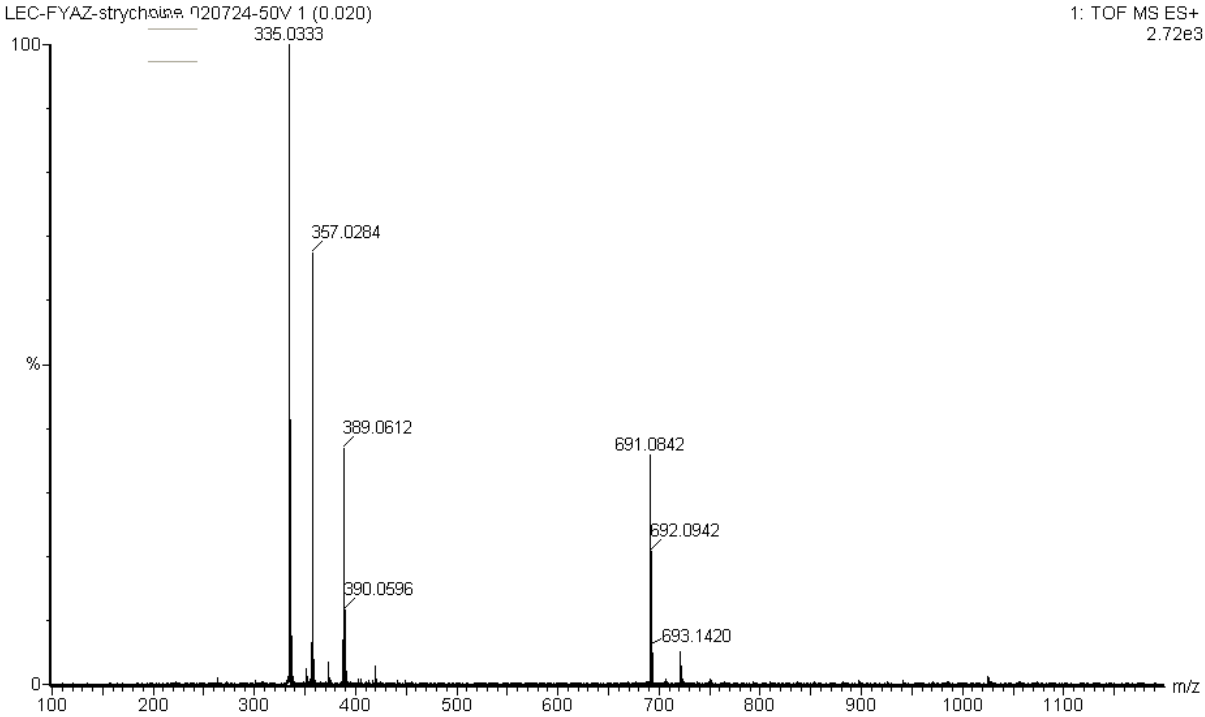


Figure 9. EI LC-MS of strychnine, run in positive ion mode – at 50 volts.

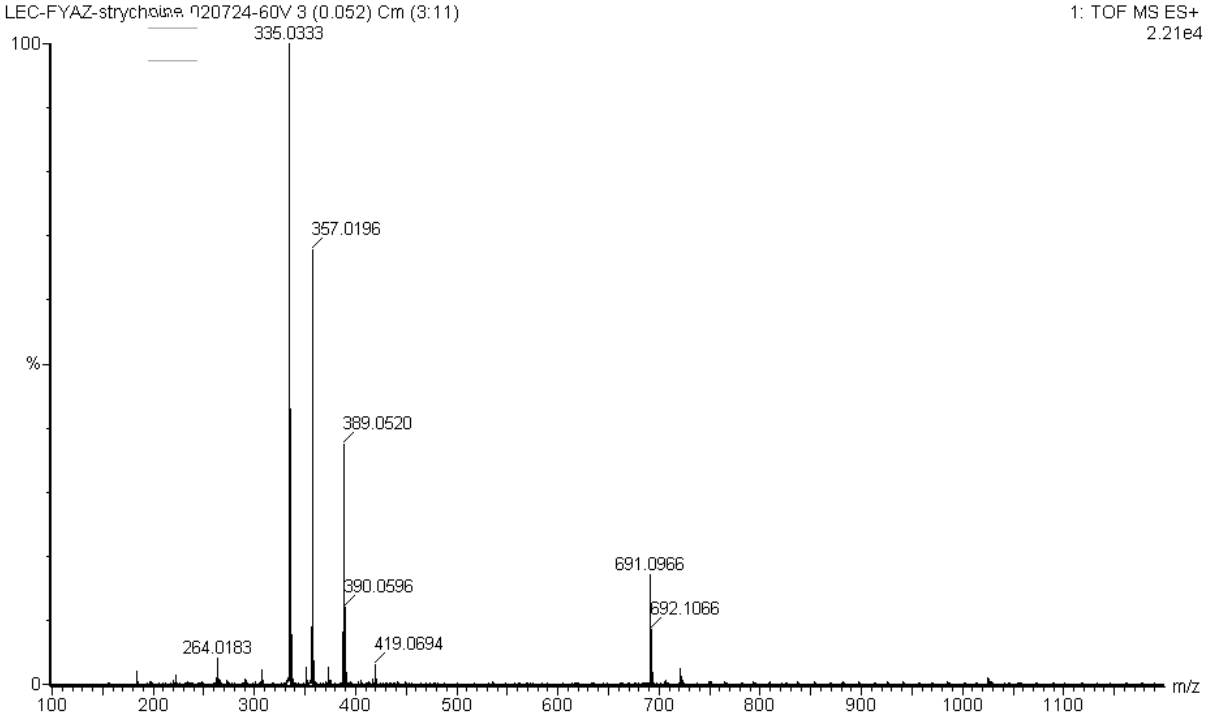


Figure 10. EI LC-MS of strychnine, run in positive ion mode – at 60 volts.

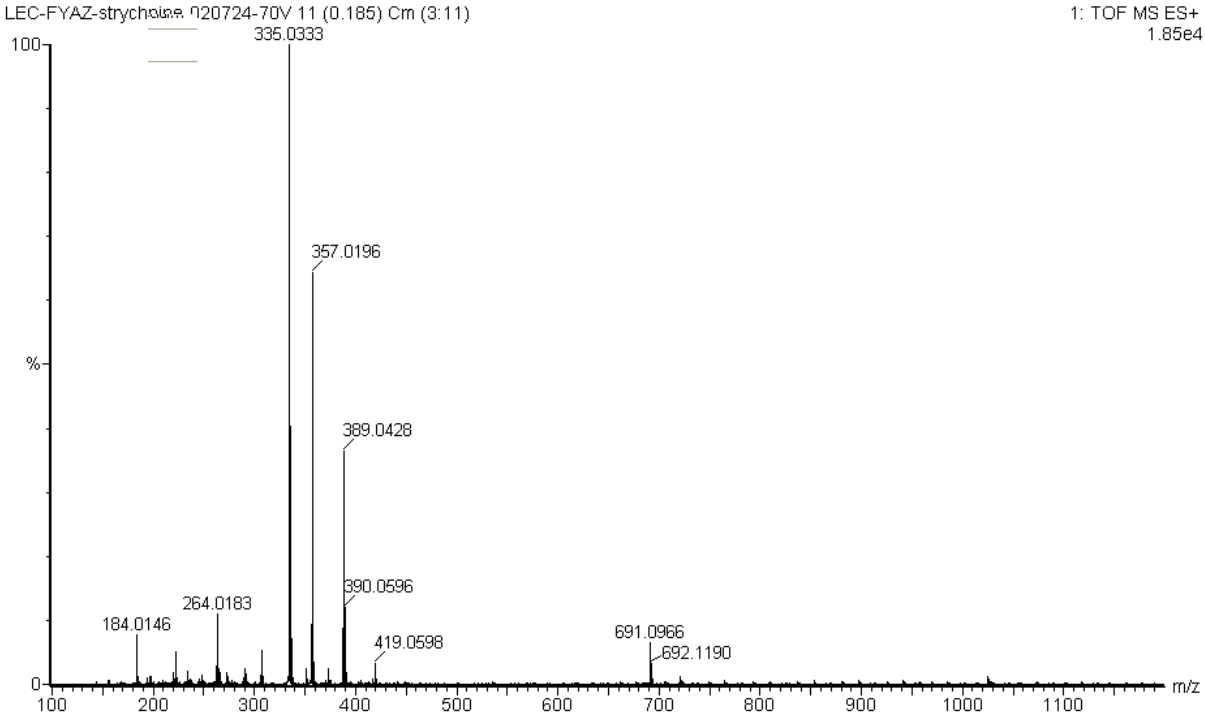


Figure 11. EI LC-MS of strychnine, run in positive ion mode – at 70 volts.

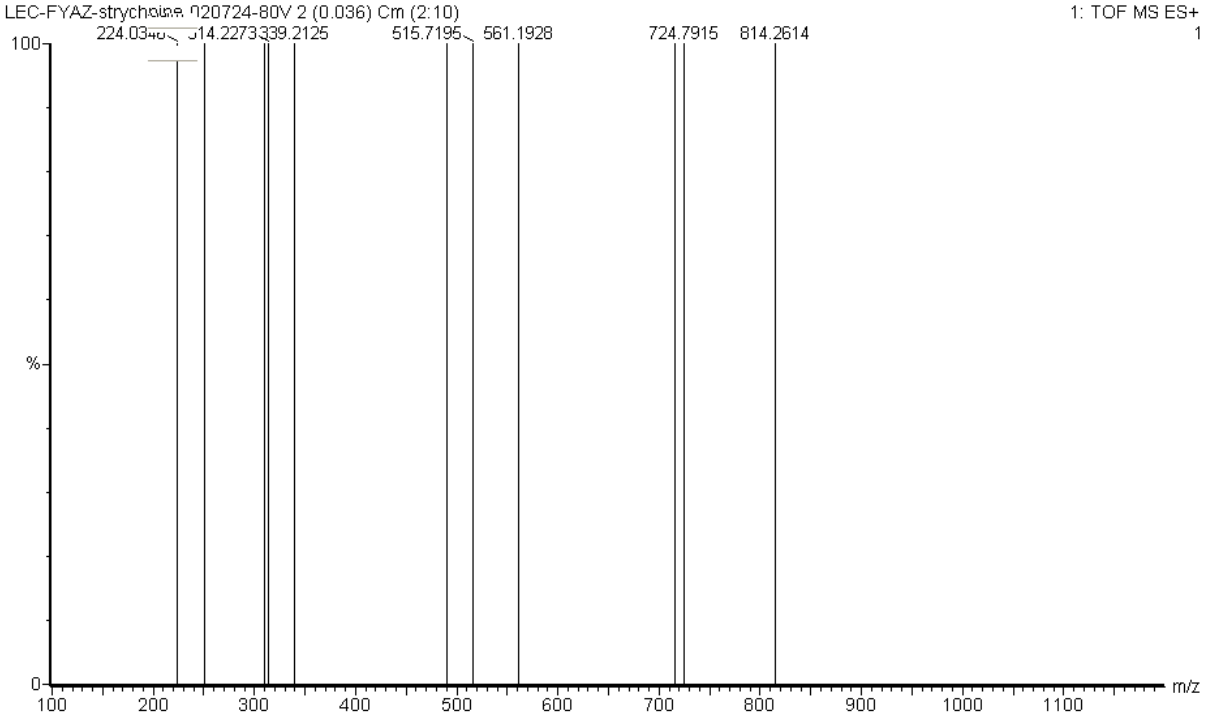
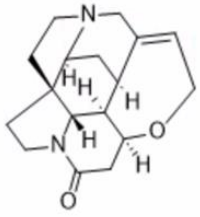
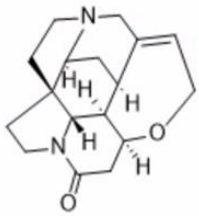
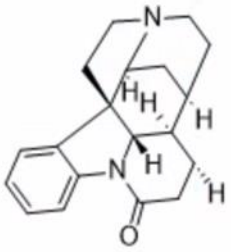
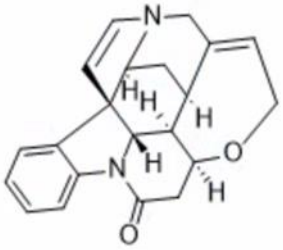
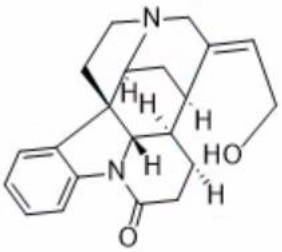
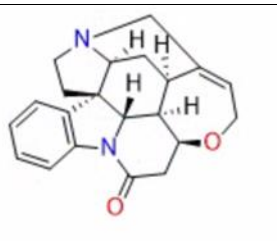
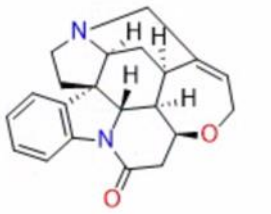
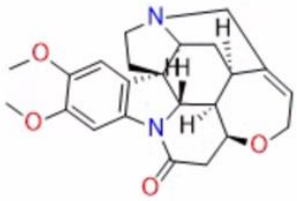
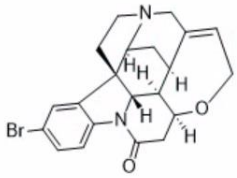
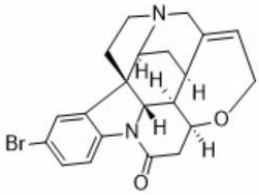


Figure 12. EI LC-MS of strychnine, run in positive ion mode at 80 volts.

Table 1. This table shows some ion peaks (m/z) present throughout the several degradations of strychnine, and the suggested structures.

Ion Peak (m/z)	Suggested Structure
239	 + 23 (sodiated due to contamination)

245	 + NH4 (ammonium ion due to contamination)
283	
334	
334	

335	
367	 +MeOH (from dilution process, or from being present in the column)
395	
412	
414	

3.2 Nuclear Magnetic Resonance

3.2.1 Strychnine

Table 2. This table shows the chemical shift, multiplicity and integration of strychnine free base – 300 mg in 0.7ml of CDCl3 – 16 scans run at 600 MHz from proton NMR spectra.

Chemical Shift (δ ppm)	Multiplicity
7.99	Doublet
7.26	Singlet
7.0	Multiplet
5.79	Singlet
4.14	Triplet
3.99	Quartet
3.92	Quartet
3.72	Triplet
3.55	Doublet
2.95	Multiplet
2.72	Quartet
2.54	Quartet
2.20	Doublet of triplets
1.72	Triplet
1.29	Doublet
1.12	Doublet
0.46	Singlet

Table 3. This table shows the chemical shift, peak direction and multiplicity of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 512 scans run at 600 MHz - DEPT Q NMR spectra.

Chemical shift (δ ppm)	Peak direction	Multiplicity
169.22 (C1)	Positive	Singlet
142.02 (C2)	Positive	Singlet
140.78 (C3)	Positive	Singlet
132.77 (C4)	Positive	Singlet
128.45 (C5)	Negative	Singlet
127.33 (C6)	Negative	Singlet
124.48 (C7)	Negative	Singlet
122.35 (C8)	Negative	Singlet
116.45 (C9)	Negative	Singlet

77.44 (C10)	Negative	Singlet
64.48 (C11)	Positive	Singlet
60.03 (C12)	Negative	Doublet
52.54 (C13)	Positive	Singlet
50.21 (C14)	Positive	Singlet
48.01 (C15)	Negative	Singlet
42.23 (C16)	Positive	Doublet
31.79 (C17)	Negative	Singlet
26.73 (C18)	Positive	Singlet

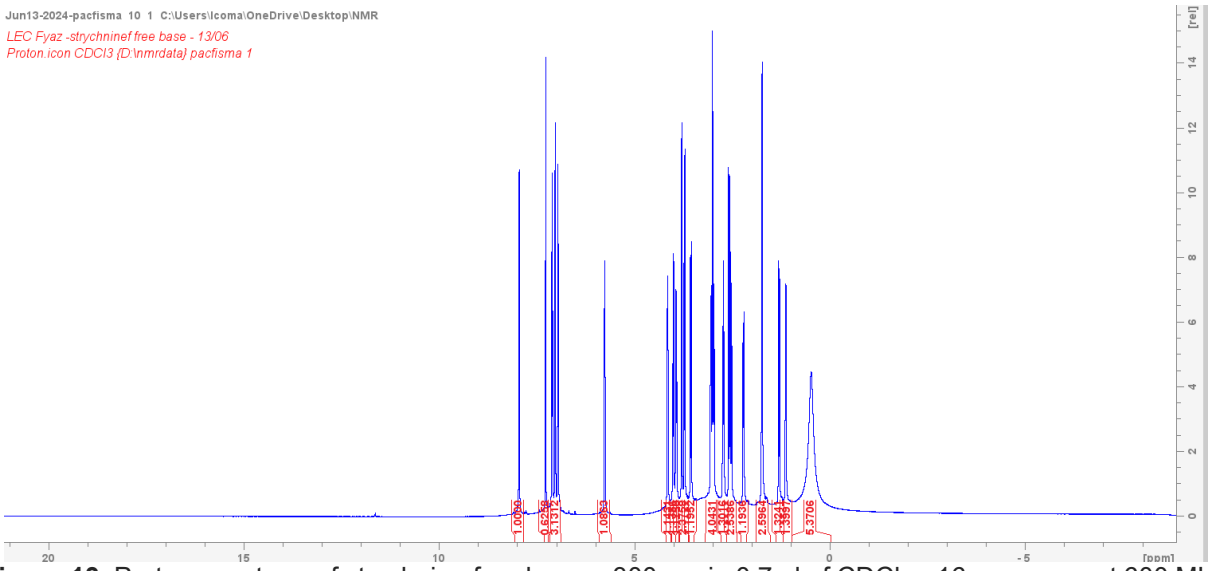


Figure 13. Proton spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 16 scans run at 600 MHz

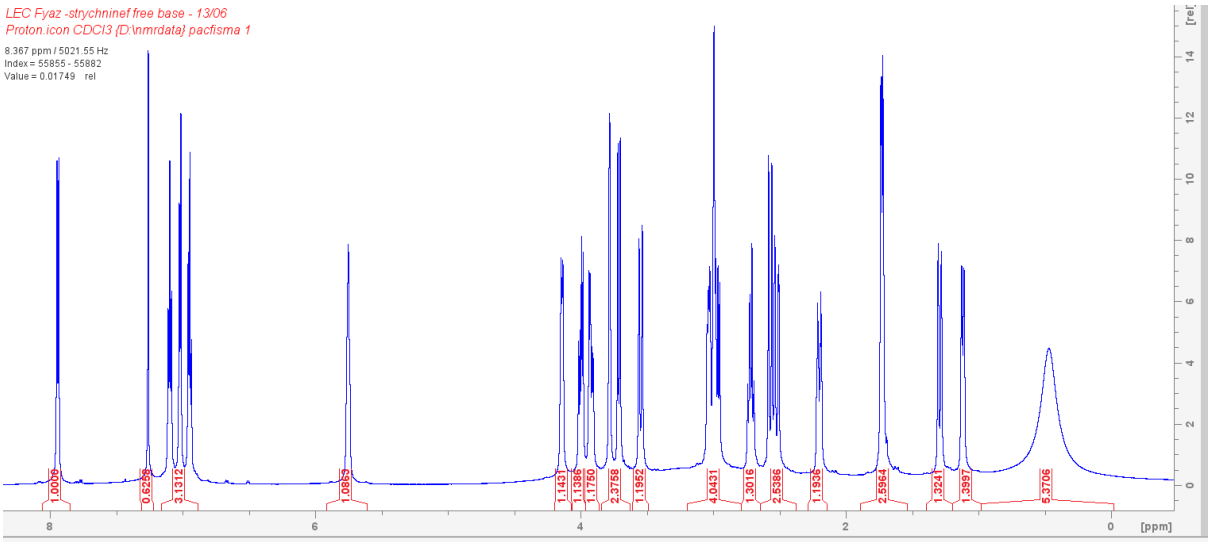


Figure 14. An expansion of the proton spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 16 scans run at 600 MHz

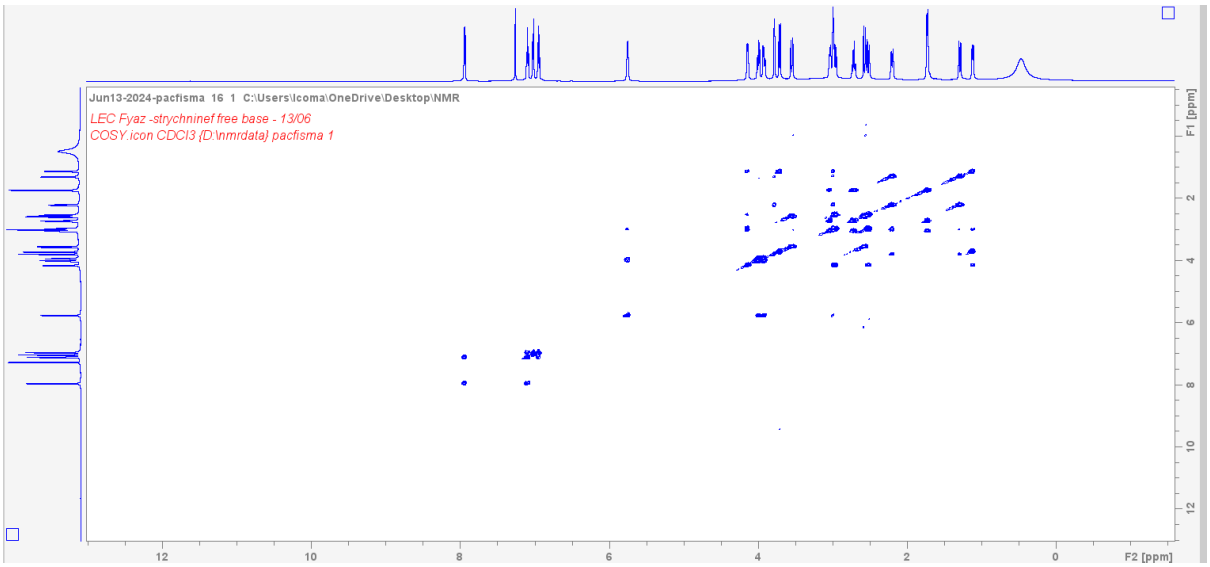


Figure 15. COSY spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 1 scan ran at 600 MHz

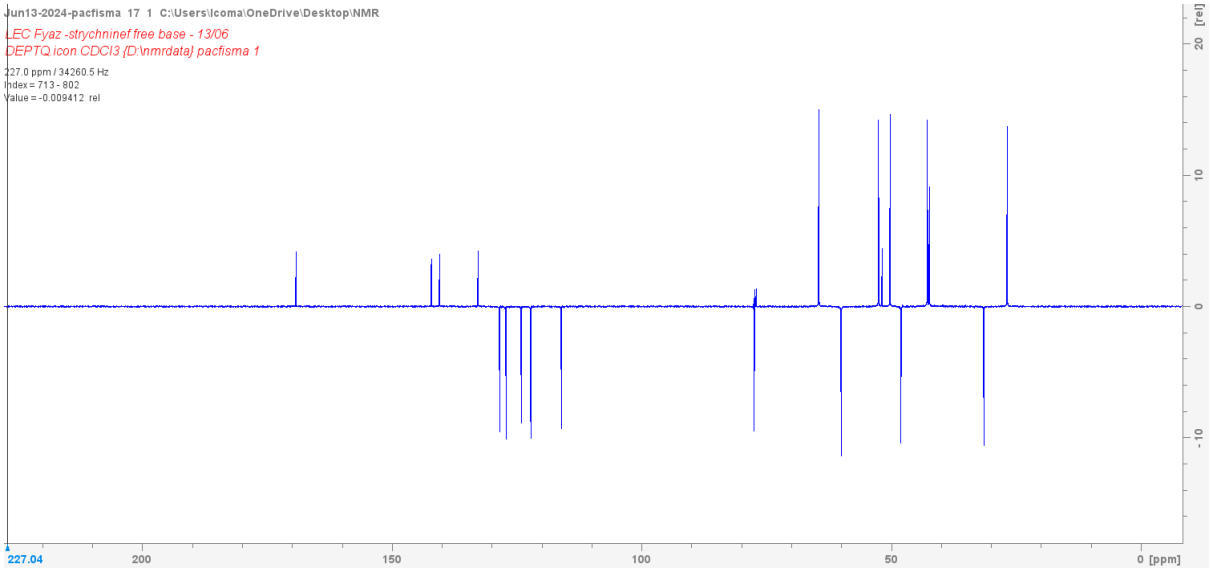


Figure 16. DEPT Q spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 512 scans ran at 600 MHz

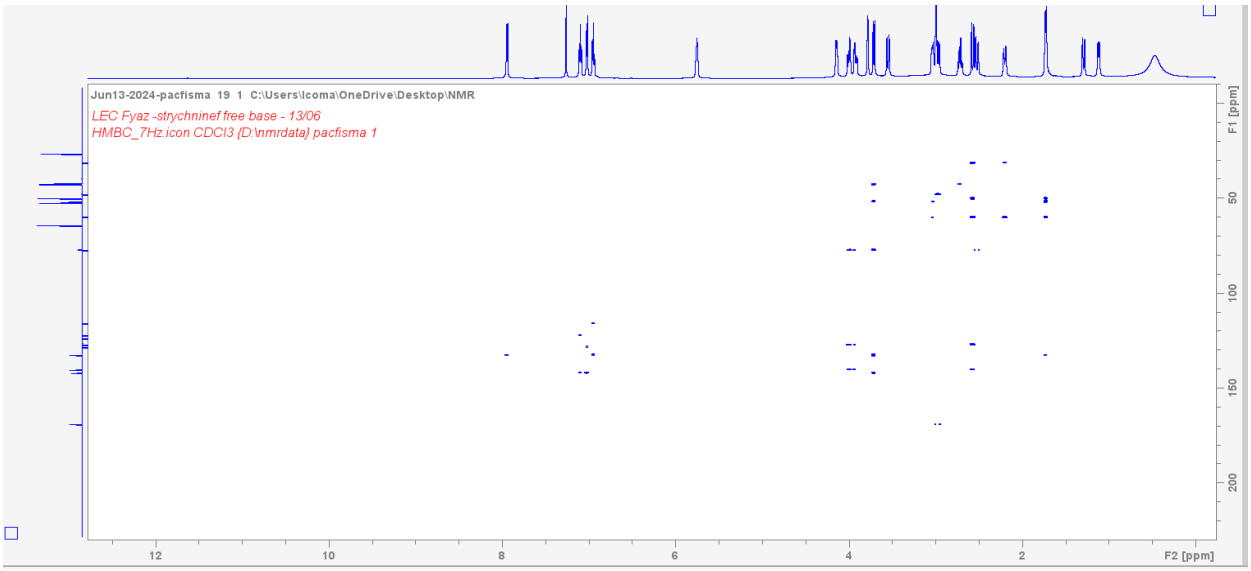


Figure 17. HMBC spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 2 scans run at 600 MHz

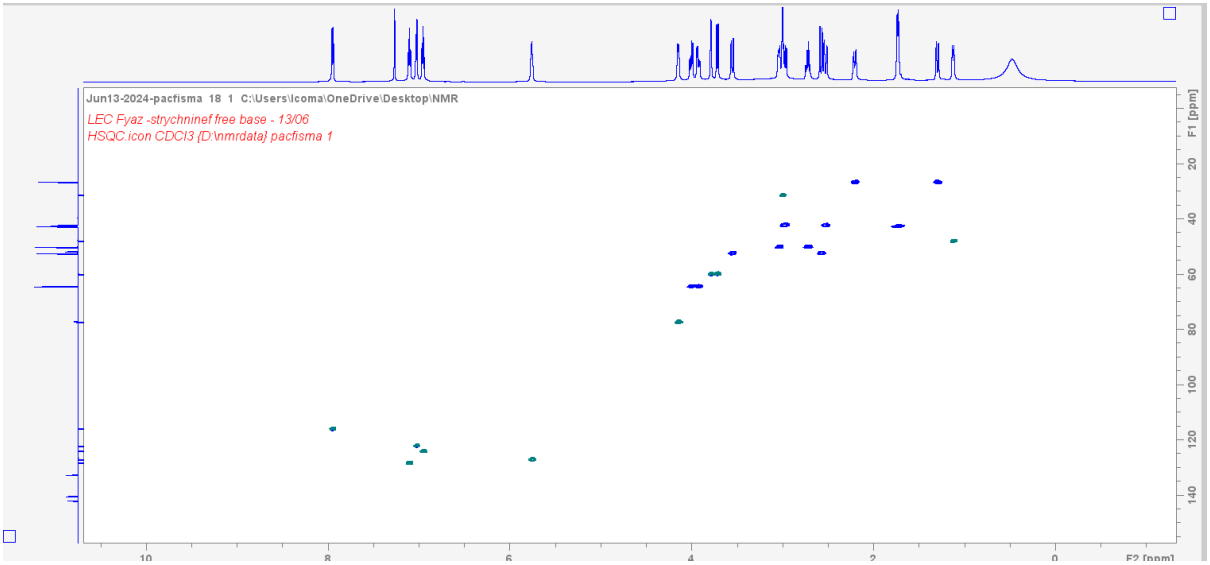


Figure 18. HSQC spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 2 scans run at 600 MHz

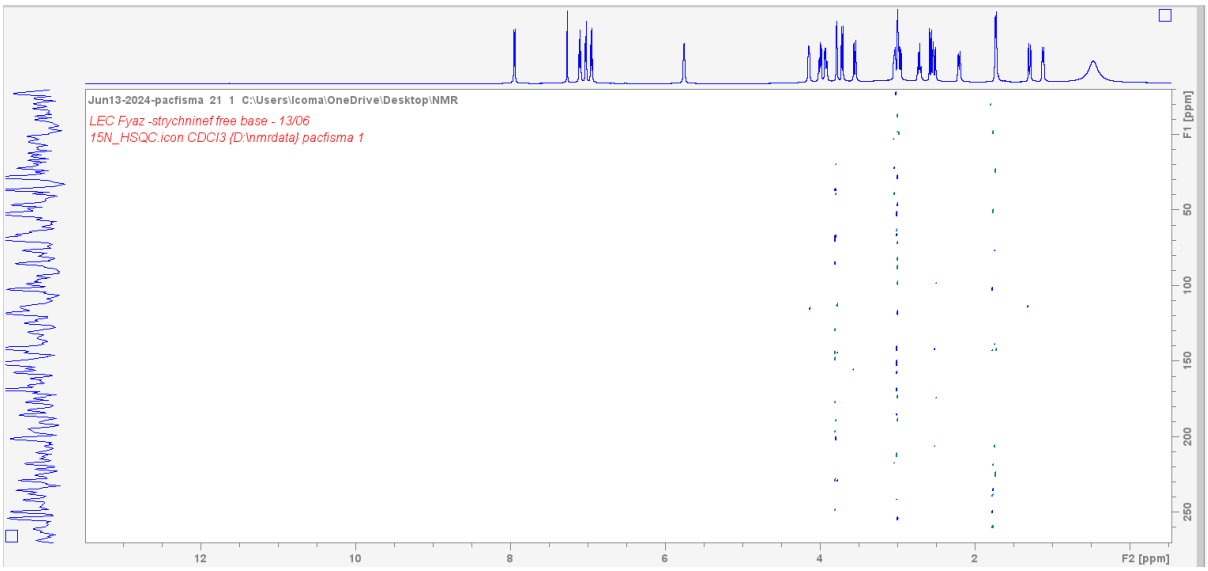


Figure 19. 15N HSQC spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 2 scans ran at 600 MHz

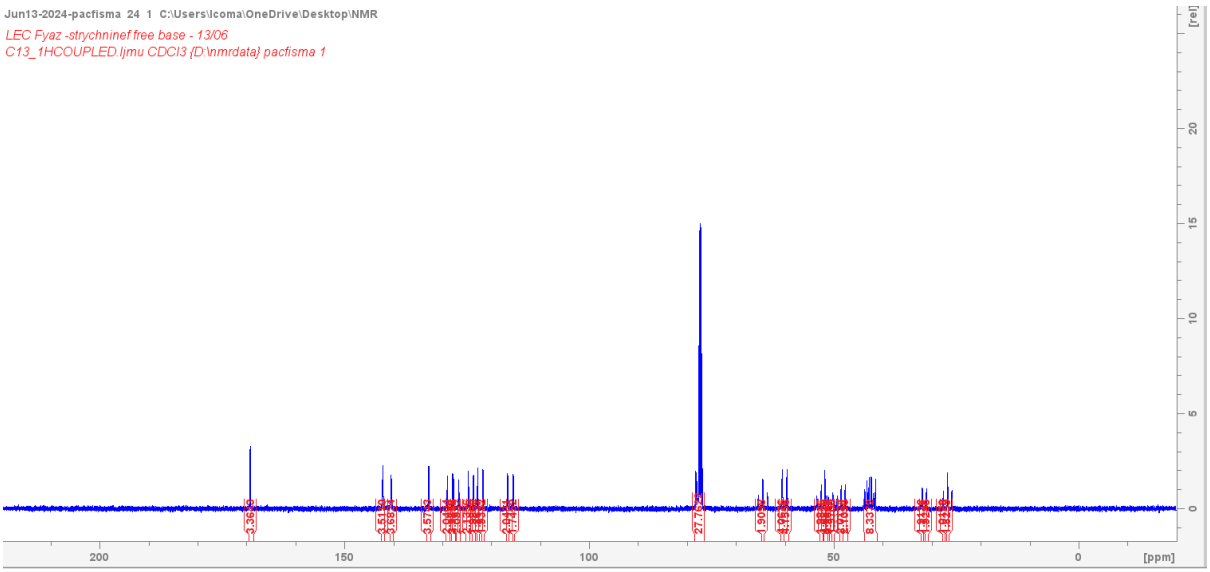


Figure 20. C^{13} proton coupled spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 64 scans run at 600 MHz

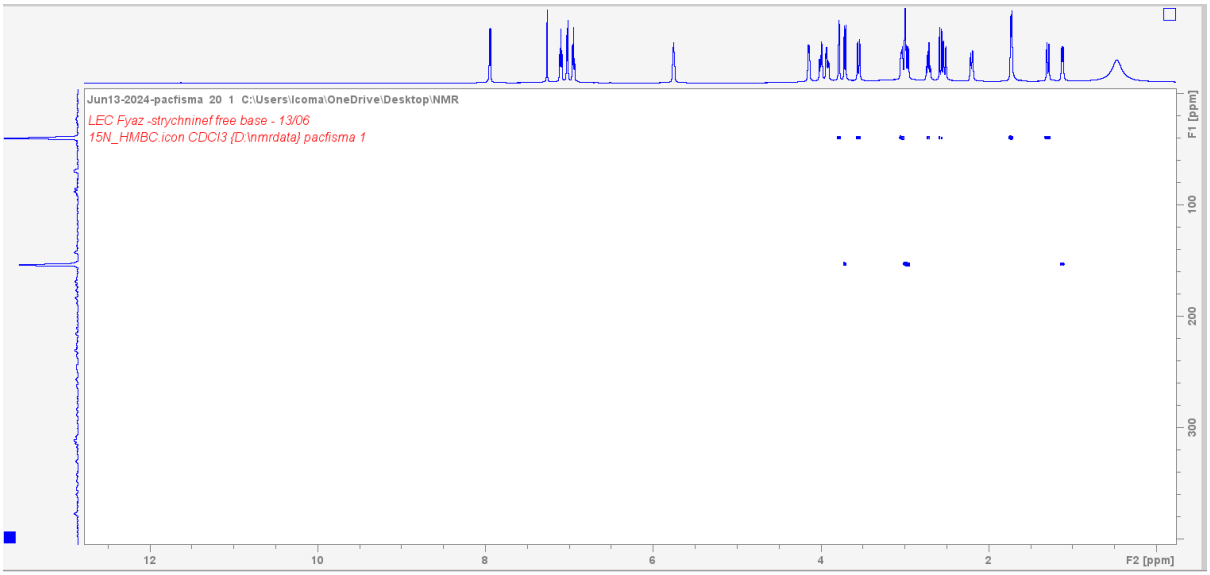


Figure 21. 15N HMBC spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 8 scans ran at 600 MHz

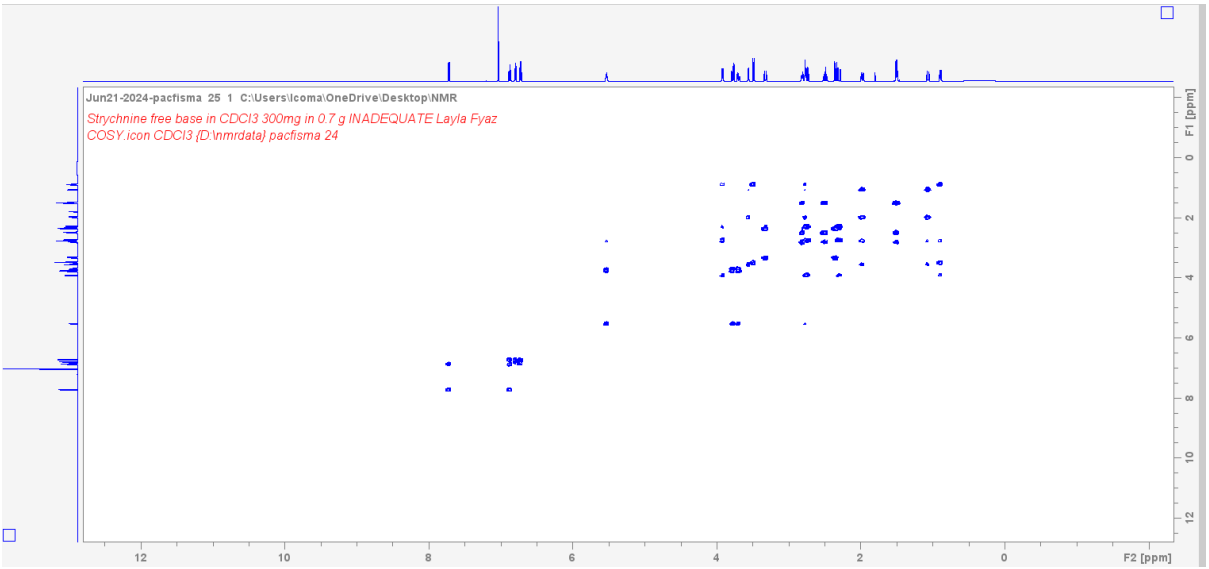


Figure 22. COSY spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 4 scans run at 600 MHZ

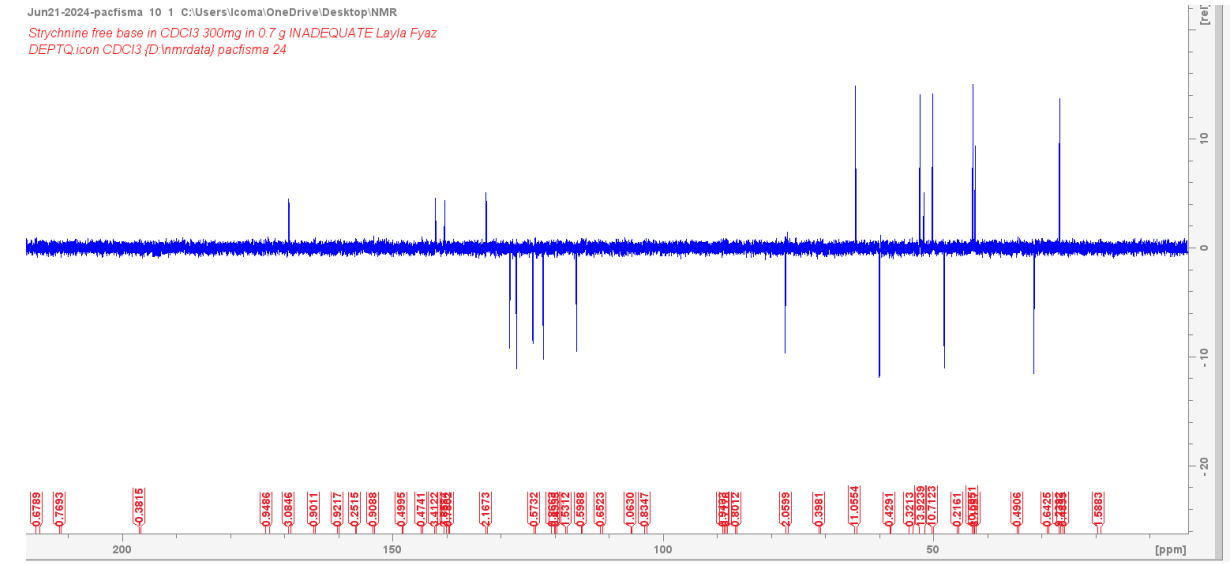


Figure 23. DEPT Q spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 1 scan ran at 600 MHz

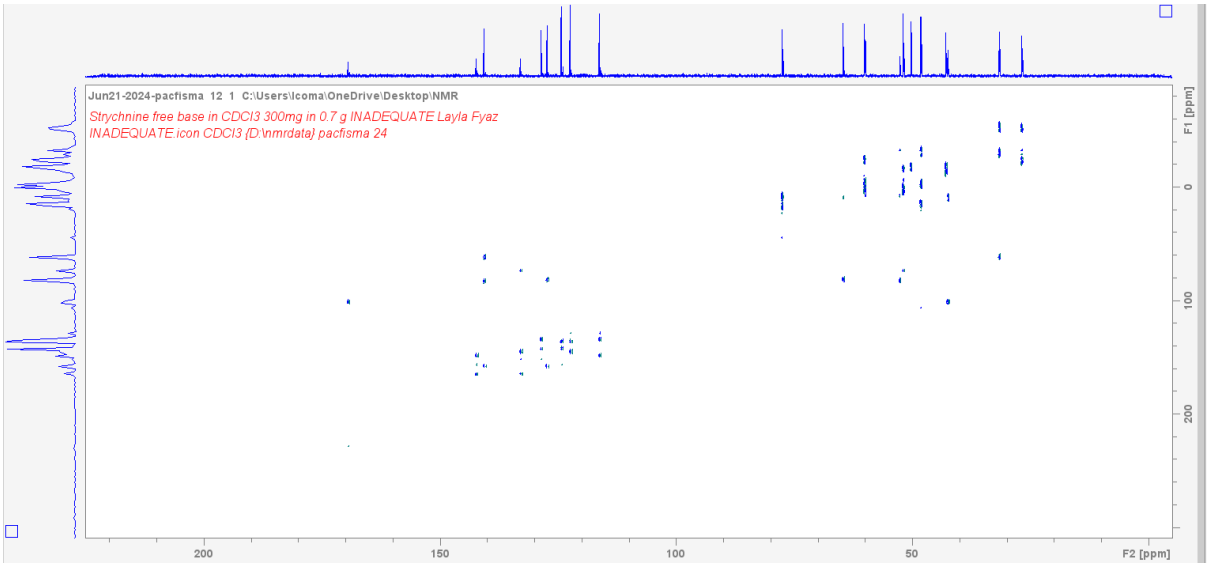


Figure 24. Shows an INADEQUATE spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 256 scans run at 600 MHz

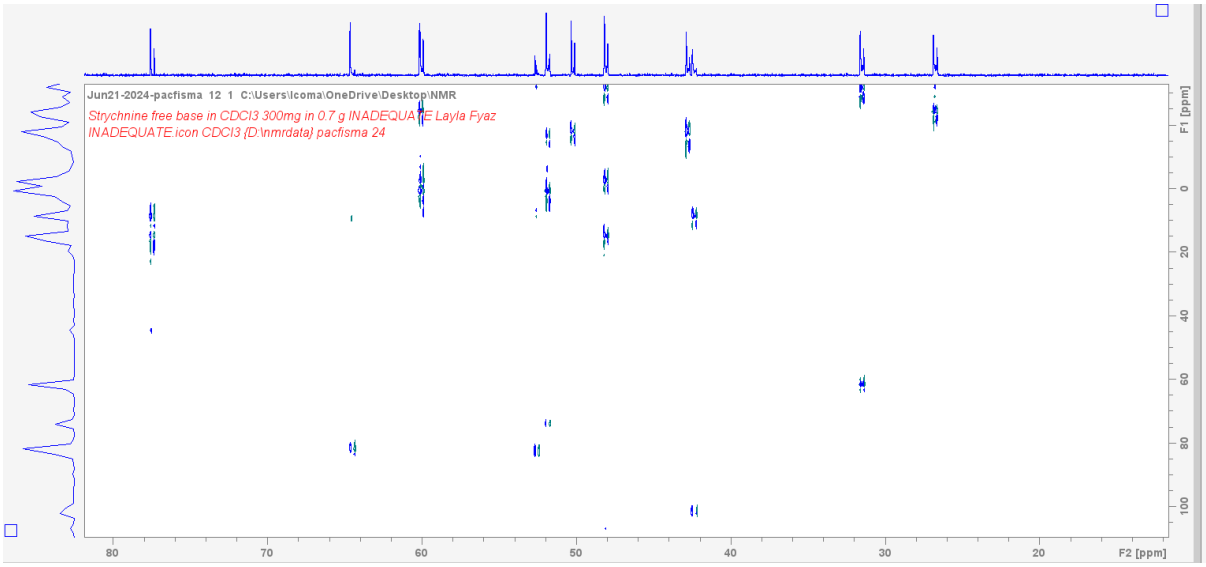


Figure 25. Expansion of an INADEQUATE spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 256 scans run at 600 MHz

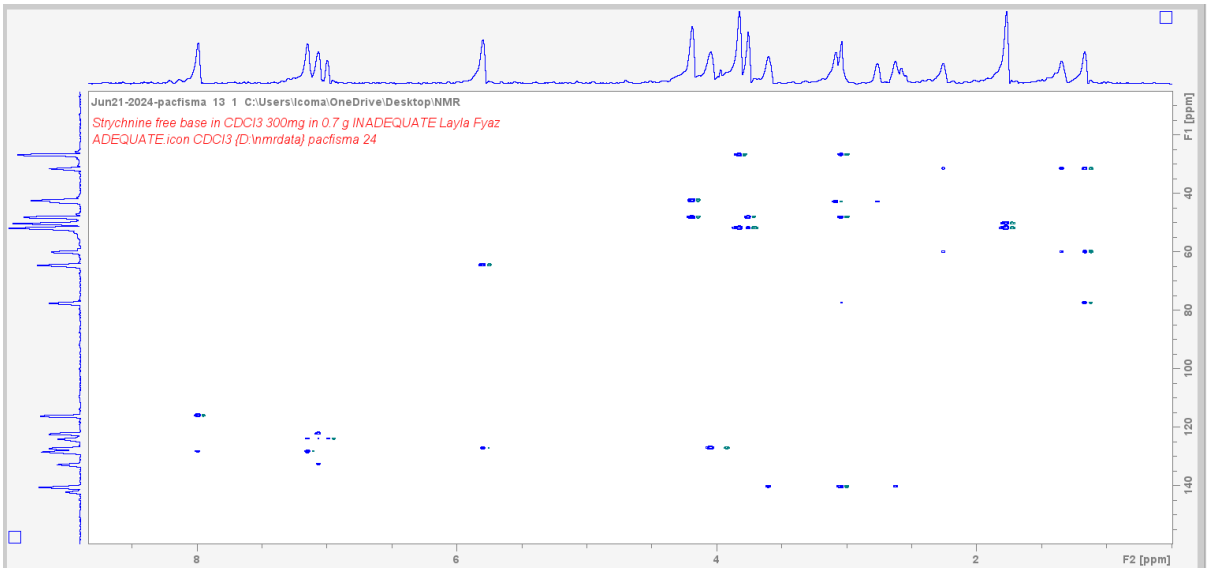


Figure 26. ADEQUATE spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 64 scans run at 600 MHz

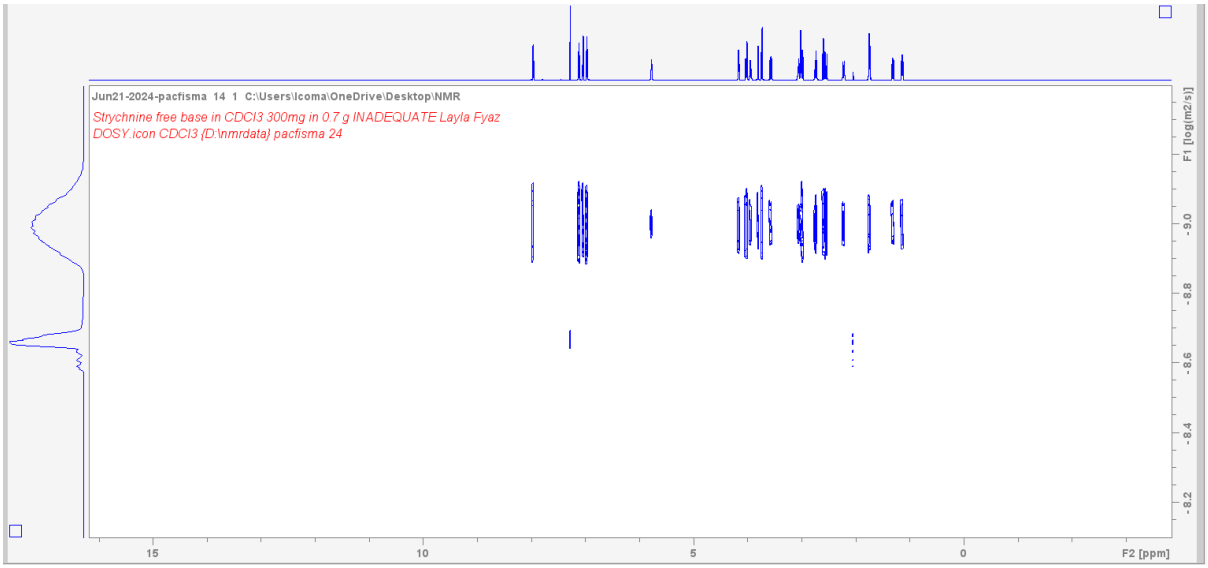


Figure 27. DOSY spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 16 scans run at 600 MHz

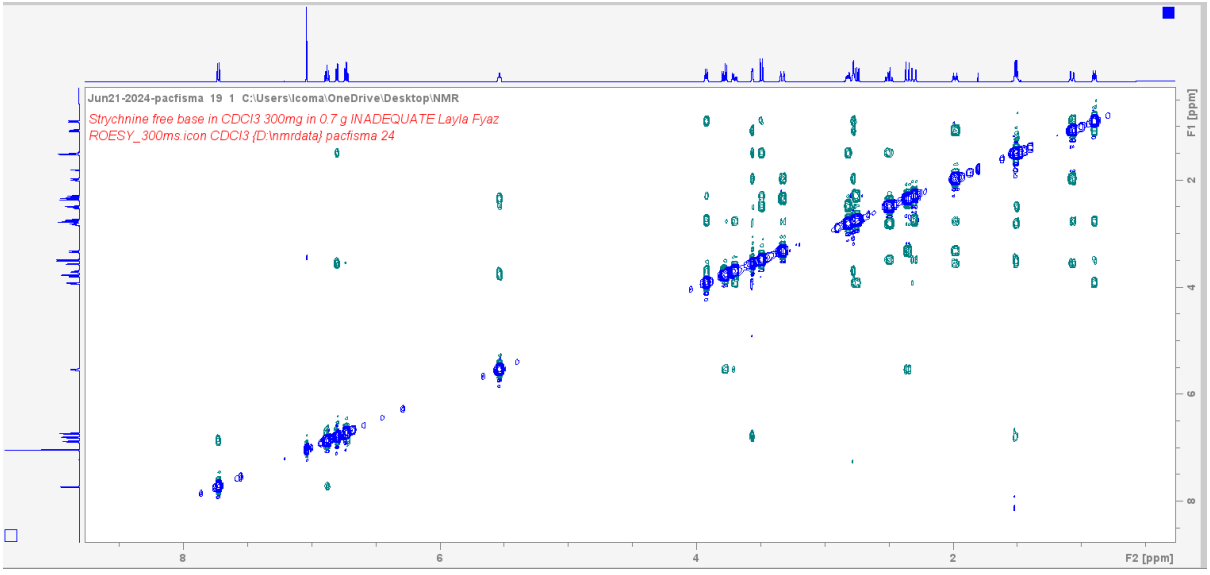


Figure 28. ROSEY spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 4 scans run at 600 MHz

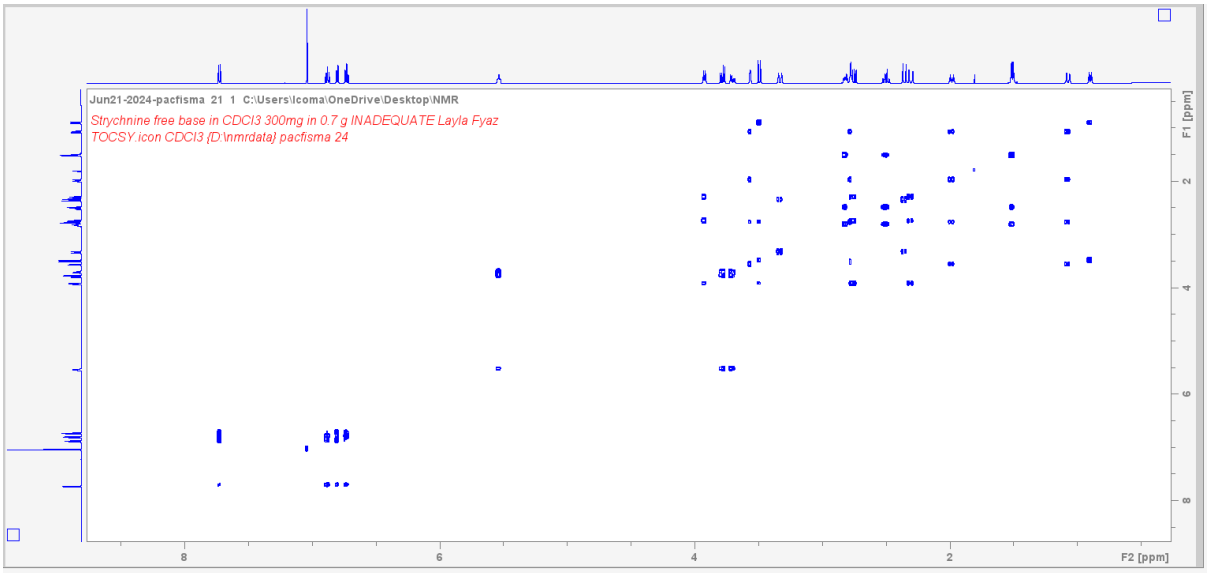


Figure 29. TOCSY spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 2 scans run at 600 MHz

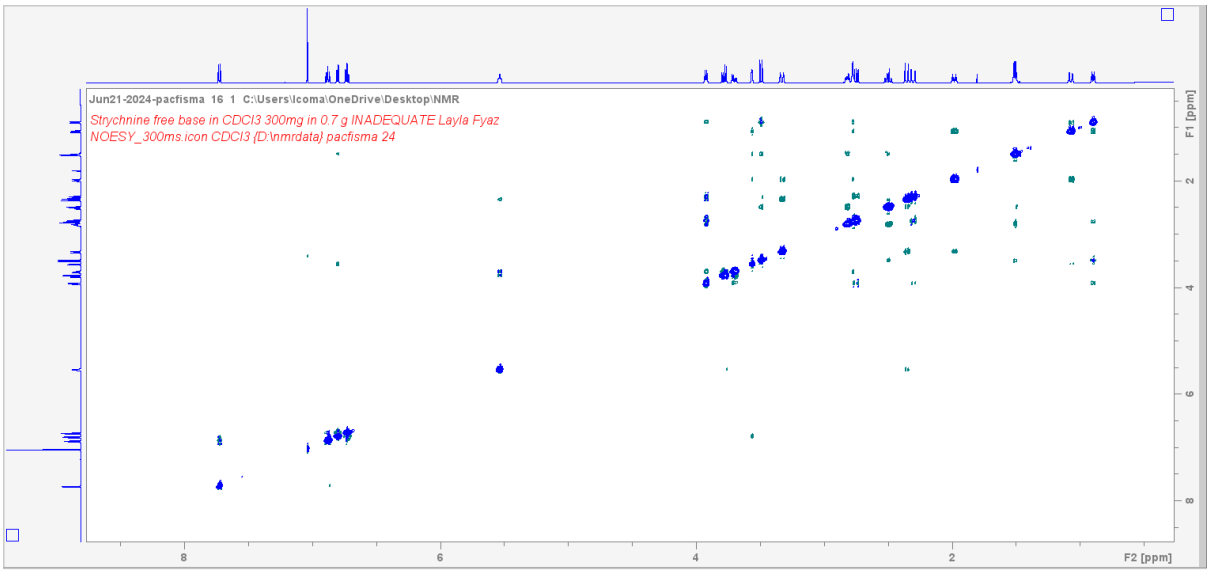


Figure 30. Shows a NOSEY 300 ms spectrum of strychnine free base – 300 mg in 0.7ml of $CDCl_3$ – 2 scans run at 600 MHz

3.2.2 INADEQUATE spectra of alkaloids

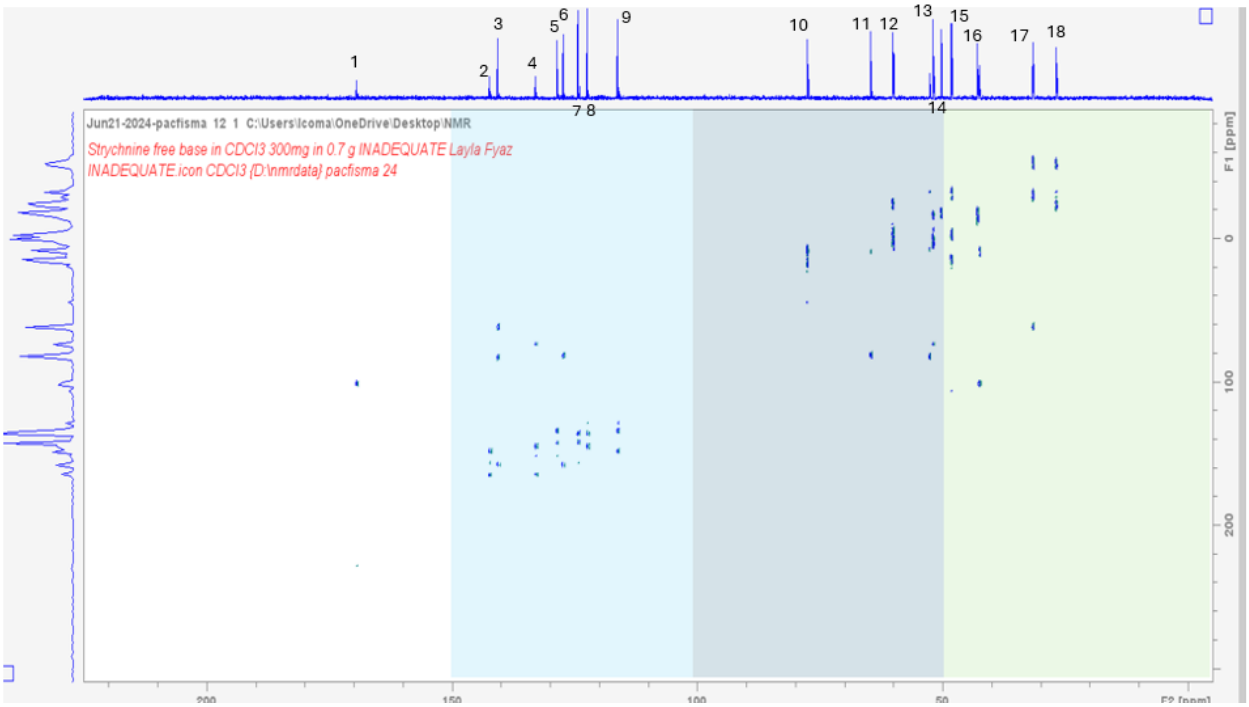


Figure 31. This shows the interpreted INADEQUATE spectra of Strychnine – 300mg; 256 scans – ran at 600 MHz. The light blue area indicates aromatic region; the dark blue area indicates nonaromatic carbonyls, and the green area indicates the alkane region.

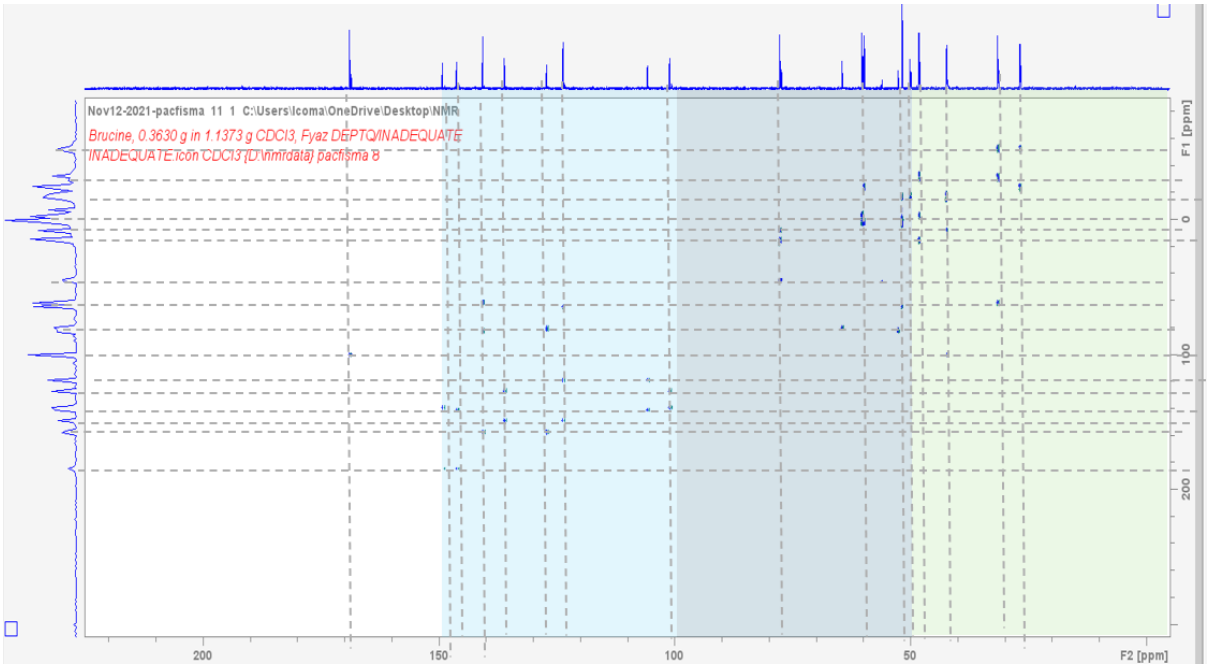


Figure 32. This shows the interpreted INADEQUATE spectra of brucine – 300mg; 256 scans – ran at 600 MHz

The light blue area indicates aromatic region; the dark blue area indicates nonaromatic carbonyls, and the green area indicates the alkane region.

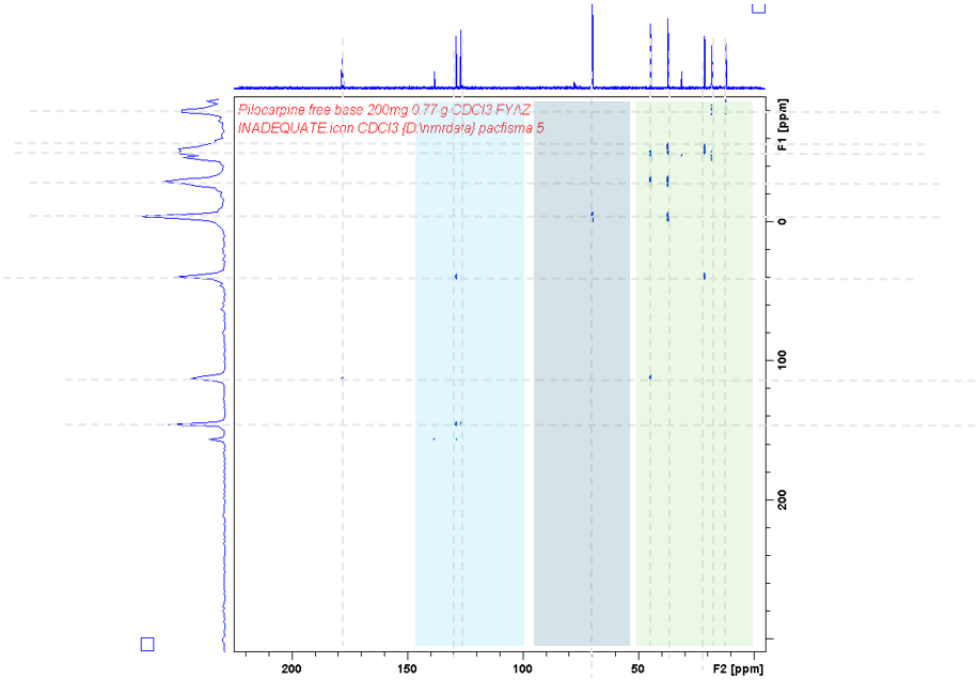


Figure 33. This shows the interpreted INADEQUATE spectra of pilocarpine – 300mg; 256 scans – ran at 600 MHz.The light blue area indicates aromatic region; the dark blue area indicates nonaromatic carbonyls, and the green area indicates the alkane region.

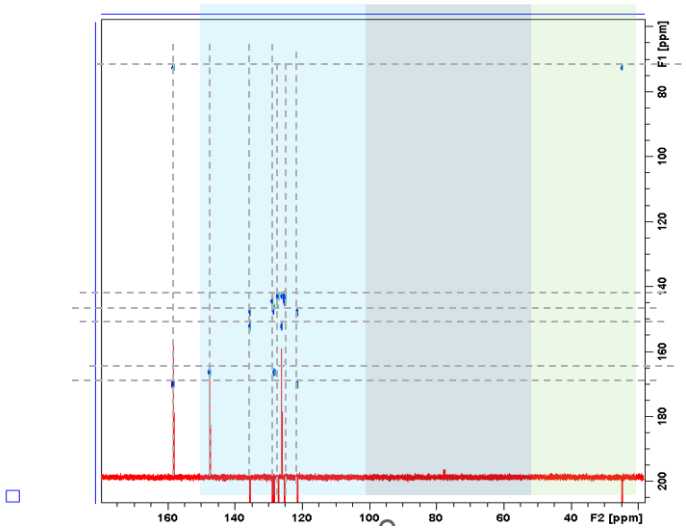


Figure 34. This shows the interpreted INADEQUATE spectra of 2-methylquinoline – 300mg; 256 scans – ran at 600 MHz. The light blue area indicates aromatic region, the dark blue area indicates nonaromatic carbonyls, and the green area indicates the alkane region.

4 DISCUSSION

The investigation and evaluation of previous methods for structure elucidation's impact on strychnine degradation and structural information fulfilled the goals of the study. Additionally, experiments using

contemporary methods were conducted to demonstrate the effectiveness and significance of modern chemistry. Furthermore, to progress the available structural information on strychnine.

The results clearly show that the previous degrading techniques were important in determining the structure; there are problems with many of the methods. The exact alteration to the structure is more challenging to observe with these approaches alone, but the degradation techniques demonstrate that a change has occurred based on colour, effervescence, or phase alteration. The understanding of complex structure and molecular interactions is advanced by the fusion of cutting-edge analytical instruments with the pioneering methods of chemists like Woodward.

4.1 Degradation

Numerous degrading procedures were used; some yielded predicted results, while others produced unexpected
outcomes, but they all produced important insights into the structures of brucine and strychnine. For all degradations, EI LC-MS spectra were generated from a range of different voltages. This displayed how the samples were fragmented as the voltage increased. It also revealed peaks that may not be present at certain voltages due to them requiring a larger voltage to be ionised. A range of peaks was present throughout all spectra, indicating degradant products. Some of these products may have slightly different peaks than expected due to interactions with contaminations / common ions such as sodium or potassium; some of these common ions are highlighted in a study by (Varghese et al., 2012).

4.1.1 Base hydrolysis

The first method was base hydrolysis; multiple conditions were tested to see if this affected the alkaloid's breakdown process. The alkaloids, solvents, base strengths, and whether or not the reactions were refluxed or stirred were the factors that were modified. All this is evident from the EI LC-MS in figures 86-133 and 334 - 343 in the appendices.

Strychnine was first tackled with a moderate degradation technique using two different strength bases, NaOH and $Ba(OH)_2$. From figures 86 – 99, it is evident that only $Ba(OH)_2$ efficiently broke down strychnine, due to the lack of the 335 m/z peak. The two most recurring peaks in the mild degradation of $Ba(OH)_2$ were 283 m/z and 255 m/z. In spectra 100-111, it is clear that sodium hydroxide caused no degradation of strychnine due to the prominent 335 m/z peak. It was predicted that NaOH would still break down strychnine even though barium hydroxide is a stronger base since it contains two hydroxide ions for every barium ion. This is because, to break down strychnine, two equivalents were added to make up for the excess hydroxide ion. This result emphasises that for NaOH to affect strychnine, there must be an excess rather than an equivalence.

From the literature base, hydrolysis with sodium hydroxide is established as an effective method in degrading compounds, specifically alkaloids. A paper produced by (S.T. Narenderan et al., 2021) determined that sodium hydroxide was a successful base in degrading nilotinib. As this alkaloid has the same level of complexity and structural similarities, it would be expected that sodium hydroxide would be able to successfully produce degradant products of strychnine. However, it was unsuccessful in degrading strychnine or brucine; it was concluded that sodium hydroxide degradations were subjected to the wrong conditions. The base needed to be in greater excess to cause an effect.

Altering the alkaloid was the second factor that was measured. Two molar equivalents of NaOH were added, and over the course of a week, both brucine and strychnine trialled a moderate deterioration. Nevertheless, based on the mass spectrum in figures 100-111 and 334-343, NaOH was unable to break down either alkaloid. The major peak in the brucine spectrum stayed at 395 m/z, whereas the strychnine peak in the spectra remained at 335 m/z. It was anticipated from the initial set of degradations that the degradation might not be successful due to prior results in this study. Nevertheless, based on the structure of brucine, it was expected that it might degrade more easily than strychnine.

In order to ascertain whether adding a solvent to the alkaloid and base affected degradation, this was the third parameter that was changed. It is clear from the results in figures 112–114 that adding a solvent to a moderate degradation does not accelerate strychnine degradation. According to earlier research, barium

hydroxide was the most effective base for breaking down strychnine. It was predicted that the increased quantity of hydroxide ions would interact with specific functional groups within the alkaloid, further breaking down strychnine when a solvent, like water, was added. A big peak persisted at 335 m/z, indicating that the combination of a strong base and solvent did not induce any degradation.

The addition of refluxing the degradations instead of letting them spin for a week at room temperature was the fourth constant that was altered. To find out if the rate of reaction increased, the same moderate degradations may be performed with a solvent at a higher temperature by refluxing the reaction. As shown in figures 115-125, strychnine was degraded as a result of refluxing the reaction. Several notable peaks were displayed at various voltages. There were three primary peaks from voltages 0 to 50 volts: 239 m/z, 501 m/z, and 549 m/z. Based on previous degradations with barium hydroxide as the base, the peak at 239 m/z was predicted, indicating that this is a significant degradant product. The barium hydroxide variation that has connected to strychnine but not broken down the alkaloid is indicated by the peak at 501 m/z. Longer refluxing of the reaction could have led to the deterioration. The last notable peak, which occurred at 549 m/z, was not predicted; it could have resulted from an instrument-related strychnine/barium hydroxide adduct. The 549 m/z peak was largest at lower voltages, but as the voltage rose, it started to decrease, and 501 m/z became more noticeable. The strychnine peak appeared, and the primary peak fluctuated to 391 m/z as the voltage reached 60 volts. Voltage increased from 60 to its maximum point, with the strychnine peak remaining the most apparent peak throughout. Since the m/z gives information on the molecular weight, some of these adduct/degradation structures can be approximated using this information. On the other hand, the ability to run the samples with accurate mass would be more beneficial. This would be more helpful in determining the structure of the unidentified adduct or degradant product since it would give a precise molecular weight. Overall, this shows how refluxing degradation improves the process of degradation and offers additional insight into the structure of strychnine.

In the literature, several studies were conducted by (Datsyuk et al., 2008) and (Yin et al., 2015), determining the effect reflux had on chemical degradations. From the data, the conclusions drawn were that in all cases, reflux positively impacted the rate of degradation, as well as enhancing the structural information via degradant products. This correlates with the trends from this research, as the addition of refluxing resulted in greater structural insights, as evident in figures 115-125 and table 1. Refluxing allowed the same reaction to be performed at higher temperatures, ultimately using a combination of chemical and thermal degradation to gain knowledge about the structural insights of strychnine.

The solvent utilised during reflux was the last adjustment to the strychnine breakdown process. Water and ethanol were the two solvents that were assessed, and the base was $Ba(OH)_2$. As previously shown, adding water while refluxing has a beneficial impact on degradation, disclosing multiple degradant products. According to the spectra run at lower voltages (0–30V), the addition of ethanol under reflux resulted in some degree of deterioration, exhibiting important peaks at 239 m/z and 245 m/z; regardless, as the voltage increases, the strychnine peak at 335 m/z becomes more pronounced. Since ethanol boils at a lower temperature than water, it was thought that ethanol would be a preferable solvent for reflux because both solvents induced strychnine to begin to degrade. The solvent can condense and enter the process when it boils at a lower temperature.

A study was performed by (Donoso et al., 1986) evaluating different solvents for base hydrolysis under reflux, and the most effective solvent for this reaction. It was concluded that the most efficient solvent system was a combination of ethanol and water with a pKa value of 6.1. In comparison to the current study, the use of water as a solvent showcased much better results in degrading and providing structural information about strychnine. From this investigation, it was concluded that water was the most appropriate solvent for refluxing the reaction compared to ethanol. Therefore, if run again using different ratios of ethanol/ water and compared to water alone as a solvent may provide greater degradation and structure information.

It was confirmed if degradation had occurred based on electrospray MS. When the original degradations occurred, this instrument was not available. Not all reactions presented a physical change, even though, from analytical data, it demonstrated degradation took place. Nevertheless, certain reactions displayed a visible change to indicate degradation, such as ethanol under reflux, which turned from clear to a dark yellow solution.

In summary, base hydrolysis was a useful technique for breaking down alkaloids since it revealed a variety of structural details about strychnine and other alkaloids. Nevertheless, not all reactions were successful. Figures 86-99 indicate that barium hydroxide was the ideal base to employ for degradation and that adding a solvent has no effect if the reaction is not conducted under reflux. Water, however, is the most useful solvent when the reaction is conducted under reflux.

4.1.2 Dehydrogenation

The second degradation technique involved looking into the double bonds within the molecule by removing hydrogens. This technique was explored in 2 ways via brominating the molecule to displace hydrogen and sulfonating the molecule to create more double bonds within strychnine and kicking out hydrogen atoms.

Upon brominating the molecule, the solution turned from a rich brown to a colourless state, confirming the existence of a double bond within strychnine. This reaction's favourable outcome made it possible to complete a bromination in order to alter the alkaloid and provide structural details. Both bromination reactions were effective, according to the data in figures 134–139, however not all of the material was brominated because a sizable strychnine peak at 335 m/z remained. The two bromine peaks at 412 and 414 m/z represent the two signs of dehydrogenation. When bromine is introduced to a molecule, the two peaks constitute a frequent isotope pattern because bromine has two highly prevalent isotopes of Br, 79 and 81. This gives structural data, which was further enhanced by the use of NMR, a secondary analytical technique. To determine if the reaction was effective and precisely where in the compound dehydrogenation occurred, a range of NMR methods were performed including proton, C^{13} and COSY.

From the NMR spectrums in figure 81-85, it is clear compared to the original strychnine spectrum, that this is less pure due to the high congestion in the alkane region. The two spectra are overlaid against each other in figures 84-85 From this it is clear the two spectra have many similarities, but the peak spectrum as a whole is slightly more deshielded. This is most likely due to the addition of the bromine. Another key indication of the presence of the bromine molecule is the prominent peak at 3.35 ppm, which falls in the desired region. Nevertheless, the spectrum is overcrowded so if run again, a pure shift NMR would be run to remove any multiplet and make the key peaks clearer. In the DEPT Q peak the peaks are within a similar expected range to original strychnine DEPT Q, with the addition of some extra peaks. In the alkane 0-50 ppm region 5 peaks are displayed rather than 4. The peak at 40.62 ppm is a clear indication of strychnine being successfully brominated. A COSY spectrum was run, however provides little information on location of bromine atoms or confirmation of strychnine being brominated. If repeated further 2D NMR techniques would be performed to decipher exactly the position in strychnine that was brominated.

The literature provides many insights into the bromination process and how successful it is in brominating alkaloids. An experiment produced by (Berti and Marsili, 1966) revealed how the structure and stereochemistry caused an effect on the position in the alkaloid that would be dehydrogenated and brominated. Based on the chemistry of strychnine the nitrogen in the indole ring is an optimum position for bromination. This is because it is extremely electron rich as well as being prone to being attacked by electrophiles. This is coherent as from the NMR and MS data there is indication that the compound was brominated. The position of the bromination would need to be further investigated via 2D NMR methods.

A study carried out by (Alka, et al, 2021) highlights how bromination is an effective method in providing information about the structure of compounds. As it reveals knowledge on a variety of characteristics such as its redox ability, spectral data, information about the skeleton of the compound and physical properties. This coincides with the conclusions made from the current research as the 2 brominated compounds displayed a variety of structural information especially when run on MS and NMR.

For various reasons, the procedure of sulfonating the molecule to introduce double bonds into strychnine was applied. First, the purpose of this reaction was to determine whether the compound could be dehydrogenated. Figures 140-218 clearly show that strychnine was successfully dehydrogenated. To find out if adding greater molar equivalence of sulphur would result in the molecule containing more double bonds, five reactions were conducted each time increasing the molar equivalence by one. Although strychnine can have up to five double bonds added to it, this could cause problems because of the molecular strain and the compound's potential difficulty dehydrogenating with sulphur and diphenyl ether.

The typical strychnine peak was absent, and a sizable peak at 334 m/z indicates that the sulfonation process successfully dehydrogenated strychnine with one equivalent of sulfur. This suggests that the molecule likewise added a double bond to the molecule, although the dehydrogenation process took the longest out of the 5 reactions to display a colour change. It is suggested that increasing the quantity of sulfur equivalencies does not result in the addition of more double bonds; rather, because there is more sulfur present, the rate of reaction is increased. Based on the 334 m/z peak, two equivalents of sulfur were dehydrogenated, and one double bond was successfully introduced. While there was a peak at 334 m/z, when the voltage rose, 364 m/z emerged as a notable peak. Since the bulk of the strychnine was dehydrogenated, the 364 m/z could be the result of an ethyl group that was added from diphenyl ether. The first two comparable replies followed this pattern. Once more, the third equivalent dehydrogenated more quickly than the lower equivalent reactions and provided more structural information. As a higher quantity of sulfur dehydrogenated strychnine more quickly than the less equivalent processes. A large peak around 280 m/z was prevalent at lower voltages, which might be a sign that the strain from the additional double bond started to break down part of the strychnine. At 40 volts, the first two reactions were followed by the presence of the dehydrogenated strychnine. Again, the fourth equivalency showed that the notable peak in the previous voltages was 280 m/z, and that 383 m/z dehydrogenated more quickly than the lower comparable processes. Based on peaks seen in the spectra, some of the structures for the expected degradant and adducts are given in Table 1. Between 50 and 100 volts, the expected Strychnine peak at 334 m/z emerged as the prominent peak. Another important high during the same voltage era was 367 m/z. The fifth equivalent reaction followed a similar fragment pattern and yielded information comparable to that of the fourth equivalency. The primary distinction is that 419 m/z is the most common peak at 50 volts, while 334 m/z is present in smaller quantities and doesn't become the major peak until 90 volts.

The process of sulfonation is a promising method, as research performed by Wang et al., 2020) demonstrates how powder sulfur had a beneficial effect in converting cyclohexanones to aromatic species. This correlates with the data found in this experiment, as another double bond was successfully added to strychnine via a similar process. Highlighting how sulfur is a useful tool in dehydrogenation. To pinpoint the exact location of where the double bond was added, 1D and 2D NMR techniques could be run.

Sulfonation, just like bromination, provides a colour change to indicate dehydrogenation has occurred. The reaction changes from a cream / pale yellow solid to a deep yellow/brown solid.

Increasing the sulfur equivalency of the aggregate observations indicates multiple tendencies. It showed that while raising the equivalency of sulfur may not result in the molecule acquiring more double bonds, it does speed up the dehydrogenation of strychnine. This is because there is an overabundance of sulphur rather than strychnine. Additionally, the amount of strychnine dehydrogenated reduces as the amount of sulfur increases in each reaction due to the 334 m/z being less noticeable. Higher Sulfur concentrations not only dehydrogenated the molecule but also caused it to break down, as shown by peaks like 280 m/z. The compound's internal strain from the extra double bond and greater ratios of sulphur, still attempting to dehydrogenate strychnine, is most likely the cause of this. Additionally, it resulted in unanticipated side products, such as 367 m/z, 383 m/z, and 419 m/z, based on adduct peaks. Therefore, additional structural information is obtained by boosting the reaction with higher sulphur concentrations.

Dehydrogenation proved to be a successful technique since, even in the absence of current analysis, both approaches offered valuable insights into the structure. In contrast to alternative degrading methods, such as base hydrolysis, which do not require analytical tools, it is more difficult to determine whether the reaction was successful. However, the incorporation of contemporary analysis increases the information through molecular and structural relationships.

4.1.3 Oxidation

In order to break down the molecule or change certain functional groups, the third degradation process involves oxidising strychnine using a variety of oxidising chemicals. Potassium permanganate was used in the initial series of oxidation processes; the first reaction was a classic strychnine degradation. From figures 219-227, it is clear that the reaction successfully degraded strychnine since there was only a little strychnine peak present, at 335 m/z, and this peak only appeared at 60-70 volts. Additional proof of the degradation's efficacy is a noticeable peak at 245 m/z that exists between 0 and 50 volts. Table 1 contains the proposed structure for this degradant product. Nonetheless, there were two notable peaks at 522 m/z and 550 m/z

between 50 and 70 volts. These peaks are most likely sodium hydroxide, potassium permanganate, and strychnine adducts, suggesting that the reaction may have been agitated longer to promote further breakdown.

A moderate deterioration with acetonitrile was the result of the second permanganate reaction. In comparison to the traditional degradation process, this degradation proved less successful in breaking down strychnine, as evident in figures 228-238. The fact that the strychnine signal at 335 m/z is still noticeable in the majority of the spectra from the two samples that were run indicates this. Despite the fact that both samples had significant levels of unaltered strychnine, a big peak at 239 m/z emerged, indicating the presence of some degradant product in the samples. The samples ranged in voltage from 0 to 30 volts.

From research (Jiang et al., 2012) is apparent that potassium permanganate is considered an adequate oxidising agent, which is consistent with the data retrieved as proficiently broken down strychnine. However, a study produced by (Tibor Dankházi et al., 1993) drew the conclusion that breaking down alkaloids in the presence of acetonitrile had a positive effect on degradation. This is the opposite of the results retrieved from this set of tests. The addition of acetonitrile with potassium permanganate caused little degradation of strychnine. This may be due to an ineffective ratio between the reactants.

Hydrogen peroxide was used as the oxidising agent in an alternate oxidation reaction, which was refluxed with water as the solvent for a duration of 72 hours. To track the efficacy of the reaction, samples of the solution were collected after reflux for one hour and compared to three days. Figures 239-257 demonstrate how little the reflux time changed either sample or how ineffectively both reactions broke down strychnine. The bulk of the spectra show a prominent strychnine peak at 335 m/z, which is indicative of this. There was a consistent peak at 239 m/z in both samples, despite the fact that there were still significant amounts of strychnine present. This seems to be a typical strychnine degradant product based on data from previous reactions. Given that the reaction was less impactful than other oxidation reactions, the reflux could be prolonged, a different solvent could be used, or a higher equivalent of hydrogen peroxide could be used, resulting in an excess of hydrogen peroxide when compared to strychnine. Chloroform was used to separate the reflux after it was run for three days to remove any strychnine or degradant product from the solution. After analysing each layer, strychnine was predicted to end up in the organic chloroform layer. However, given the presence of 335 m/z in figures 258-276, strychnine was visible in both layers. Strychnine could be entirely pushed into the organic phase by introducing an acid to change the pH and force it into one layer.

(Targhan, Evans and Bahrami, 2021) demonstrates how hydroxide peroxide is an advantageous method for oxidation; however, in the current research, it is insufficient, as evident from the data obtained. Nevertheless, two studies conducted by (Mika and Jaakko H. P. Rämö, 2001) and (Wang et al., 2018) suggest that hydroxide peroxide is efficient in alkali conditions when degrading compounds. Therefore, if repeated trialling, alkali conditions would be used to determine if this increased degradation.

Another method of oxidation that was utilised to break down strychnine was the use of chromic acid. The data presented in Figures 277-285 indicates that the reaction was partially successful, since there was evidence of a recurring degradant product of strychnine within the voltage range of 0-40 volts. The strychnine peak was quite noticeable even at lower voltages, indicating that the oxidation was not very successful. This was surprising because chromic acid is a potent oxidiser and should break down strychnine quickly; if this were to happen again, a lot of chromic acid would be needed to make sure there was an excess.

A study performed by (ALESSANDRO, 1932) investigates the oxidation of strychnine. From the results, it displays that chromic acid is a useful reagent in oxidising strychnine to an acid with a molecular formula of $C_{21}H_{22}O_4N_2$. On the contrary, chromic acid had minimal effect on strychnine in the current set of data. As a result, the method used in (ALESSANDRO, 1932) would be considered if rerun rather than the current method used.

A singlet oxygen process using methylene blue is the last oxidation technique carried out for deterioration. It is evident from the spectra in Figures 286-293 that it followed a pattern that was comparable to previous oxidation degradations. Similar to a prominent peak at 239 m/z at lower voltages, the strychnine peak at 335 m/z rose with voltage. Consequently, demonstrating that the deterioration was not particularly successful, like other ways. According to the literature, the initial process was carried out over 24 hours,

whereas this one was run over 48 hours. However, it is clear from the other oxidation processes that strychnine is less susceptible to oxidation reactions. Using various equipment and oxidising chemicals of varying strengths, a range of oxidising techniques was carried out.

A key indicator of oxidation would be a prevalent peak at 334 m/z, showcasing that the ketone group has undergone oxidation to an alcohol group. As ketones usually present as a hard functional group to oxidise. As a result of this require strong oxidising agents are required for this reaction to be successful; the expected structure can be seen in Table 1.

The majority of oxidation reactions had less of an impact on the degradation of strychnine than the other degradation procedures examined. A recurring pattern in the spectra obtained at low voltages is the presence of a modest amount of degradant product. The oxidising chemicals used in the processes were either slightly in excess or in equal molar equivalence. This indicates that a substantial amount of the oxidising chemical is needed to significantly affect strychnine. This might be brought on by the molecule's intricacy. On the other hand, the method continued to offer valuable structural insights into strychnine; however, as with base hydrolysis, this would be difficult to discern without more recent studies. All things considered, it is clear that there are more effective ways to break down strychnine than oxidation.

4.1.4 Amination

Aminating strychnine with a variety of amines in the presence of boric acid was the last degradation method used. To determine which was most successful, four separate examinations were conducted. A meta and an ortho isotope of phenyl diamine were used for the first two aminations. Different versions of the amine groups were used to see if this affected the way strychnine degraded. Figures 293-313 demonstrated how the positional variation of the amine had a noteworthy impact on strychnine breakdown. Based on a high concentration of strychnine that persisted in the sample from the 335 m/z peak present at all voltages, phenyl diamine (ortho) did not affect strychnine. There was no indication of any degradation in the spectra. Figures 303-313 show that, in contrast, the amination of phenylene diamine (meta) was effective. The primary peak was observed at 368 m/z when the voltage grew from 40 to 80 volts, whereas the noticeable peak from earlier levels of 0 to 30 volts was 239 m/z. Further evidence that the degradation was successful comes from the fact that both peaks recur during many degradations as degradant and adduct peaks. Also, there was no evident strychnine peak until 30 volts; even at higher voltages, the strychnine peak remained small. The meta isomer of phenylene diamine was likely to have a greater effect on strychnine because of the stereochemistry of the compound. The 2 amine groups are closer together; therefore can both attack a particular point of strychnine simultaneously, hindering the molecule and causing more extensive degradation. Whereas in ortho-phenyl diamine, the amine groups are too far apart to interact with each other or attack the same point of strychnine. This ultimately caused it to have minimal effect on degrading strychnine.

Morphaniline is the third amine that is utilised to assess amination; a significant base peak at 335 m/z, in figures 313-324, persisted for most of the voltages that were run. Therefore, showing that the amination had only a minimal impact on strychnine degradation. From a peak at 239 m/z, there is some indication of a decline in the lower voltages.

T-butyl amine, the last amine tested, showed a pattern that was comparable to the previous amines looked at, as displayed in figures 325-333. There was a degree of degradation demonstrated based on a recurrent peak at 239 m/z and a new peak at 230 m/z, which has not been present in any other degradations. These two peaks only occurred at lower voltages. Strychnine peak progressively grew more common as the voltage rose, and there was a consistent peak at 368 m/z across the whole set of data. The lack of effective degradation is most likely caused by the fact that it is a very bulky and sterically hindered molecule. Therefore, this makes it difficult to interact with other bulky compounds, such as strychnine, resulting in poor degradation.

The amination process can provide key structural understandings about molecules, as in a study produced by (Dawood et al., 2019), which determined that indole alkaloids could successfully be aminated, in which this process could be considered when totally synthesising strychnine. In the current research, it is also apparent that amination is a successful method for providing structural insights into strychnine. As evident in figures 303-313 via animation with meta – phenyl diamine.

From the data, it is clear that the change in position of the amine group had an influence on how successful the amination reaction was. The study (Kalendra and Sickles, 2003), it shows how the meta isomers of compounds are more reactive and better for substitution reactions than the ortho versions. This is due to the groups being further apart, so being unable to react.

Since all reactions showed some degree of degradation, the amination process was a good tool for learning about strychnine. Nevertheless, the most efficient amine for breaking down strychnine was the meta isomer of phenyl diamine; however, without the aid of analytical tools, it was challenging to ascertain when the reaction had finished. This is because there is no proof that there was a physical alteration that would have suggested a structural change. This is consistent with the conclusions discovered from the data in the current study.

4.2 Woodward's original work

This work was performed based on the original work by (Woodward, Brehm and Nelson, 1947). Solving the structure of this complex molecule without the use of modern analysis demonstrates the qualities of an outstanding chemist. However, there are critics of Woodward's work. The main issue is that the work is hard to reproduce in today's society, making it difficult to determine if there were any inaccuracies, as he was not fully sure of his work until it was published. He determined the structure in 1947; however, publication and certainty of the structure did not occur until 1948. The full determination of strychnine was confirmed via x-ray crystallography, 4 years later in 1950. Then, not until 7 years later was a final confirmation of the structure via total synthesis, which was completed in 1954.

A study performed by (Seeman and Tantillo, 2020) examined how fast the problem of strychnine structure can be solved today with all the new technology. As well as how easy it would be to rule out the wrong proposed structure of strychnine with access to 1H NMR and 13C NMR. If this study included 2D NMR techniques, the problem could be solved at a faster rate.

4.3 Modern instrumental analysis

The power of these modern approaches and the progress made in analytical chemistry since the first structure elucidation of strychnine are demonstrated by the use of current analysis alone to investigate the structure, functionality, and molecular relationships of alkaloids.

4.3.1 EI LC-MS

With the help of electrospray ionisation liquid chromatography mass spectrometry, one can verify the identity of compounds, determine whether reactions were effective, and quantify sample purity by analysing the spectra generated. This is accomplished by using m/z peaks, where the base peak, which is the strongest peak, indicates the sample's molecular weight. The busyness of the spectrum can be used to assess purity; if there are multiple m/z peaks, this may indicate that the sample is impure. Additionally, by adjusting the voltage and mode, several peaks can be revealed. The compounds' functionalities determine which mode is required for the compound to ionise; therefore, the instrument can be operated in either positive or negative mode. A greater variety of molecules can be employed with the instrument because of its adjustable voltage; the higher the molecular weight, the higher the voltage required to ionise the substance. As an alternative, the voltage can be raised gradually to determine the ideal voltage at which the molecule will ionise. Additionally, by progressively increasing the voltage, it can highlight how the product is degraded by ionisation as the voltage increases or reveal elements present in a sample with varying molecular weights.

The technique's sole limitation is that the instrument won't be able to generate a spectrum if the molecule cannot be ionised. It will also be challenging to ascertain whether the base peak represents the molecular weight of the molecule plus one if the molecular weight of the sample is unknown. The reason for this could be contamination or interactions between the base peak and other ions, including sodium, which would result in a m/z of +23.

In the literature, it is presented that EI LC-MS is a highly beneficial technique compared to other analytical methods. An experiment carried out by (Stefan Kg Grebe and Singh, 2011) determines that EI LC-MS is the most suitable spectrometry method, particularly for small molecules. Therefore, this makes it an

extremely useful technique when assessing alkaloids under 500 da. Also, research performed by (Abonamah, et al, 2019) showcased how it had many positive attributes. They included characteristics such as being able to obtain a large range of structural information via altering different parameters and gaining knowledge on the purity of the compound.

On the other hand, the literature, it evaluates some of the negatives of the technique. A study carried out by (Nasiri et al., 2021) stated that this type of instrument is prone to becoming affected by matrix effects and susceptible to blockages. This can then lead to further issues with ion enhancement and suppression. Overall, this can influence how sufficient the spectrum is.

4.3.1.1 Strychnine

From the data in figures 4-12, strychnine was run at voltages from 0 to 80 volts, as 80 volts was the maximum voltage at which strychnine could be ionised. Since strychnine's known molecular weight is 334 g/mol (PubChem, 2024), 335 m/z remains the most prominent peak in all spectra and supports the mw + 1 criterion. The spectra show very few tiny peaks, indicating that the strychnine material is comparatively clean. Based on the molecular weight, the best voltage range to measure strychnine is between 40 and 60 volts, which yields the cleanest spectra. At larger volts there is an example of some of the samples being sodiated with the peak at 357 m/z.

As presented in (Guo et al., 2022), it shows the LC-MS data of strychnine which demonstrates a peak at 335 m/z. This correlates with data retrieved from this study, as strychnine was run at a range of voltages, all present at the m+1 peak of 335 m/z.

4.3.1.2 Brucine

From the data in figures 63-70, brucine was run at a range of voltages starting at 0, with the maximum voltage being 90 volts. The highest voltage at which brucine could be ionised was slightly larger than strychnine; this is most likely due to it having a slightly bigger molecular weight of 394 g/mol (PubChem, 2024a). From 0-90 volts, the brucine peak at 395 m/z was the most prevalent peak. Similar to strychnine, the spectra display few peaks, indicating a pure compound.

In a study by (Guo et al., 2022), it shows the LC-MS data of brucine show a peak at 395 m/z. This correlates with data retrieved from this study, as brucine was run at a range of voltages, all present at the m+1 peak of 395 m/z.

4.3.2 NMR

Compared to all other methods, nuclear magnetic resonance offers the most structural information about a molecule, making it an incredibly useful tool for elucidating a molecule's structure. One can establish a compound's structure and molecular relationships using a variety of 1D and 2D methods. The main benefit of this method is that only a small quantity of sample is needed to produce a sufficient spectrum. Many overlapping clusters of peaks and unexpected peaks in the spectrum can be used to analyse the purity. The drawbacks of this method are that the molecular formula is required in order to accurately infer the structure of the unknown.

NMR is a highly praised technique, as evident from studies by (Aritro Sinha Roy and Srivastava, 2023) and (Williamson and Marquez, 2016). They determined that NMR provided precise molecular connections between atoms, high reproducibility and was extremely effective when retrieving information on small molecules. Although it is a groundbreaking technique when it comes to eluding structures, it does have some flaws. In studies by (Cherni et al., 2019) and (Giraudeau, 2014) it is made clear that NMR is a time-consuming method, especially for more complex 2D techniques. Also, research produced by (Bucher et al., 2020) determined that in comparison to other analytical methods, NMR's molecule number sensitivity is of lower quality.

4.3.2.1 Strychnine

In figures 13-30, NMR spectra confirm strychnine's structure. Strychnine's chemical formula is $C_{21}H_{22}N_2O_2$. In order to assign the structure, the double bond equivalent was first computed using the formula. DBE = C – (H/2)-(N/2)+1. Due to the fact that rings equal four double bonds and carbonyl groups equal two double

bonds, it was discovered that the expected structure included eight double bonds, which matches the structure of strychnine.

Many of the peaks from the proton spectra in figures 13-14 are located in the alkane area, which is between 0-2.0 ppm and corresponds with the structure's many C-H bonds. Additionally, there are many peaks in the aromatic area between 6.0 and 8.2 ppm, which is predicted given the molecule's strong conjugation and aromatic ring. The amide and ether groups provide further significant functions. Two distinct peaks based on each of the neighbouring hydrogens show the presence of the methoxy group. At 3.5 ppm, a doublet is found, as expected given that it is next to an unsaturated C-H bond. Additionally, a triplet is seen at 4.1 ppm, which is predicted given that the other hydrogen is next to a CH2. Both peaks are located in the 3-5 ppm ether range. The multiplet at 2.99 ppm confirms the existence of the amide group, which is consistent with the strychnine structure because it is adjacent to x2 CH2 groups.

Before determining peaks, the orientation of the CH and CH3 groups is first determined using the DEPT Q spectra. The triplet peak from the CDCl3 solvent, which is present at 77 ppm, can help with this. Since this peak is pointing upward, any peaks in the upward region are thought to represent the CH or CH3 groups. The peak in the positive direction at 169.22 ppm is one of the important peaks. This indicates the presence of a ketone group on one of the heterocyclic rings; it does not have any neighbouring hydrogens, but because the carbon adjacent is not quaternary due to being attached to a nitrogen atom, it indicates a positive direction. After that, there are three aromatic peaks in the positive direction at 142.07 ppm, 140.78 ppm, and 132.77 ppm, which are located in the aromatic zone between 150 and 100 ppm. These peaks line up with the structure's aromatic ring. The group's degree of symmetry and the way it is joined to the rest of the structure limit the number of C-H environments to three. Two peaks, one in the positive direction at 64.48 ppm and one in the negative direction at 60.03 ppm, can be used to establish the existence of the ether group. All of this is consistent with the strychnine structure: the peak in the positive direction is a CH, which is attached to two carbons, one oxygen, and one hydrogen; the peak in the negative direction is CH2, which is attached to two carbons and two hydrogens. The peak at 26.73 ppm in the positive direction confirms the presence of the amide group. There are nine peaks in the negative direction of the spectrum, indicating six CH2 groups and three quaternary carbons, and these peaks overlap with the strychnine peak overall.

Several 2D NMR techniques were run to establish molecular interactions between hydrogens and carbons. Methods such as HSQC. HMBC, COSY, ROSEY, TOSCY, and NOSEY were used to confirm structural information about strychnine as evident in figures 13-30. Techniques such as INADEQUATE were run to advance structural data on strychnine.

Strychnine is a complex molecule, and confirming its structure via NMR is the most accurate and efficient method. As studies by (Núria Marcó, Gil and Parella, 2018) and (Martin et al., 2008) display several 1D and 2D spectra run in the same conditions, which correlate with the spectra in the current study.

INADEQUATE studies interactions between two coupled carbons as opposed to a hydrogen-carbon coupled interaction using the same methodology as INADEQUATE. It is possible to deduce information about a compound's carbon backbone from these cross-linked interlinks. Figure 31 illustrates the process of unravelling the strychnine INADEQUATE spectra, while figures 32-34 show the INADEQUATE spectra of other alkaloids.

Before synchronising any cross peaks, the spectrum was compared to the DEPT Q. Based on this comparison, the direction of the peak from the DEPT Q was noted, and the carbon peaks on the horizontal axis were numbered. Next, peaks exhibiting an interaction and aligned along the horizontal axis were combined and recorded. The set of interactions, which are shown in the table, starts to create a route for strychnine's skeletal structure. Finding a peak, in this case, peak 1, that has no other carbon interactions makes it the most useful place to start. It must be near the end of the chain or a group on a ring, as it cannot be in the centre of the pathway.

This pathway began with peak 1, which interacted with peak 15, from 15 the pathway could move down to a different interaction with 15 and across to peak 10. From 10, there was a small interaction with peak 16, which then moved down and interacted with 14. Peak 14 interacted with 13, then horizontally moved to another 13 interactions across to 12. 12 then has an interaction with 18, which has a short interaction with 17. The first half of the pathway remained in the lower chemical shift range, the alkane region. From peak

17, there is a long interaction with peak 3, and the pathway begins to move into the more deshielded peaks. From peak 3, there is an interaction with 11 and 11 also has an interaction with 6. Peak 6 then interacts with peak 4, and peak 4 interacts with peak 2. Peak 2 then demonstrates an interaction with 9, which reacts with peak 8. The final set of interactions occurs from 8 with 7 and ends the sequence 7 with peak 5.

This displays a complete pathway map indicating the chemical interactions and bonding between carbon atoms. Because the entire skeleton carbon framework can be determined from this 2D NMR data alone, this is a very helpful technique that is particularly helpful for complex molecules like strychnine. Because of the potential for interactions and interferences to oversaturate 2D spectra. The main drawbacks of this approach are that it requires more samples—at least 300 mg—long run times, a high number of scans, and interpretation difficulties compared to other NMR methods to obtain a clear spectrum.

As INADEQUATE is determined as a more intricate spectrum to interpret when it has complex small molecules, there is less data available. A range of studies produced by (Sylvian Cadars et al., 2007), (Orendt et al., 1995) and (Horne, 1986) establishes that they are advantageous methods when it comes to aromatic systems, but come with complications. As various parameters may need to be altered to maximise the clarity of the spectra.

A study performed by (Sakas and Dušan Uhrín, 2022) demonstrated how the use of ADEQUATE 2D NMR advances the skeletal data of structures, specifically complex structures such as strychnine. Therefore, highlighting how impactful these new and upcoming spectroscopy techniques are in modern structure elucidation.

4.3.2.2 NMR of other alkaloids

Using the same principle, several other NMR techniques have been run on different alkaloids of different levels of structural complexity. This was carried out to showcase how the same principles of structure determination can be applied to any compound.

In figures x-x, a range of spectra is presented for 2-methylquinoline, in figures x-x, spectra of pilocarpine and in figures x-x, spectra of brucine.

All spectra confirming previous work, the INADEQUATE spectra of the alkaloids are displayed in figures 31-34, interpreted via the same steps as the strychnine spectrum.

4.4 Overview

Both collections of methods provided insights into structure determination, and analytical methods proved to be much more efficient and effective. As degradation methods often didn't work, required trial and error to find optimum conditions or required modern analysis to determine if the reaction was successful.

Modern analysis displayed both confirmation of Woodward's original structure elucidation, provided molecular knowledge on degradant products, and advanced the current spectral data on strychnine.

If rerun, a range of different degradations would be run rather than just chemical degradations. From the literature, thermal degradation and bacterial degradation are showcased as effective methods in breaking down alkaloids based on studies conducted by (Vera-Baquero, et al, 2022) and (Wada, 1957).

As well as the use of other degradation techniques, more analytical instruments would be used to expand the knowledge further on the alkaloids. In combination with EI LC-MS, accurate mass could be used to confirm the exact molecular weight of the degradant products and be able to differentiate between certain fragments.

A study performed by (Cody, et al, 1992) examines the benefits of using accurate mass in hybridisation with other techniques. It is useful for determining molecular masses of molecules.

Another improvement, if the experiment were carried out again, would be to employ statistical tests. A t-test could be employed to evaluate if the size of the molecule had a direct effect on the maximum cone voltage at which it can be ionised. By comparing two alkaloids, such as brucine and strychnine, that are derivatives of each other, it can be determined if, due to the higher molecular weight, they can withstand being ionised by a higher cone voltage.

5 CONCLUSION

In conclusion, the aims of the research were met as the original methods used for structure determination were trialled, tested and modified to find the optimum method. The methods overall were considered highly helpful steps in the synthesis process when it comes to providing structural details about a chemical. However, the information that can be gleaned from these methods is not very extensive. Dehydrogenation is the most efficient type of degradation since it may be used to verify whether the reaction is finished without the need for analytical tools. Additionally, when combined with EI LC-MS, the two dehydrogenation methods—bromination and sulfonation—provided the greatest structural details regarding strychnine. By adjusting certain parameters, base hydrolysis and amination were able to decide which version of the procedure worked best; yet strychnine was unaffected by most of the methods. Since most of the reactions underperformed, and it became clear that more oxidising agent was required to have an impact on strychnine, oxidation proved to be the least successful degradation process. It was discovered that, in contrast to strychnine, the oxidising agent must be in excess. The aims were also met as modern techniques were reviewed and analysed in comparison to older procedures. Both EI LC-MS and NMR proved to be extremely beneficial instruments in providing structural information when paired with degradation techniques and as solo methods. The techniques not only confirmed current structural information but also provided advancements. This was done via m/z peaks of degradant products, ADEQUATE and INADEQUATE NMR spectra. Evidently, modern analysis is much more efficient and precise; however, the methods do have drawbacks. EI LC-MS can be prone to blockage and outside matrix effects, and NMR can suffer molecule number sensitivity, affecting the quality of the spectrum. If the research were to be repeated without time limitations, different degradation methods would be trialled, accurate mass would be implemented to confirm the actual masses of the degradant products, and a wider range of complex alkaloids would be examined.

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