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A Reinvestigation of Salvarsan and Related Arsenic

Chemistry.

A thesis submitted in fulfilment
of the requirements for the degree of

Doctor of Philosophy

in

Chemistry

at

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by

Nicholas Lloyd



THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

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“Life is a chemical incident,

A normal oxidation,

A fluorescence of the brain”

Paul Ehrlich

1872

Abstract

In 1910 the first deliberately targeted search for a new chemotherapeutic agent came to fruition when Paul Ehrlich introduced Salvarsan for the treatment of syphilis.

This thesis presents a detailed review of the history and literature leading up to and following on from Ehrlich's discovery and thoroughly investigates the chemistry of Salvarsan and related species.

A series of arylarsonic acids was prepared and fully characterised by electrospray mass spectrometry, NMR and X-ray crystallography for six examples.

A detailed analysis of the hydrogen bonding in crystals of these molecules showed that they adopt several characteristic motifs which govern the packing in the crystals. Two of the examples containing NH_2 groups crystallised as zwitterions while one NH_2 containing example containing other bulky groups was in its molecular form.

Salvarsan (*cyclo* 3-amino-4-hydroxyphenylarsenic(I)) was prepared by several different methods and analysed in detail using high resolution electrospray mass spectroscopy.

This showed that Salvarsan consists of small cyclic species of the type $(\text{RAs})_n$ where R is 3- NH_2 -4- OHC_6H_3 and n is three or greater. The dominant species in an aqueous solution of Salvarsan were found to be $(\text{RAs})_3$ and $(\text{RAs})_5$.

A detailed analysis is presented of impurities in Salvarsan prepared by different methods and also in a sample of original commercial Salvarsan.

Mixtures of $(\text{RAs})_n$ and $(\text{R}'\text{As})_n$ exchange R groups in aqueous solution at room temperature, as shown by ESI-MS.

ESI-MS studies are reported for the oxidation product of Salvarsan, $\text{RAs}(\text{OH})_2$ (commercially known as Mapharsen) and related As(III) compounds. Oligomers

involving As-O-As linkages were found in solution and one tetrameric example (R = 3-NO₂-4-OHC₆H₃) was isolated and structurally characterised.

Preliminary ESI-MS studies showed that As(III) species bind to thioredoxin, a possible target for the pharmaceutical activity of Salvarsan and its derivatives.

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1 Chapter One - Introduction

The history of an element and the history of a disease may seem like two unrelated topics but as is the pattern for many unrelated things in this world, they came together in a spectacular fusion before separating and going their separate ways again.

The purpose of this introduction is to tell the story of arsenic as it was used as medicine and poison and to tell the story of syphilis - its abrupt appearance as a horrific disease, evolution to something milder and the story of its eventual cure.

More importantly, I hope to tell the story of the people who brought arsenic and syphilis together – the many scientists who have long passed into obscurity and the few who have been remembered.

This history is not written in a linear fashion; rather the threads have been linked as they emerge.

There are problems in writing an account like this including – lack of information, misinterpreted information and misleading information. Many earlier scientific papers contain unattributed or unreferenced claims and these are impossible to verify. I have tried to highlight these problems throughout.

1.1 Some background on arsenic

1.1.1 Pre-alchemy

Arsenic is sometimes found as a native element in nature, but this is uncommon and it is unlikely that this material would have been recognized as “Arsenic” to the early chemists.

Arsenic was known to the ancients as its sulfides, yellow arsenic – As_2S_3 –orpiment (or arsenikon) and red arsenic As_4S_4 – realgar (or sandarach).



Figure 1-1 - Realgar and Orpiment

Both the sulfides are mentioned in Dioscorides and they are also mentioned in Chinese medical and alchemical tradition since the Han Dynasty (second century B.C.) in the *Tzu-hsu fu* by Ssu-ma Hsiang-ju.¹

Arsenic also occurs in association with many other ores² which meant that as people began to smelt copper and bronze and later iron, the oxide would have been found as a white powder on the chimneys of the vessels that were used for smelting, where it would deposit from the smoke. This became known as white arsenic and enters history in written records in the West sometime around the 8th century when it reported to have been “discovered” by an Arabian alchemist (possibly Geber) by distilling orpiment.

It is likely that this white arsenic was known earlier in folk medicine but this is hard to substantiate.

1.1.2 Discovery of elemental arsenic

Preparation of arsenic as a pure element is widely attributed to St Albertus Magnus²⁻³ (1193 – 1280AD) but it was probably prepared earlier by several earlier alchemists – with Zosimos (~300AD) and Geber (721-815AD) both being contenders.

Holmyard paraphrases Zosimos in his book 'Makers of Chemistry'⁴ as follows

“The 'second mercury', [arsenic], he says can be obtained from sandarach [arsenic sulphide], by first roasting it to get rid of the sulphur, when the 'Cloud of Arsenic' [arsenious oxide] will be left. If this is heated with various [reducing] substances, it yields the second mercury [metallic arsenic], known as the 'Bird', which can be used to convert copper into silver [copper arsenide is a white metallic or metallic looking compound not altogether unlike silver].”

Provided that the interpretation of the alchemical terms is correct and that these records are not actually falsely attributed from a later date this seems to provide a very early written method detailing preparation of elemental arsenic and also white arsenic, which seems to have escaped the notice of Dioscorides⁵ (40-90AD) only a few centuries earlier when he compiled his pharmacopoeia of known remedies at the time. Thomson⁵ points out that this omission is unusual as if the substance had been known its high toxicity would have earned it a mention.

Zosimos⁶ lived in Panopolis at the end of the third century or the beginning of the fourth and is one of the earliest alchemists to have left identifiable written records. His diagrams show equipment which, although primitive, would still be recognizable today. Zosimos was influenced by Gnostic teachings and Greek Hermetic beliefs and those writings which are available in English are more mystical in nature than anything we would consider to be scientific today. Incidentally, (according to Holmyard) the word “Chemeia” occurs for the first time in his writings – this being the one of the possible origins of “Chemical” and “Chemistry” as they are used today.⁴

1.1.3 Geber or Pseudo-Geber?

The other alchemist who possibly mentions elemental arsenic prior to Magnus is Geber, or Abu Musa Jābir ibn Hayyān (~721-815). He was an alchemist of the Islamic school, which appears to have evolved from Greek alchemy as Islam started to rise in the East.

Thomas Thomson⁵ mentions that Geber was familiar with metallic arsenic and that he mentions it several times in his books in particular his treatise on furnaces.

Sadly this body of work is now considered to have been written by a later writer (known as Pseudo-Geber)⁷ in the 13th century and so the authenticity of this claim is unknown and still debated.⁴ It was common practice for lesser known alchemists to attribute their writings to some of the more famous alchemists, so the authenticity of many manuscripts is disputed and these problems will probably never be solved.

1.1.4 Chinese alchemy

Alchemy wasn't confined to the Greek and Arabs though – the Chinese had been studying alchemy and medicine for thousands of years and knew that they would prepare arsenic oxide by roasting realgar in air.³ The Chinese were the first to recognize that the substance they could prepare was the same as the arsenic oxide they found naturally as Arsenolite. It is unknown whether the Chinese prepared elemental arsenic as the records of the Chinese alchemists are less accessible than those of the Eastern and Medieval schools.

The links between Chinese alchemy and Greek (and later Islamic) alchemy are also hard to determine but there was known to have been some crossover of information between the different cultures, carried by traders and wandering teachers. Both disciplines had a strong connection to mysticism and the different schools of thought were influenced heavily by the different worldviews of the surrounding culture.

1.1.5 Problems

These descriptions of early history have many flaws – the alchemists in both the East and the West seemed to enjoy writing their manuscripts in what many today refer to as a code – understandable only to the initiated. This coupled with the problem of language and the lack of original manuscripts means that it is very much a speculative history.

Did Geber actually discover arsenic the element or did he merely discuss the preparation of arsenikon, the oxide? One imagines that maybe he would have thought his white powder (the oxide) to be as pure as he could get it and cease further work, or did he perhaps mix it with dung or soap and try and distil it again? - In which case he may well have prepared the element.

Did he really author all the attributed manuscripts, or did he merely borrow from earlier writings?

One also wonders if the white arsenic found around bronze and iron smelters was not kept and used as a folk remedy of some sorts before it passed into written knowledge, without realizing that it was actually arsenic or connecting it with any of the known arsenic compounds of the time.

1.1.6 Albertus Magnus

Needless to say, by the time Albertus Magnus (1193 – 1280AD) was doing his alchemical experiments arsenic oxide was well documented and was a common remedy for many ills.

Albertus Magnus seems to give the most definite preparation for elemental arsenic, although he was still very much influenced by the alchemical beliefs of the time. His

records suggest that he distilled arsenic trioxide with soap (Cullen³ suggests perhaps soot was used – but I would imagine that either would work as a reducing agent) Some sources suggest he may have used orpiment which would have given similar results.

Sadly the works of Albertus Magnus are not readily available in English, nor do they make much sense without knowledge of how the universe was understood at the time – often containing observations of the natural world combined with theological reasoning and mystical nonsense. His contribution to theology however is great - he was the one of the first scholars to apply Aristotle's philosophy to Christian thought. He was also interested in all the sciences although doubt remains as to how much alchemy he was actually involved with, as it seems he also could have had many works falsely attributed to him. It could be said that this merely makes the study of the history of alchemy more interesting as there comes a point where it is impossible to actually determine with any accuracy who wrote what - further blurring the lines between fact and fantasy which alchemical writings are well known for doing. Analysis and debate on this subject still continues to this date.

Whatever happened, elemental arsenic was definitely known to the alchemists by the 13th century. At this time alchemy and chemistry were still firmly woven together.

To put some point of reference in, the periodic table was not formulated until 1869.

1.2 Syphilis

1.2.1 Early medicine

From early on arsenic, in the form of its various compounds, was used as medicine – in the broadest sense of the word. Medicine at the time was based on the Greek and

Roman ideas of Humorism – this concept postulated that the body was four basic substances, yellow bile, black bile, blood and phlegm. Disease arose when there was an excess or lack of one of these substances. These substances were also connected to the four elements – fire, earth, air and water, respectively.

Cures were designed to balance out the excess or lack of these humors.

Reading some of the reports written by physicians of the time, one gets the idea that they applied remedies with a sort of hit or miss abandon. If something didn't work, something else was tried.

Humorism was debunked by many people – most notably when Ibn al Nafis (1213-1288)⁸ discovered pulmonary and coronary circulation, but its influence on medicine lasted much longer. To give one example, the practice of blood-letting was still practiced well into the 19th century.⁹

The study of medicine however, was evolving at a similar rate to other scientific ideas – remember that the germ theory of disease wasn't widely accepted until after 1875 and the periodic table was not formulated until 1869.

1.2.2 A new disease

Syphilis, like arsenic, has origins which although more recent, are lost in the clouds of history and subject to much debate today.

Syphilis first appeared in written records as a distinctive disease in 1495 when Cumano, a military doctor to the Venetian troops who pursued Charles VIII in his retreat after the Battle of Fornovo related that he saw

“several men-at-arms or foot soldiers who, owing to the ferment of the humours, had “pustules” on their faces and all over their bodies. These looked rather like grains

of millet, and usually appeared on the outer surface of the foreskin, or on the glans, accompanied by a mild pruritis (itching). Sometimes the first sign would be a single “pustule” looking like a painless cyst, but the scratching provoked by the pruritis subsequently produced a gnawing ulceration. Some days later, the sufferers were driven to distraction by the pains they experienced in their arms, legs and feet, and by an eruption of large “pustules” (which) lastedfor a year or more, if left untreated.”¹⁰

**De Balafra morbo galloꝝ
preservatio ac Cura a Bartholo-
meo Steber Viennensi artium &
medicinis doctore nuper edita.**



Figure 1-2 - An early engraving showing doctors treating early syphilis patients¹⁰

Another doctor Benedetto, who served at Fornovo was quick to note the nature of the disease.

“Through sexual contact, an ailment which is new, or at least unknown to previous doctors, the French sickness, has worked its way in from the West to this spot as I write.

The entire body is so repulsive to look at and the suffering is so great, especially at night, that this sickness is even more horrifying than incurable leprosy or elephantiasis, and it can be fatal.”¹⁰

Benedetto’s comments are interesting in three respects – Firstly he notes the sexually transmitted nature of the disease (remember this is about 400 years before the germ theory of disease). Secondly he notes that it was a new condition – one which he had not observed before. Thirdly, in an era where plague, smallpox and leprosy were commonplace he notes that this new disease caused terrible suffering.

This new disease very quickly spread across Europe – called “the French sickness” by the Italians, “the Neapolitan disease” by the French and finally, after everyone had named it after their least favourite enemy it came to be known as “the Great Pox”.

1.2.3 Origins

As it spread across Europe people started to debate over its origins. Early theories involved things like “the intercourse of a leprous knight with a courtesan”, “the coupling of men with monkeys (or other animals)”, “mixing leper’s blood with Greek wine” and the Neapolitans poisoning wells. Some observers were quick to note that it must be a symbol of Divine anger, a fact made more obvious by the venereal nature of the disease. It is interesting to note that all of these explanations in slightly different guises have been theorized for the appearance of the disease which has taken the place of syphilis in our own culture - AIDS – the mating of men with monkeys, a disease made by vengeful American scientists, a disease spread by disliked minorities (this time Haitians and homosexuals) and (of course) the wrath of God.

Some doctors of the time considered it to simply be a new form of leprosy – one paper mentions

“An early European writer Gordonio as reporting an epidemic of “lepra”, or leprosy. He described the infection as highly contagious with a short incubation period. Gordonio stated that children were often born with “Lepra” and that it was acquired venerally.”¹¹

The authors of the paper go on to conclude that the disease must have been syphilis.¹¹ If this was the case, then the comments made by the military doctors at Fornovo that the disease was new were rather odd – they would have presumably have known many of the diseases of the time as many diseases appeared during and after times of war due to the conditions.

The rapid spread, extreme symptoms and mortality rate of the disease seems to suggest an immunologically unprepared population which adds credence to the idea that syphilis was something new.

The possible link between the discovery of the Americas by Columbus (in 1492, although he arrived back in Spain in 1493) was pointed out in the 1520's by Gonzalo Fernandez de Oviedo y Valdes and then later, in 1539 in a treatise on syphilis by Diaz de Isla.

The link between Columbus's voyage and the battle of Fornovo is unmentioned but syphilis arrived and spread very quickly, so it is possible that the disease was present before the battle, but not noted. Maybe some of the troops at Fornovo had been to the Americas with Columbus – sadly history omits to mention this.

Incidentally, the captain of Columbus's ship Martin Alonso Pinzon (and/or his brother Francisco) is reported to have died of syphilis in 1493 – two years before it was noted at the battle of Fornovo.

The theory that syphilis was already present in the old world had its supporters too – with some people suggesting that some of the diseases mentioned in the Bible and other ancient documents were possibly syphilis.

This debate has been going ever since – with people producing archeological corpses showing bones with possible syphilitic lesions and so on and shows no signs of being resolved in the near future.

1.2.4 A complicated disease

Things are complicated by the fact that *Treponema pallidum* has four very closely related subspecies each causing a different disease.¹²

T. pallidum pallidum causes syphilis and is spread through sex or transmission of infected body fluids.

T. pallidum pertenue causes yaws and is found in tropical areas and is spread by skin to skin contact with infected lesions.¹³

T. pallidum endemicum causes bejel which is spread by mouth to mouth contact or contact with infected eating utensils.

Finally *T. pallidum carateum* (often considered a species in its own right) which causes pinta, which was confined to the New World and is spread by skin to skin contact – no known specimens of this disease remain and no cases have been reported since 1988¹⁴ so it is not known if it still exists. It was the mildest out of the four *T. pallidum* subspecies causing changes in skin pigmentation and is considered a rare disease. *T. p.*

careteum has already been hypothesized as the disease from which yaws and syphilis evolved.¹⁴

The four diseases are serologically identical. Hasselmann¹³ provides useful comparisons between syphilis and yaws.

Bejel, yaws and pinta have apparently evolved together with humans for a very long time – all three have similar but different symptoms, none of which are as severe as syphilis was when it was initially observed at Fornovo.

A recent paper¹⁵ has studied the phylogenetic relationships between the subspecies and forms. This paper concluded that the closest relatives to venereal syphilis were from the New World, while the Old World had known yaws for much longer. It also suggests that syphilis arose comparatively recently in human history. This fits very well with the early records of a new disease. Another recent study examines some of the historical bones available and comes to a similar conclusion.¹⁶

Yet another study has noted that the virulence of syphilis has subsided considerably since it was first documented and attributes this to rapid evolution in order to ensure the survival of the disease.¹⁷

Sadly *T. pallidum careteum*, which was the New World *Treponema*, was not available to be studied by the group – seeing how the genome of this species fits into the equation might provide some more useful answers.

Flannery¹⁸ suggests that maybe the combination of the New World and the Old World strains of *T. pallidum* resulted in the formation of the new disease and notes that it is of interest that the disease arose in the Old World and not in the New World.

If a gene transfer between two populations of bacteria was to occur (and gene transfer is well documented in bacteria) such a combination would only have to occur once for a disease as contagious as syphilis to spread very rapidly in an unprepared population. I

have to wonder about the mathematical possibilities of this happening in the human population with a disease like syphilis – for this to happen once must be a rare event, let alone the same mutation occurring in both the Old and New World simultaneously to give the same new disease.

This still raises questions of how a skin disease evolved to take advantage of a venereal method of transmission.

One can only imagine however, the scenario in which this could occur – a sailor returning from the New World carrying a New World strain of *Treponema*, visiting a brothel and picking up an older strain. Maybe the prostitute came to be carrying two strains of *Treponema* which did not cause noticeable infection. Untreated they continued to multiply until some gene transfer took place giving rise to a new combination and a much more virulent disease. Another soldier had intercourse with the woman and took the new strain with him and, not realizing what the primary chancre, was spread the disease to other prostitutes who became infected. And so on.

One can however see a form of poetic justice taking place – as Columbus and his men colonized the Americas, raping, pillaging and spreading smallpox among a vulnerable and unprepared population,¹⁸ syphilis seems some small revenge for the many atrocities committed by the colonialists. Of course, this is never mentioned in history, which seems only to remember that smallpox was God's way of clearing the American lands of indigenous (and viewed by the colonists as inferior) people to allow the white conquerors to take hold of their destiny.

1.2.5 New disease needs new cure

The new disease called for a new cure. The doctors of the time were quick to decide on gaiac (a decoction made from the bark and wood of the New World tree, *Guaiacum officinale* – see below) and mercury as important cures for the new disease.

Some of the early recommendations are very interesting – Juan Almenar who in 1502 wrote a treatise on the new disease recommends that

*‘if the penis is ulcerated and infected, you must immediately wash it with soft soap, or apply it to a cock or pigeon plucked and flayed alive, or else a live frog cut in two’.*¹⁰

As the sexual nature of the disease was quickly noted, many doctors recommended simply avoiding sexual contact with infected women, but as the initial infection in woman was often internal, this may or may not have been useful advice as it was not easy to determine if a woman was infected or not. Most of the early treatment assumes that the sufferer is male probably because the initial chancre in the female was internal and not easily observed. The difficulty in treatment was compounded by the fact that the initial symptom - the primary chancre, would often go away without (and presumably also with ineffective) treatment. Two prominent treatments arose from an initial diagnosis that the disease was untreatable. Initially mercury was used, both as compounds applied to the skin and ulcers and internally.¹⁹ Mercury was also abused by some ‘doctors’ after fast money (one hesitates to use the word “charlatan” as most of the doctors of the time would be covered by the modern definition of that word). It was around the 16th century when people started to doubt the benefits of mercury which had to be carefully administered and had many horrible side effects. In 1500, Pintor recommended mercury ointments used as frictions, as well as bloodletting. The doctors

found just about every possible way of introducing mercury into the body – ranging from mercury salts taken internally, mercury baths in which the patient would inhale mercury vapour, all manner of ointment and pastes right through to ‘aphrodisiac chocolate’ – which was chocolate laced with corrosive sublimate. There were also many disagreements between doctors about the best way to treat patients – it seems that mercury was administered to patients in just about every way possible. Figure 1-3 illustrates the popular conception of mercury treatment at the time.



Figure 1-3 - Picture showing various mercury treatments for syphilis²⁰

1.2.6 Competing cures

Ulrich Von Hutten wrote a book in 1519 called “*De Morbo Gallica*” (On the French Disease) which is considered to be one of the first patient narratives in the history of medicine. He wrote about his treatment including the use of mercury which was horrendous. He is one of the first to mention the use of gaiac, which he claimed to have been cured by, presumably where mercury had failed. Gaiac was derived from a tree,

Guaiacum officinale (and possibly *Guaiacum sanctum*) found in subtropical and tropical regions of the New World.

It was the source of “*Lignum Vitae*” or the wood of life. It has a very dense wood and has been used to make police battens, cricket balls and even ball bearings in the past. It is also very resinous and contains many phenolic compounds including guaiacol which is the trivial name for 2-methoxyphenol.

To cure syphilis, a decoction was made of the powdered wood and it was simmered. The cure involved putting the patient in a warm room, keeping them on a strict diet and administering mild purgatives. Every day they had to drink a large dose of the gaiac decoction and then were wrapped in blankets to sweat.¹⁰ In light of some more modern treatments for syphilis one wonders if the phenolic compounds in Gaiac had any therapeutic effect or if any effect observed was simply due to the fevers it caused killing the *Treponema*.

Oil of Gaiac can also refer to an essential oil from the related *Bulnesia sarmientoi* tree. It is possible that this could have been used instead or as well as *Guaiacum officinale* extracts in some treatments, making it harder to determine what the “Gaiac” used in treatment actually was. I suspect that other tropical (and probably local) woods could have found their way into the various concoctions used.

Often mercury and gaiac were combined for treatment – the two treatments together succeeding where a single treatment might have failed.

Other remedies were often called for and as would be expected these were often bizarre and unpleasant.

1.2.7 Arsenic

Arsenic was another substance known to doctors of the time and was recognized to be toxic in small amounts. An interesting early case of the use of arsenic is the ‘arsenic eaters of Styria’ who were reported to ingest quantities of arsenic trioxide to enhance the complexion, make breathing easier at high altitudes, act as an aid to digestion, act as a prophylactic against infectious disease and increase courage and sexual potency. They were reported to build up a tolerance to arsenic, to the point where the quantities ingested would be toxic to an ordinary person.²¹ The first person to suggest arsenic could be used as a medicine was Villalobos in 1498.¹⁰ He objected to the extensive use of mercury, presumably on the grounds of its horrible side effects and recommended instead lenitive (analgesic) medicines. He also favoured purges, clysters (enemas), baths and bloodletting. He does recommend the use of mercury ointment but only as one part in six.

Villalobos recommends arsenic to be used as an ointment as follows.

“Take equal parts of both arsenics (presumably red and yellow, or maybe white), of flowers of sulphur, black hellebore, pine resin and garlic ash; mix with myrrh, incense, aloe, nickel, dull mercury, axungia, citron juice and lemon juice; add oil and apply to the scabs”

Arsenic never seems to have been a regular remedy for syphilis and is mentioned few times along with other common medicinal compounds such as antimony and lead.

Mercury however, remained the mainstay of syphilis treatment and was still in use well after the invention of Salvarsan.¹⁹

Arsenic gained popularity in medicine during the 18th century – so much so that it was branded the “therapeutic mule”.²¹ Fowlers solution, which was defined as:

*“a 1% solution of arsenic trioxide dissolved in potassium carbonate with a little tincture of lavender added ‘merely for the sake of giving it a medicinal appearance’”*²¹

was added to the London pharmacopoeia in 1809.

1.3 More on syphilis

Initially syphilis had appeared out of nowhere and despite the efforts at treatment still caused much suffering – suffering so great that syphilis was known as “the great pox” to distinguish it from smallpox,²² - another fatal disease which was common at the time.

As the disease spread the early doctors observed (as early as 1508) that it was getting milder in nature. This was possibly caused by a simple form of natural selection – as the more virulent disease wiped out its host it was less likely to be transferred to another host.¹⁷ Therefore strains that didn’t cause the host to die quickly were more likely to be passed on to other individuals and so on. Von Hutten notes that the first seven years of the disease were its most virulent. Fracastor (Girolamo Fracastoro, 1483 – 1553) notes in 1546 that the nature of the disease had changed

*“Although this pestilential sickness is at present still fully active it is no longer the same as it was at first....the sickness is in decline, and very soon it will no longer be transmissible even by contagion, for the virus is getting weaker day by day.”*¹⁰

Some doctors even went further and gave the dates they expected the disease to have disappeared by.

Syphilis continued to occur though and had a similar stigma to that which AIDS would have today – an unpleasant and difficult disease which was often fatal.

As it continued to spread in the population it became known as “the great imitator” as its symptoms were many and often imitated those of other diseases, causing it to have a difficult diagnosis.

Fracastor, who was a much respected doctor and scholar of the time, was the first person to call the disease Syphilis. His poem “*Syphilis sive morbus gallicus*” (Syphilis, or The French Disease) tells the story of the shepherd Syphilus who offended the Sun God by overturning his altars and replacing them with altars to King Alcithous, whose flocks he tended. As punishment, the Sun God sent a nasty venereal disease.

Syphilis wasn't widely used as a name though as most people preferred to stick with the old word “Pox” until the end of the 18th century.

Fracastor's treatise on syphilis talks about American origins and conjunctions of stars as well as recommending mercury and gaiac as cures.

Fifteen years later, in 1545, he published another book - this time on contagious disease where he suggests that disease could be caused by the multiplication and spread of tiny invisible living things – hundreds of years before germ theory was recognized.¹⁰

1.3.1 More confusion

Understanding syphilis in history is far from simple. There are three main problems which added to the confusion – both to the doctors of the time and the scientists and historians who seek to understand the disease today.

1.3.1.1 Many diseases – one name

The “pox” was often used as a blanket diagnosis for many forms of venereal disease including gonorrhoea. Some physicians even went as far as to say that all venereal disease was caused by different manifestations of the same poison. This confusion persisted way into the 19th century. The first person to provide evidence that this was not the case was Balfour, in 1767 when he published a thesis claiming two different diseases. The experiments to prove this seem to have involved his students and leave little to the imagination. In reply, Hunter went so far as to inject himself with gonorrhoeal pus which apparently produced syphilis chancres.¹⁰

One imagines the confusion could be further added to by the fact that prostitutes could carry both diseases at the same time and presumably which one was caught was a matter of chance.

1.3.1.2 Different stages

Syphilis also has several different and distinct stages.

Congenital syphilis could also occur when the baby was infected within the mother’s womb. Such children were often born deformed and brain damaged and rarely survived.

Primary syphilis is the first sign – usually a chancre at the point of infection.

This is usually a lesion which appears several weeks after the infection.

Commonly only one chancre is found. They can occur anywhere on the body – usually on the genitals as this is commonly the point of infection. This creates an added point of confusion as the lesion can remain invisible in females as it is internal.

The chancre usually heals spontaneously, but the syphilis bacteria continue reproducing in the body.

Secondary syphilis occurs 1-6 months after the primary infection and usually manifests itself as a rash on the body as well as many other symptoms including fever, headaches and sore throat. Like the primary stage it will often appear to clear up on its own.

Latent syphilis can occur at any stage and is defined as having serological proof of infection with no symptoms – this was not recognized until the Bordet-Wassermann test was discovered in 1906.

The early, secondary and latent stages are considered to be the most infective stages but the disease is transmittable at any stage.

Tertiary syphilis is the final stage and can occur up to 50 years after the initial infection. This stage is recognized by the formation of gummas which are soft tumour-like inflamed balls. At this stage the disease has invaded almost all the organs of the body and can lead to heart failure (where the bacteria has invaded the heart and vascular tissue), neurosyphilis (where the bacteria has invaded the brain and CNS), bone problems (where the bacteria has caused lesions on bones) and general bodily decay.

These stages had implications on the treatment as prior to the test there was no way of knowing if the disease had in fact been cured or just passed into latency.

Did mercury actually cure syphilis or did the disease just clear itself up and disappear until the patient was much older and had forgotten the episode, not to link it in with the disease and bodily decay that could have passed for early aging?

It should be mentioned that these stages are generalizations, any symptoms can appear, or none at all which leads into the third point of confusion.

1.3.1.3 Syphilis – the great imitator

Due to the variation of symptoms of syphilis it was dubbed “the great imitator” as it could imitate the symptoms of many other diseases, especially as it continued into the later stages.

This is no longer a problem as the disease can be detected in the blood but prior to the discovery of the Bordet-Wassermann test, syphilis could be suspected with the onset of many different diseases.

Today this has had the interesting result of many people speculating about which historical figures in fact had syphilis and which merely went insane or suffered other diseases. Hitler, Stalin and Van Gogh are among the suspected group as well as many other historical figures.²³

Figure 1-4 illustrates the popular conception of syphilis during the nineteenth century – the patient, complete with his vial of mercury prepared to enter the hospital, turning his back on debauchery, luxury and cavorting woman in the background. In the foreground a snake is eating his child - representing the effects the disease had on those unfortunate enough to have been born with it.



Figure 1-4 - Engraving by Rambert dating from 1851¹⁰

1.3.2 Other interesting points on syphilis

Syphilis has many other interesting historical interludes. With the discovery of vaccination by Jenner in 1796 it wasn't too long before someone came along with a

vaccine for syphilis. This, in 1844 led to Dr Auzias-Turenne promoting the idea of “syphilization”.²⁴

Syphilization involved repeatedly injecting the contents from a syphilitic chancre into another person. It was reported that as these injections continued, that the lesions formed became less and less serious until further injections gave no lesions at all.

This was, predictably, a failure and one wonders how many healthy people were infected by this procedure – Dr. Auzias-Turenne clung to the idea that gonorrhoea and syphilis were the same disease and he also tried the procedure on himself (something which was also popular at the time). Syphilization was deemed to be dangerous by 1852 in France. However it appears to have remained popular elsewhere for longer.

Another episode of mention is the Tuskegee study of untreated syphilis in the Negro male²⁵ which was only ended in 1972 when its continued existence was exposed to the press. This study was exactly as the title said it was. In 1932, 399 African American sharecroppers seeking treatment for syphilis were selected and instead of receiving treatment which was readily available at the time, were simply monitored as the disease progressed. By the time the study was finished 28 participants had died of syphilis, and 100 were dead from complications of syphilis. 74 of the test subjects were still alive. During the course of the study, wives had been infected and children had been born with congenital syphilis.

1.3.3 Advances in treatment

As science advanced, the understanding of syphilis also increased. By the middle of the 19th century syphilis had come to be seen as a disease separate from gonorrhoea and other venereal disease. The idea of different diseases being caused by different forms of virus

(the word virus was originally used to denote a poison, or substance rather than organisms) began to gain popularity and eventually led to germ theory. In 1837, Donne claimed to have observed a spiral microbe in syphilitic lesions, but this appears to have passed unnoticed although several authors mention Donne's discovery without providing a reference.²⁶

In 1890 Koch published his famous postulates on the relationship between microbes and disease – this was one of the key advances leading to a modern understanding of the nature of disease.

In 1903, Metchnikoff and Roux proved that monkeys could be infected with human syphilis and then that rabbits could be used for *in vivo* studies into syphilis. This finally proved that a microbe was responsible and in 1905 Schaudinn and Hoffman discovered a small spirochaete in syphilitic lesions and other diseased tissue.¹⁰ This microbe was named *Treponema pallidum*.

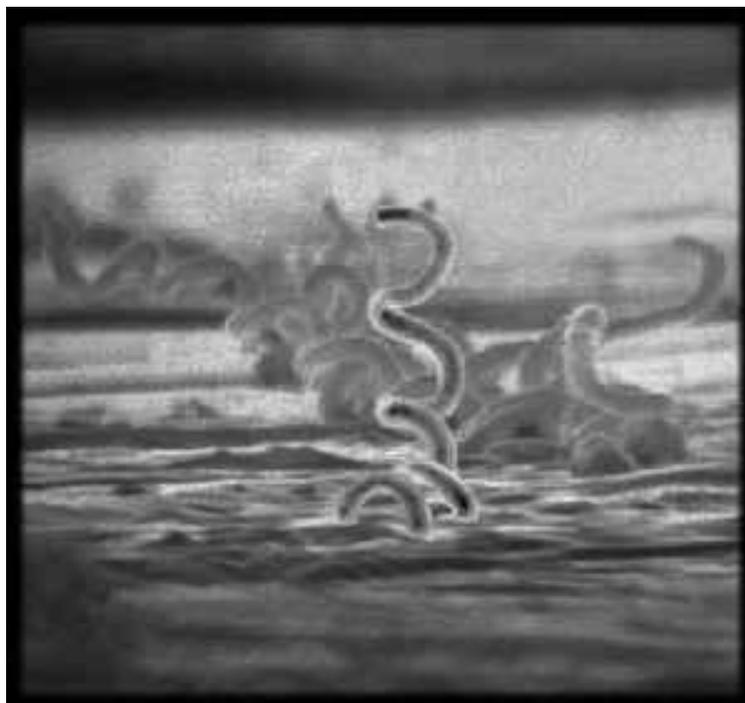


Figure 1-5 - *Treponema pallidum* – the microbe that causes syphilis.

In 1906 a test was finally discovered for syphilis, the Wasserman test (which was also referred to as the Bordet-Wassermann test, as Bordet had discovered the technique used).

By 1906 the nature of the disease was known, the causative microbe had been discovered and people were working on culturing *T. pallidum in vitro* and finally a test had been discovered to prove more or less (the Bordet-Wassermann test had a reputation for giving false results¹⁰) conclusively if one had syphilis. At this time mercury was still used as a cure although people were well aware of its toxicity and mercury baths had passed out of use.¹⁹ All that was needed now was a reliable cure.

In late 1909 (or early 1910), Paul Ehrlich announced that he had produced a substance which had successfully cured 24 syphilitics. Arsenic and syphilis had collided and Salvarsan made its way onto the world's stage, albeit ephemerally.

1.4 Paul Ehrlich

To study Paul Ehrlich and his contemporaries is to study an era of science and humanity which has long passed. It was an era of heroic discoveries motivated by interest and a desire to help other people. It was also an era of faith in science in which every problem looked solvable and the future looked bright.

1.4.1 Beginnings

Paul Ehrlich was born in 1854 into a prosperous Silesian-Jewish family living in Strehlen, Lower Silesia. According to his biography

“Paul Ehrlich was born on a beautiful day in early March.... A day when refreshing breezes laden with the fragrance of newly ploughed soil gave a foretaste of spring and warmth and sun.”²⁷

Unfortunately the biography is written rather romantically which somewhat spoils its usefulness when it comes to facts – the discovery of the effectiveness of Salvarsan is sandwiched between reminiscences of burnt mice, sick dachshunds and cigar boxes. Very little seems to have been written about his childhood although most point out that his interest in the sciences started early, most notes about his life seem to leave it out altogether. It is known however that he was inspired and mentored by his mother’s cousin – Carl Weigert²⁸ who was a well-known pathologist. By all accounts the young Paul Ehrlich was a good student at school and one story which is mentioned in several different biographies is the German composition he wrote for his final exams in 1872 on the topic “Life, A Dream” – Ehrlich’s essay concluded “Life is a chemical incident, a normal oxidation, a fluorescence of the brain” which needless to say was not what his teachers had been expecting.²⁷⁻²⁸ He went on to study medicine and pathology and had an interest in microscopy and staining of biological material.

Ehrlich was studying at the University of Breslau when he first met Robert Koch – the biography says he was introduced to Koch as “Little Ehrlich - He is very good at staining, but he will never pass his examinations”²⁷ - Ehrlich was later to collaborate with Koch who was doing ground breaking research with the Anthrax bacillus and tuberculosis. His MD was awarded from the University of Leipzig in 1878 and his thesis was entitled “Contributions to the theory and practice of histological staining”.²⁹ His interest in the way different compounds bound to cell walls would be a theme

throughout his life, indeed the concept that guided most of his work was that “chemical affinities govern all biological processes”.³⁰

1.4.2 Chemistry in Ehrlich’s time

Staining was in vogue at the time – William Perkin had discovered “Mauve” in 1856 and this had led to a rush of interest in dyes and how they could be used.³¹ This is simply another example of how science seemed to work at the time - Perkin had set out to synthesize quinine and failed, discovered mauvine in the process and successfully marketed it as a dye for fabric. This led to other people working to create other artificial dyes with the goal of emulating Perkin’s success and in this process many new dyes were discovered. Other scientists working with the new dyes discovered that some could selectively stain and kill some microbes (microbes also being a very new concept to science). Therefore the product which was mistakenly developed in the attempt to prepare a medicine turned out to have non-medicinal uses and some of the products which attempted to emulate this non medicinal discovery turned out to have valuable medicinal uses.

Ehrlich had arrived at a time in history when science and medicine were changing rapidly worldwide and at the time, Germany was leading the world in chemical innovation and industry.

1.4.3 Paul Ehrlich and Robert Koch

After completing his MD, Ehrlich went on to do his clinical education and habilitation for which he wrote the thesis “The need of organisms for oxygen”, at the Charite in

Berlin.³² His biography makes mention of Ehrlich having an “anatomical collection – which included, among other things, “an extirpated primary chancre”.²⁷

Ehrlich attended a lecture by Koch in 1882 where Koch set forth his discovery of the tuberculosis microbe. Soon after Ehrlich started working on staining tuberculosis bacteria and became friends with Koch.

In 1985 Theodor Frerichs, who had been a long-time supporter and inspiration for Ehrlich died and Ehrlich was transferred to clinical duty in the Charite. Several authors²⁷⁻²⁸ state that Ehrlich ended up suffering from tuberculosis sometime before he started work at the Koch Institute in 1892 but this date is contradicted by other sources,³² it is certain however that at some stage in this period Ehrlich suffered from tuberculosis and needed time away to recover.

At the Koch Institute, Ehrlich started working with Emil Von Behring on diphtheria. Ehrlich was unhappy with his position in the Koch institute and his disagreements with Von Behring are well documented.²⁷

In 1896 Ehrlich was named director for the newly established State Institute for serum research and serum testing.²⁷ This was set up by the controversial politician Friedrich Althoff who wanted to make Prussia’s universities and research centres the best in the world and who had recognized Ehrlich’s talent and genius.³⁰

This proved short lived however as in 1899 Ehrlich moved to Frankfurt to head the new Royal Institute for Experimental Therapy, which had been set up with funding from the city, the Prussian state, private donors and industrial support – all working towards Althoff’s dream of scientific superiority.

In 1906 the Georg-Speyer Haus was established with donations from the widow of a wealthy Jewish banker.³⁰ (I’m deliberately trying to avoid mentioning the racial and religious tensions present at this time of history– they tended to permeate everything in

this era and to discuss these in detail would be beyond the scope of this thesis – for more information see Fritz Stern’s book “Einstein’s German World”.³³⁾

This was the final step for Ehrlich and it was in Frankfurt that he did most of the immunological and chemical research which was to make him famous. Ehrlich was appointed director of the Georg-Speyer Haus and started to work almost exclusively on chemotherapy – a word he had coined earlier.

This institute had links to several major German dye works - the Hoechst dyeworks and Casella and Co which were used for manufacturing chemicals.³⁴ This further demonstrates the links that Ehrlich’s institute had with chemical industry. Ehrlich would test samples of new dyes and derivatives made – later on, the Hoechst dye works would become the exclusive manufacturer of Salvarsan.

1.4.4 The Japanese link

During this period several Japanese scientists were working with Ehrlich.

The association started with Kitasato Shibasaburō who arrived in Germany in 1885 to study under Robert Koch and ended up working on diphtheria with Von Behring until 1891 when he returned to Japan and founded the Institute for the Study of Infectious Diseases. Kitasato is remembered (and also forgotten) for his work on the diphtheria antitoxin with Von Behring and his discovery of the plague bacterium, independently but almost simultaneously with Alexandre Yersin.³⁵

As a side note, Koch was to visit Kitasato in Japan in 1908 and delivered a memorial lecture for which Kitasato acted as the interpreter. Koch is reported to have fallen in love with Japan and wanted to “take a Japanese maid back to Germany”.³⁶ Kitasato arranged this and a girl called Ohana-san “faithfully cared for Koch for two years until he died”.³⁶

Kiyoshi Shiga was a student of Kitasato and is best remembered for his discovery in 1897 of the dysentery bacterium *Shigella spp.* He moved to Germany to work with Ehrlich from 1901 to 1905. After his time in Germany he returned to Japan to work in Kitasato's laboratory.³⁷

Whilst in Germany, Shiga was working with Ehrlich trying to find drugs which would cure Trypanosomes. Shiga was able to grow the Trypanosomes in lab animals and Ehrlich would suggest chemicals to try to cure them.

Ehrlich published a paper on the treatment of Trypanosomes in 1907³⁸ which included results on Trypan red and 50 derivatives of that dye. Trypan red had been created in the Casella dye works by sulfonation of a benzopurpurine dye in 1903.³⁹

The last of the Japanese in this story is Sahachiro Hata who also studied under Kitasato in Japan and arrived in Germany in 1909²⁷ to work with Ehrlich on Syphilis. Hata had been very successful in culturing *Treponema pallidum in vivo* using rabbits as subjects. This allowed new drugs to be tested against the spirocheate, something Ehrlich was very interested in at the time.

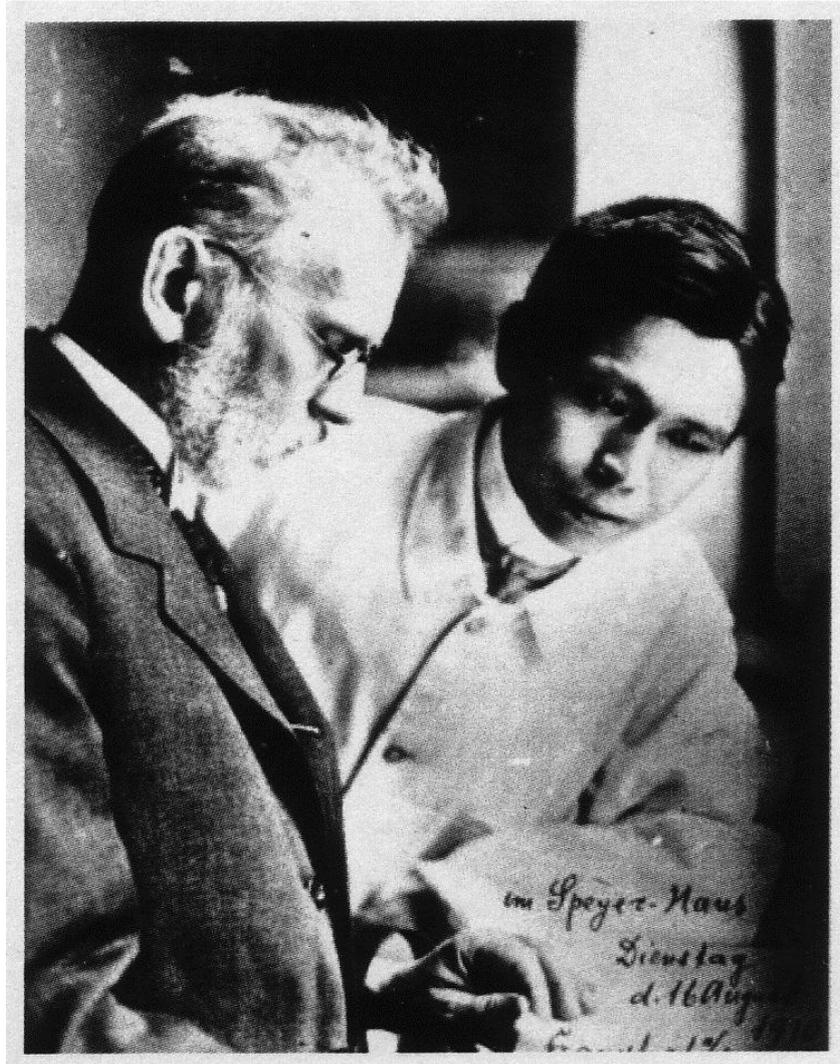


Figure 1-6 - Sahachiro Hata and Paul Ehrlich

Another Japanese scientist who deserves mention in passing is Hideyo Noguchi who first observed the *Treponema* bacterium in the cerebral tissue patients with neurosyphilis in 1913 at the Rockefeller Research Institute – proving that late stages of syphilis were caused by the *Treponema* bacterium.³⁶

1.4.5 *Trypanosomes and Arsenic*

In the late 19th century Africa experienced an epidemic of sleeping sickness – caused by two subspecies of the protozoa *Trypanosoma brucei* and spread by the tsetse fly. In the epidemic between 1896 and 1905 an estimated 300,000 to 500,000 people died causing the colonial governments to call for people to work on a cure.³⁹

In 1856 David Livingstone wrote a letter to the British Medical Journal observing that he treated a horse with “two grains of arsenic in a little barley, daily for a week” and that its condition temporarily improved.⁴⁰

Possibly this observation caused other researchers to investigate other arsenic compounds and research was done using sodium arsenite to kill trypanosomes in laboratory animals. Recognizing the toxicity of inorganic arsenic compounds researchers quickly moved from inorganic to organic arsenic compounds.

In May 1905 Thomas, who was also working with Trypanosomes published a paper in which he used atoxyl – an organic arsenic compound which will be discussed in detail in 1.4.6 – which had been

“before the profession since 1900, and various workers have recorded its worth in the treatment of various skin affections and in anemia.”⁴¹

He goes on to talk about the reduced toxicity of atoxyl and ends the paragraph with “I have tried the drug in high doses intravenously on myself without ill effect.”⁴¹ Later that year, Thomas published more data on his treatments, finding atoxyl to be effective⁴² – he also tried combining it with Trypan red, which Ehrlich and Shiga had demonstrated to be effective against Trypanosomes.

1.4.6 Atoxyl

Atoxyl was another spin off from the dye industry.

It seems to have been common practice in the middle of the 19th century to react whatever was available with aniline to see what would happen. If the product was a dye, then the creator had a slim chance of becoming rich and famous. If the product was colourless and had no obviously interesting features it was usually noted and ignored.

Atoxyl was one of these colourless compounds and was reported by Antoine Béchamp in 1863.⁴³

Béchamp had been born in 1816 in France. He studied many diseases and is mainly remembered for disagreeing with Pasteur's germ theory. Allegations surfaced many years later that Pasteur had plagiarized some of Béchamps work.⁴⁴ Béchamp continued to support a pleomorphic theory of disease – basically that bacteria are not the cause of disease but instead result from the disease and arose from tiny “microzymas” which he postulated were present in all matter and changed form in response to disease. This theory was disproved by Louis Pasteur, possibly using some of Béchamp's own experiments, which has meant that Béchamp has been largely forgotten by history.

I have been able to find no information on how or why Béchamp started working with aniline, which had been discovered in 1826 by Otto Unverdorben who obtained it by destructively distilling indigo.

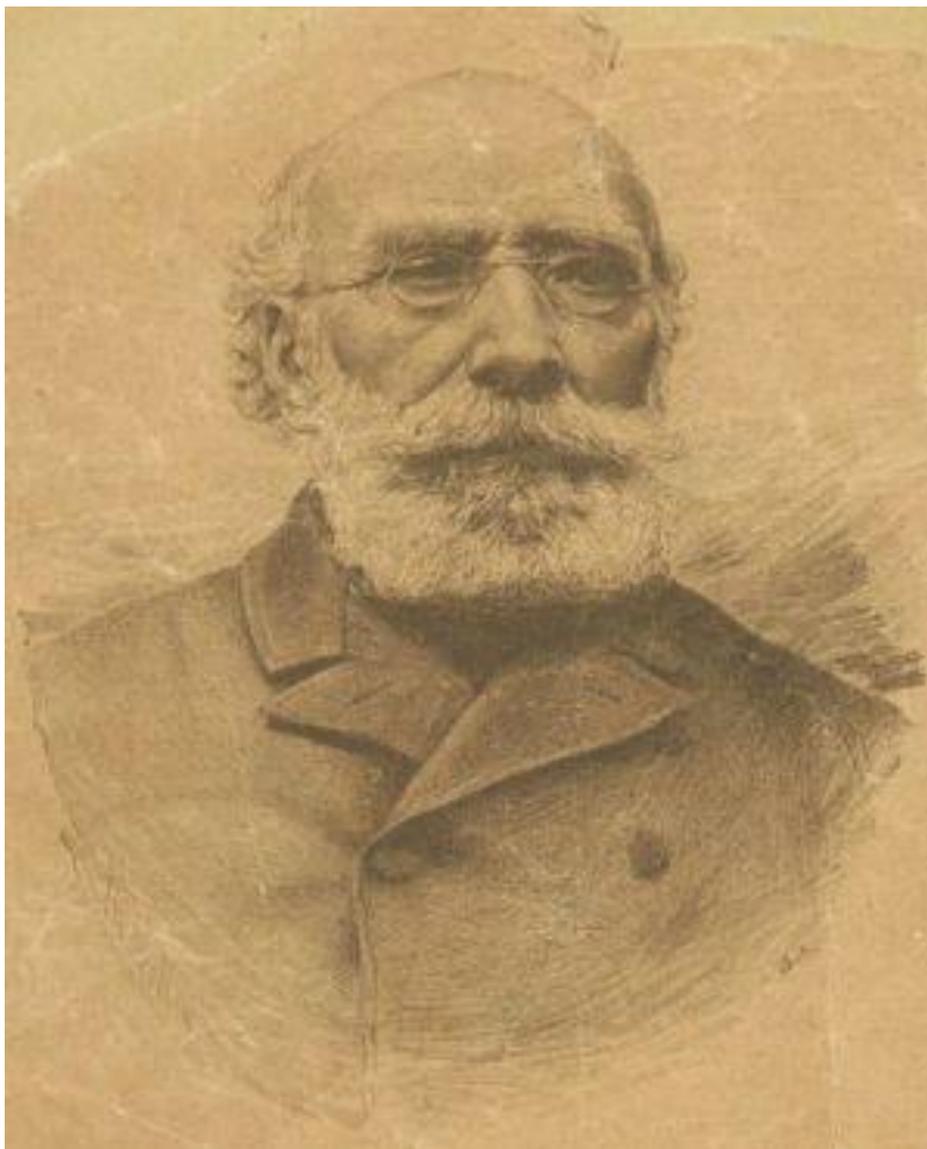


Figure 1-7 - Antoine Béchamp⁴⁵

Aniline was then isolated from coal tar but remained a chemical curiosity obtainable only in small volumes. Aniline was found to be very useful in creating new dyes such as Perkin's mauvine (1856).

Béchamp published a method for making aniline from nitrobenzene in 1854, allowing it to be synthesized in much larger quantities than was previously available – feeding the growing dye industry.

It was this process which Perkin³¹ was able to take advantage of to supply cheap aniline for his dye manufacturing plant in England.

Sometime after this, Béchamp must have done some experiments in which he reacted aniline with various substances in his lab.

Béchamp published two papers on the reaction between aniline and arsenic acid – the first paper⁴⁶ details his reactions of several acids, including arsenic acid, with aniline, forming anilide compounds

The second paper,⁴³ published in 1863, describes the formation of a second anilide of arsenic acid – which he notes was different to the compound described in the first paper. This paper then seems to have passed unnoticed until the early 1900s when the compound made its way into medicine as atoxyl.

This second paper shows that Béchamp still considered his compound to be an anilide of arsenic acid.

Thomas (*1.4.5*) didn't reference or mention Béchamp when he first started using atoxyl. The name “atoxyl” appears to have arisen in a paper by W. Schild in 1902⁴⁷ which investigated the substance for certain skin conditions and cancers and was thus given because the compound was shown to be 40 times less toxic in animals than inorganic arsenic compounds. This seems to be the paper which Thomas noted in his first communication.⁴¹

Atoxyl at this stage was still thought of as an anilide of arsenic acid, which is somewhat surprising as its properties differ markedly from what one would expect of an anilide.

Atoxyl was a brand name, marketed by Lanolinfabrik, Martinikenfelde, Berlin – it was also marketed by Burroughs, Wellcome and Co. in Britain as Soamin, which was deemed by some authors to be superior to the German atoxyl as it was a “pure commercial preparation”.⁴⁸

1.4.7 Ehrlich and atoxyl

1905 was an eventful year for atoxyl – it had been dragged out of obscurity by Thomas, discovered by Ehrlich and was about to get a makeover on the road to Salvarsan.

It is known that on August 29 1905 Ehrlich wrote to Karl Herxheimer⁴⁷ who was the professor of dermatology at the University of Frankfurt and asked for a copy of a paper on the arsenical which was known as atoxyl.

“Please be good enough to send me the paper on atoxyl, since I would like to read it in the original”.

Exactly which paper Ehrlich was requesting is unknown - I suspect it could be the paper by Thomas which was current at the time, Thomas was working in the same area as Ehrlich. However, Ehrlich could also have been referring to the Béchamp paper or even to the Schild paper.

In the Marquardt biography²⁷ it is mentioned in passing that Ehrlich was using atoxyl in tests against trypanosomes as early as 1902:

“on a written block dated December 1902 the following directions : ‘quinine derivatives, including methylhydrocuprein, some dyestuff preparations and atoxyl’”

It is hard to guess at the accuracy of this, as all other reports seem to suggest Ehrlich became interested in atoxyl only after the paper by Thomas.

One assumes that after reading Thomas’s paper, Ehrlich instructed one of his chemists to make (or obtain) this compound. As soon as this was done, Ehrlich could start testing it against trypanosomes. It seems to have still been too toxic for use in human medicine,

though, so Ehrlich embarked on a search to make a derivative which was less toxic to the host, but could selectively destroy the trypanosome.

There was one issue that seemed to stand in the way of making variants of atoxyl– the Béchamp paper had noted that reacting aniline and arsenic acid gave rise to two different compounds. Something was different about the second anilide of arsonic acid which meant this compound was useful in treating trypanosomes while the real anilide of arsenic acid was not. It is also hard to make derivatives of an anilide as it is essentially just a salt of aniline and the respective acid.

1.4.8 Enter Alfred Bertheim

Alfred Bertheim had been born in 1879 and had completed his PhD in 1901. He worked as a manufacturing chemist in Bitterfeld until he joined the team at the Georg-Speyer Haus in November 1905. Ehrlich notes in a letter to Julius Von Braun

“November 7, 1905 Dr. Bertheim, who has given up his position at the factory, has in the meantime arrived here, and since he is free, he can start to work and be of help to me, which is very welcomed.”⁴⁷

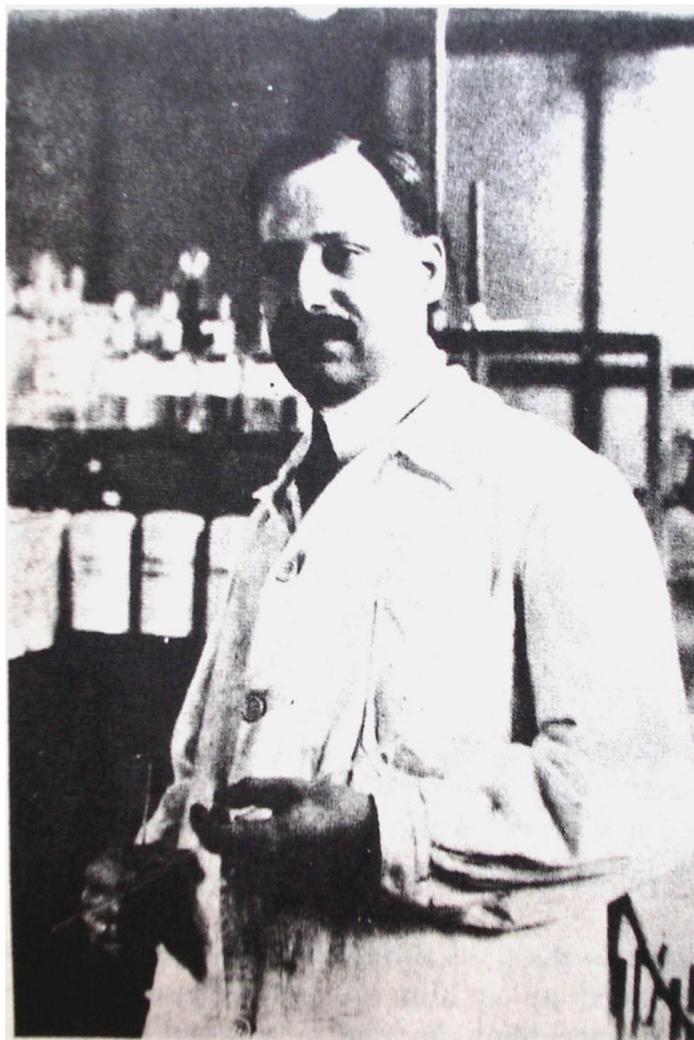


Figure 1-8 - Alfred Bertheim

Presumably in the same letter Ehrlich notes the structure of atoxyl, which his group was obviously doing some work on. His letter recalls the structure suggested by Béchamp – but Ehrlich has obviously had some difficulty trying to get the structure to match that of an anilide plus still have free amino groups which analysis had surely showed atoxyl had. Ehrlich's structure was at this stage, quite inventive (Figure 1-9).

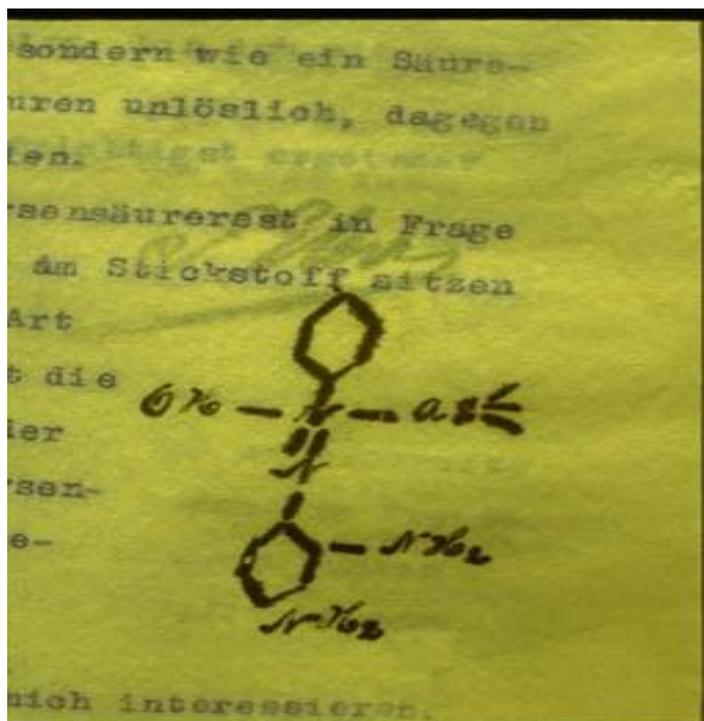


Figure 1-9 - Ehrlich's initial structure for atoxyl

One week after this letter (and the arrival of Bertheim) Ehrlich wrote again to Von Braun – November 14, 1905

“One must consider if the constitution of the atoxyl is really correct, or if perhaps it is not in fact a para-amino derivative of benzol arsenic acid.”⁴⁷

What happened in this one week to change Ehrlich’s mind?

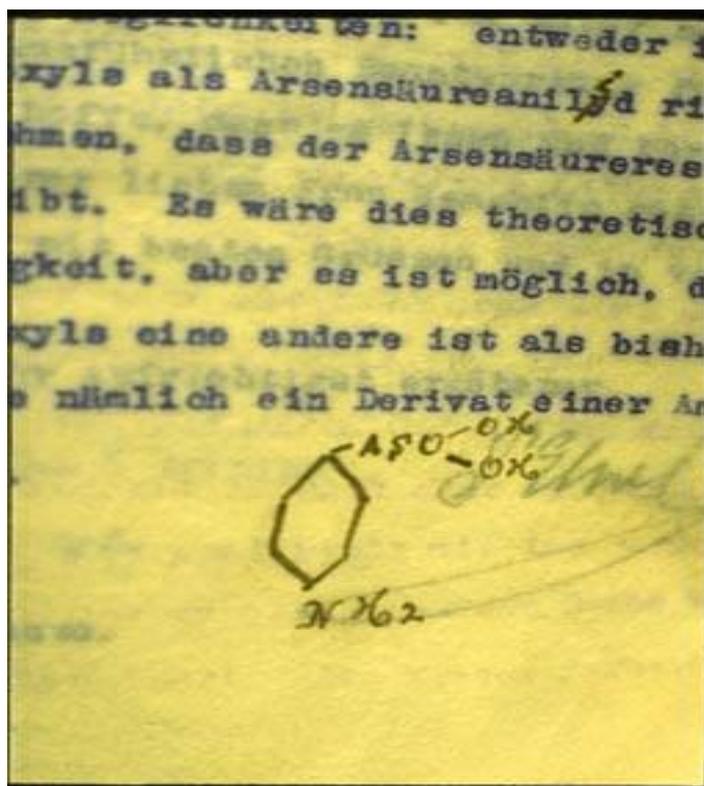


Figure 1-10 - Ehrlich's notation of the correct structure of atoxyl

The biography²⁷ suggests that the structure of atoxyl was the subject of contention – In the biography and the film based on the biography²⁹, Ehrlich is talking to his three chemists and telling them to proceed with their work on the assumption that atoxyl is an amino acid. A row ensues, and two chemists resign, leaving only Bertheim.

A different explanation and one that seems to fit the facts better is that Bertheim arrived in the lab, realized that Béchamp's structure (the structure which Ehrlich appears to have assumed to have been correct) was improbable and that the simplest structure that fit the chemical properties was that of *p*-aminophenylarsonic acid. Sadly this leaves no explanation for the resignation of the other chemists, which is mentioned in the biography.²⁷

Three weeks later, after the arrival of Bertheim, Ehrlich penned another letter to another chemist – Ludwig Darmstaedter²⁹ in which the correct structure of atoxyl was given (Figure 1-10).

It was only after the elucidation of this structure that Ehrlich and his team could embark on a serious search for more active derivatives. The correct structure of atoxyl was published by Ehrlich and Bertheim in 1907.⁴⁹ The biography strangely notes however that Bertheim did not arrive until months after Ehrlich had worked out the structure of atoxyl – seemingly contradicting the letters written by Ehrlich - As already noted elsewhere the biography is slightly hagiographic and one finds it hard to accept as the sole source of reference. The publication⁴⁹ names Bertheim as a co-author suggesting that he had a significant part in elucidating the correct structure.

The first patent for atoxyl was applied for by Ehrlich and Bertheim on October 3rd, 1907.⁵⁰

Once the structure had been solved Ehrlich was able to make many different derivatives and related compounds – starting off the first known systematic approach to chemotherapy.

The logic was simple – atoxyl was a compound which could successfully treat disease, but was too toxic for human use. If one could alter the structure of the molecule to reduce the toxicity to humans while still retaining the toxicity to the target organism you would have a drug that was safe and effective – essentially the modern understanding of chemotherapy.

1.4.9 Ehrlich's numbering system

Each compound which was synthesized in Ehrlich's lab was assigned a number which was used to track the substance throughout the process of biological testing and any further development as a chemotherapeutic product.

This system had been started years before and by the time he started working with syphilis he had a bank of different compounds – including dyes and arsenicals which had been tested on trypanosomes lined up to be tested on animals infected with syphilis. It is also known that Ehrlich revisited some numbers (Mapharsen has two such numbers – see Figure 1-11) which further adds to the confusion. Each variation or salt of a substance was assigned a different number.

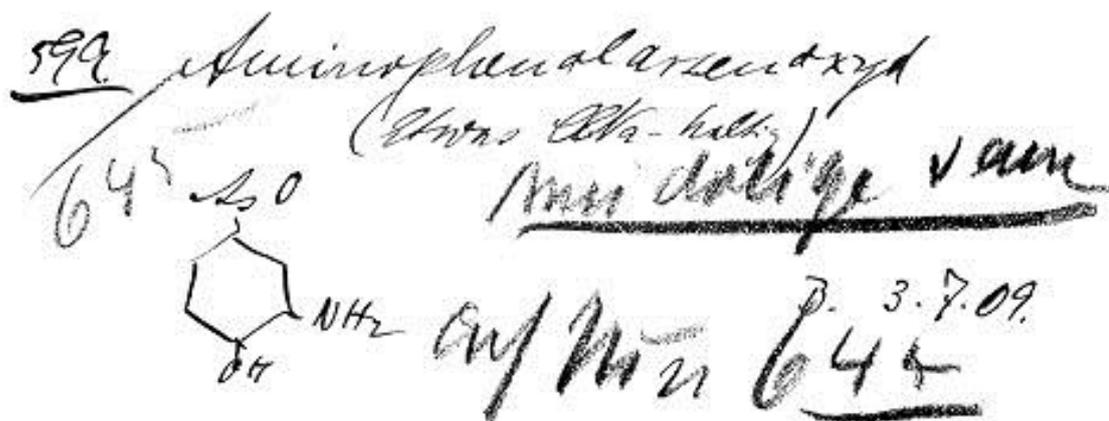


Figure 1-11 - Mapharsen from Ehrlich's notes showing the two numbers assigned – 599 and 644

1.5 Salvarsan

1.5.1 Discovery of Salvarsan

The dates in Ehrlich's manuscript seem to indicate that Salvarsan was initially investigated by Hata on the 7th of September 1909. Hata arrived in 1909 (in spring –

(March – May in the northern hemisphere) according to the biography) with the ability to grow *Treponema pallidum* in rabbits.

I have not been able to ascertain what caused Ehrlich to start working with *Treponema* rather than the trypanosomes which his lab had been working on for many years. The idea which seems most probable is that Ehrlich had heard that Sahachiro Hata had been successfully infecting rabbits with *Treponema* in Japan. Ehrlich seems to have thought that trypanosomes and *Treponema* may have been related and might respond to similar drugs and so, Ehrlich arranged for Hata to come and do some work in his lab.

Hata would infect rabbits with syphilis to use as experimental animals and then work through Ehrlich's catalogue of chemicals.

Bertheim (and probably other chemists, possibly also chemists in the dyeworks) would make compounds related to atoxyl and Ehrlich would tell them what to do (Ehrlich said that his method consisted of “examining and sweating” – his co-workers were known to joke that Ehrlich did the examining and they did the sweating.)²⁷

It seems though that Ehrlich had switched from trypanosomes to *Tremonema* very abruptly – one possible reason for this was Ehrlich's collaboration with other doctors and scientists.

Ehrlich would send samples of compounds which had been found to be active in animal models to doctors who would carry out controlled tests on patients. One such trial which was in progress at the time was for arsenophenylglycine – number 418, which had been very successful in curing sleeping sickness in animal models. Ehrlich had sent samples to Dr. Konrad Alt who had carried out the clinical trial but he had also trialled the new drug against syphilis.

“finding it easy to assume that a drug so immediately effective in animals infected with trypanosomes would also have an effect on syphilitic processes in patients with progressive paralysis”.

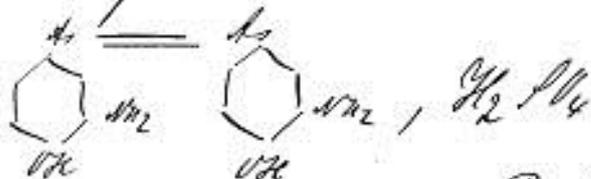
The experiment was a success and a short communication was published on the 20th of July 1909.⁵¹ It seems that this was the encouragement Ehrlich needed to start investigating syphilis.

Success was quick for Ehrlich’s group.

Some sources (including the biography which mentions that it was even patented this early – which appears not to be the case⁵²) seem to indicate that Salvarsan had been examined as early as 1907 and had been tested against trypanosomes but found to be ineffective. This seems unlikely but is possible if Salvarsan (which at that stage was simply number 606) had been produced and added to the library of compounds.

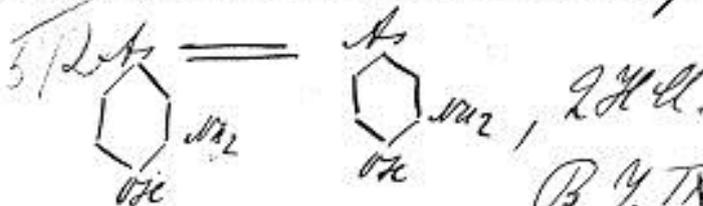
However, if the structural elucidation of atoxyl was the key and this had occurred in 1905 it seems unlikely (but not impossible as Ehrlich had considerable resources) that so many related compounds could have been synthesized so quickly.

605. *Salvarsan* *As* *HCl* *H₂PO₄*



B. 6. IX. 09.

606. *Salvarsan* *As* *HCl* *H₂PO₄*



B. 4. IX. 09.

Figure 1-12 - Salvarsan H_2PO_4 and HCl - 605 and 606

The biography makes an interesting note about the numbering Ehrlich used

*“606 is the number of the substance with which, as with all the previous ones, very numerous animal experiments were made”*²⁷

It also mentions that 606 had been tested previously by a “Dr R” who found nothing, which Ehrlich took to indicate the incompetence of this employee. Again, in this case the truth is hard to determine. At least one paper makes mention of Ehrlich initially testing with a strain of trypanosome which was later found to be non-reactive to arsenical compounds, while Thomas had used a strain which was far more sensitive to them.⁵⁰ It could simply be the case that this compound was tested against this strain and

found to be ineffective. When Hata tested it in rabbits infected with *Treponema*, it was then found to be very effective.

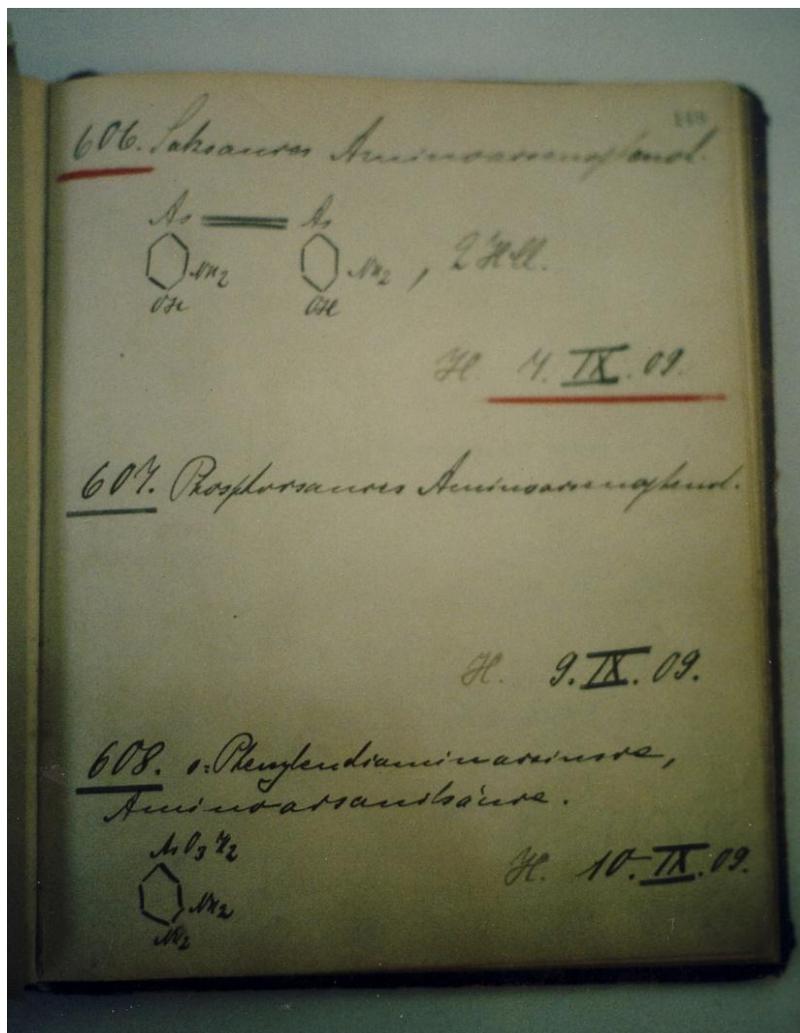


Figure 1-13 - Photograph of Ehrlich's notebook showing Salvarsan (606)

Salvarsan was tested in animals over and over again – Ehrlich is recorded as telling Hata “Then it must be repeated, dear Hata, repeated”²⁷ - and found to be relatively non-toxic to the animals treated and effective at killing the *Treponema*.

Salvarsan was tested in numerous animals before being distributed to a number of Ehrlich’s colleagues in Europe who performed more tests.

Finally it was tested in human subjects and found to be effective.

Eventually Ehrlich announced his success and published the work in 1910.⁵³

Salvarsan was patented in December 1910 and trademarked as “Salvarsan” as previously it had just been known as “606”.

It should be noted that Ehrlich’s group had also prepared other salts of Salvarsan base (such as 592) but only the hydrochloride ended up being used therapeutically.

Ehrlich received much praise for his new drug as it was the first drug that actually offered some hope to people suffering from syphilis

Ehrlich was very closely involved in the early days of Salvarsan – personally approving the people to whom it was distributed and following up any unusual cases.²⁷ He also tried to improve its administration and effectiveness through education and ensuring that strict instructions for the administration of Salvarsan were carried out.

The patents on Salvarsan were taken out worldwide on Salvarsan assigned to Farbwerke vorm Meister Lucius and Bruening⁵⁰ in 1910⁵² which caused some controversy in itself as “patent medicine” was something which was previously usually reserved for quack remedies.⁵⁴

1.5.2 The Salvarsan wars

At this point history becomes somewhat murky. Ehrlich and his collaborators had reported Salvarsan to be effective in treating syphilis but other doctors were not convinced about the new remedy. One letter to the New York Times in November 1910 reports a number of relapses and that the new remedy has become “prematurely popular”. The writer also reports that

*“people who I discharged ten or fifteen years ago as well, and who during the entire period have shown no evidences whatever of disease, who are the fathers of perfectly healthy children have been begging for a dose of “606” just to make “perfectly sure””.*⁵⁵

The British Medical Journal introduced ‘606’ cautiously – initially noting that

“Ehrlich and Hata showed much shrewdness in calling this substance ‘606’, since the mystery which surrounds this term in the eyes of the public has made people anxious to find out something about the substance which it notes”

and proceeding in a cautious tone about the new drug.⁵⁶

One can divide the controversy over Salvarsan into two parts – one part was played out in the press and the courts and was about the safety of the new drug and Ehrlich himself, whilst the other played out in the scientific literature, and was more concerned with the composition of the new drug.

However with the outbreak of the First World War, Ehrlich’s character was also under fire in the allied countries.

Ehrlich seems to have initially attracted controversy in Germany by his practice of sending samples of Salvarsan to anyone who requested it and it seems likely that in the early days of production, short supply caused him to be unable to meet the demand which causing some doctors to feel left out of the process.

When Salvarsan started commercial production at the Hoechst factory doses were sold at 10 marks – about 10 shillings in Britain⁵⁴ and 4 dollars in the USA,⁵⁷ which caused

detractors to claim that Ehrlich was profiting from the new drug. The price of Salvarsan also meant that it was too expensive for many syphilis patients.

Ehrlich was also called into court to defend his drug on several occasions. In February 1914 the New York Times reports that Ehrlich bought a suit for criminal slander⁵⁸ against Dr. Druer, who had published a report saying

*“in regard to the serious danger to life and health, and in the public interest of the country...the use of Ehrlich-Hata 606 should be forbidden, or at least its application in doses greater than the maximum official dose of arsenic”*⁵⁹

Ehrlich also attracted the attention of Carl Wassmann who published a pamphlet called “Die Wahrheit” – “The Truth” and made public accusations of ‘606’ being toxic and testing being unreliable. Wassmann was sued for libel in 1914 and lost – receiving a year’s sentence in prison for his unfounded accusations.⁶⁰ Wassmann was released after serving only two months in prison as the result of an amnesty because of the outbreak of war on the 28th of July 1914.

The controversy about Salvarsan ended with Ehrlich being summoned before the Reichstag (German parliament) to defend Salvarsan. When asked directly if the administration of Salvarsan had caused him any misgivings, Ehrlich replied

*“If the advantages did not far outweigh the disadvantages, I would not think for one moment of still permitting the drug to be used for treatment. My attitude would be the same if a drug existed which was more effective than Salvarsan!”*⁶¹

Ehrlich maintained throughout the trials that Salvarsan was an effective treatment when administered properly and most of the fatalities and side effects were caused by improper administration of the drug.⁶²

Salvarsan also attracted much favourable attention with Albert Neisser writing an open letter to Ehrlich stating

*“with absolute certainty... this new drug exerts an extreme, an astonishing action as well upon the spirochetes as upon the products of syphilis”*⁵⁷

The outbreak of World War One changed things considerably and German patents worldwide were abrogated which meant that control of Salvarsan was out of Ehrlich's hands.

1.5.3 Administration of Salvarsan

Salvarsan was not without its difficulties – it was sold as the hydrochloride in sealed ampoules and had to be made up immediately before it was administered intravenously. To prepare the compound for administration,⁶³ doctors were advised to check the ampoule carefully for signs that air had entered, rendering the Salvarsan too toxic for use.

The contents of the ampoule are then added to three or four ounces of warm saline solution (approximately 100 mL) prepared with freshly distilled water. Upon dissolution of the powder 10 mL of “double decinormal sodium hydrate” solution (sodium hydroxide) was added. This caused a precipitate to form which was then dissolved by further addition of the sodium hydrate solution – until the precipitate was totally dissolved (but no more, which would render the solution too alkaline). The solution is

then made up to ten ounces (either 280 mL or 300 mL depending on country) with saline and “once or twice” filtered through muslin or several layers of gauze.

The instructions go on to note that ten ounces “must be considered the maximum dose”. This mixture was then injected intravenously. Intravenous injection was a relatively new concept at that time and Salvarsan could also be administered subcutaneously and intramuscularly although Salvarsan was known to cause necrosis of the tissue when injected by the two latter routes.⁶⁴ Ehrlich was even quoted as saying

“606 should not be given, unless it was given intravenously”⁶⁴

Other routes of administration were also investigated including oral administration in liquid and capsule forms, which were found to have little toxic effect but little curative properties,⁶⁵ and rectal administration via suppositories,⁶⁶ an emulsion of Salvarsan in olive oil⁶⁷ or enteroclysis.⁶⁸

Special apparatus was available for the administration of Salvarsan – A pamphlet advertising injection apparatus for Salvarsan is shown in Figure 1-14.

Original-Injektionsbesteck
für alle vorkommenden
Injektionen für
Salvarsan
(Ehrlich 606)

Jedem Besteck wird die von Herrn Geheimrat Ehrlich genehmigte Technik, unter Berücksichtigung der jeweiligen neuesten Ergebnisse beigelegt.

Fig. 12. Kristallglas-Karatspritze

Fig. 8 u. 9. Mess- u. Mischzylind. u. 60 Gekugeln.

24) Komplettes Original-Ehrlich-Injektionsbesteck (Fig. 24) enthaltend: eine Kristallglas-Karatspritze oxydfrei, eine Dreiwegkanüle nach Oberarzt Dr. Schreiber, ein Reservovorderteil, eine Kanüle m. Schlauchkonus n. Weintraud, eine Kanüle f. subkutane Injektion, eine Kanüle f. intraglutale Injektion u. ein Lederetui: z. Aufnahme der Spritze u. all. Kanül., Tel.-Bez. „Ehrlich“ M. 32.—

24 a) Dasselbe Besteck aber mit Platinkanülen, Telegr.-Bezeichn. „Ehrlichpl“ „ 59.—

25) Dasselbe in sterilisierbarem Metallteil (Fig. 25), Telegr.-Bezeichn. „Ehrlichm“ „ 40.—

25 a) Dasselbe Besteck aber m. Platinkanülen, Telegramm-Bezeichnung „Ehrlichsal“ „ 67.—

Fig. 8. 1 Spezial-Mess- u. Mischzylinder mit chem. gereinigten Glaskugeln, Telegr.-Bez. „Ehrlichgl“ „ 10.—

Fig. 16. 1 Weintraudbürette mit Schlauch und Glaszwischenstück . . . Telegr.-Bezeichnung „Ehrlichbur“ „ 6.—

Die Original-Apparate für alle vorkommenden Injektionen werden hergestellt:
B. B. Cassel Präzisions-Werkstätten **Frankfurt a. M.**
f. Arztl. Konstruktionen
Tel.-Adr.: Cassel, Frankfurtmain, Hanschaus **Stiftsstraße 9—17 an der Zell.**

Figure 1-14 - Pamphlet advertising injection apparatus for Salvarsan²⁸

On reading through the instructions for administering Salvarsan to patients one cannot be surprised that fatalities and side effects occurred – even a totally non-toxic product being subjected to the work-up required and injected into the patient would be expected to cause some problems. The main problem with Salvarsan was not its toxicity but rather the complexity of its administration. On reading some of the papers and books

about the administration of Salvarsan^{64, 67} one gets the impression that Salvarsan was not seen by the doctors as the “magic bullet” which Ehrlich imagined, but rather as another remedy to add to the already considerable arsenal of substances to use against syphilis. In practice, this meant that Salvarsan was often administered in conjunction with mercury and other substances used to treat syphilis. Indeed papers were even published to this effect with one claiming that

*“It is hardly necessary now to produce further proof of the superiority of Salvarsan and mercurial treatment over exclusively mercurial treatment”.*⁶⁹

Special apparatus was designed for the administration of the drug to help simplify the process.

The intravenous injection of Salvarsan often entailed a 24 hour hospital stay and “normal” side effects were known to include nausea and vomiting.⁵⁴ Salvarsan was known to have other side effects and patients were evaluated for suitability before being treated with Salvarsan, even so, side effects were sometimes unexpected and serious.⁷⁰

Side effects were sometimes also confused with the continuing effects of syphilis which could re-occur if the dose of Salvarsan was too little – in some cases deafness was reported which was found to have been caused by a regrowth of the *Treponema* as too small a dose of Salvarsan had initially been administered.²⁷

It is also mentioned that when Salvarsan was given intramuscularly or subcutaneously

*“an arsenical depot is formed, on which the organism draws”*⁶⁴

and that when given intravenously that

*“arsenical depots are formed in the internal organs”*⁶⁴

which seems to show that even in 1912 the doctors recognized that Salvarsan had some longer term activity in fighting infection.⁶⁴

1.5.4 After Salvarsan

Ehrlich was well aware of the difficulties in preparing and using Salvarsan and thought that there was room for improvement - mainly because of solubility issues and the fact that it often needed more than one dose to successfully cure syphilis. His team continued to work on these problems and in 1912⁷¹ released Neosalvarsan which was more soluble and had fewer complications on injection.



Figure 1-15 - Photo of vial and packaging of commercial Neosalvarsan

The biography notes that Neosalvarsan was a disappointment to Ehrlich who saw it as less effective and against his desire of “*therapia sterilisans magna*” as Neosalvarsan often required a course of treatment rather than a single injection of Salvarsan, which had cured many cases of syphilis.²⁷

Neosalvarsan was simply a derivative of Salvarsan and was supposed to be more stable to oxidation and easier to administer. It seems to have attracted considerable less attention than Salvarsan.

Bertheim continued to work with Ehrlich until the war and published a handbook of organic arsenic compounds in 1913.⁵²

1.5.5 Commercial preparation of Salvarsan

Initially Salvarsan was commercially prepared at the Georg-Speyer Haus, but demand quickly outstripped supply. Ehrlich had strong commercial connections with the Farbwerke – Hoechst dyeworks⁷² and his team was able to oversee the process of scaling up facilities for commercial production of Salvarsan with production starting in December 1910.²⁷ Liebenau notes that

“Salvarsan was developed at the Speyer Haus, controlled by and with the backing of the government institute, and patented, produced and marketed worldwide by Hoechst”⁷²

Salvarsan was prepared by reduction of 3-nitro-4-hydroxyphenylarsonic acid with sodium hyposulfite (sodium dithionite). The resultant solid was filtered off and redissolved in a solution of hydrogen chloride in methanol followed by precipitation of the hydrochloride with ether.

This was the standard method of commercial preparation however once Salvarsan came to be made in other countries other methods were evaluated. Some sources state that every batch of Salvarsan was tested for toxicity before being sent on to doctors for administration as there were known to be more toxic contaminants in some of the batches of Salvarsan.

When the First World War broke out, the extent of the world's dependence on Germany's synthetic drugs came to be known as many countries were no longer able to obtain Salvarsan.

In America the price of Salvarsan rose to 35 dollars per ampoule as German supplies of the drug had been shut off. Raiziss succeeded in making the first American Salvarsan and was distributing his product before patents were abrogated in the USA.⁷³

Physicians petitioned congress to abrogate the German patents on the drug and on November 27, 1917, the Federal Trade commission licensed three firms to manufacture and sell the drug under the American name "Arsphenamine". The war led to many German patents being abolished in other countries and Salvarsan came to be made worldwide.⁷³

The start of the First World War was the beginning of the end of Germany's superiority in the medical and chemical industry fields.

1.5.6 The end of Ehrlich

Ehrlich continued to promote Salvarsan and Neosalvarsan until his death.

War broke out in July 1914 and Ehrlich's laboratories were requisitioned by the military for production of sera.²⁷ Ehrlich is reported to have said

"But this war is pure madness! No good can possibly come of it!"

Ehrlich's health was also starting to deteriorate – caused by years of smoking strong cigars and paying little attention to his personal nutrition. Around Christmas 1914 he had a mild stroke. Although he recovered from this quickly, his general health

continued to deteriorate and in August 1915 he had a second stroke. Paul Ehrlich died on the 20th of August 1915.

Ehrlich was honoured by his country – the street in which his institute was situated was named Paul Ehrlich Strasse, but this was removed when the Nazis came to power.

After the Second World War when his birthplace of Strehlen came back under Polish authority it was renamed Ehrlichstadt in honor of Ehrlich. The Paul Ehrlich Institute also suffered when the Nazis came to power – in 1935 all Jewish employees were fired from their jobs and Richard Otto was appointed as director and had all writings of Paul Ehrlich removed from the library in an effort to remove any links to the Jewish Ehrlich.

The institute was renamed the State Institute for Experimental Therapy. In 1947 Ehrlich's name was returned to his institute.⁷⁴

Today the Paul Ehrlich Institute carries on his work and produces and tests sera and vaccines.

Ehrlich received a Nobel Prize in 1908 and was nominated for a second when he died.

In 1940 a movie was made about Ehrlich's discovery of 606 titled Dr Ehrlich's Magic Bullet and starring Edward Robinson as Ehrlich The movie is reported to be very loosely based on the Marquardt biography^{27, 47} with much artistic license thrown in for good measure.

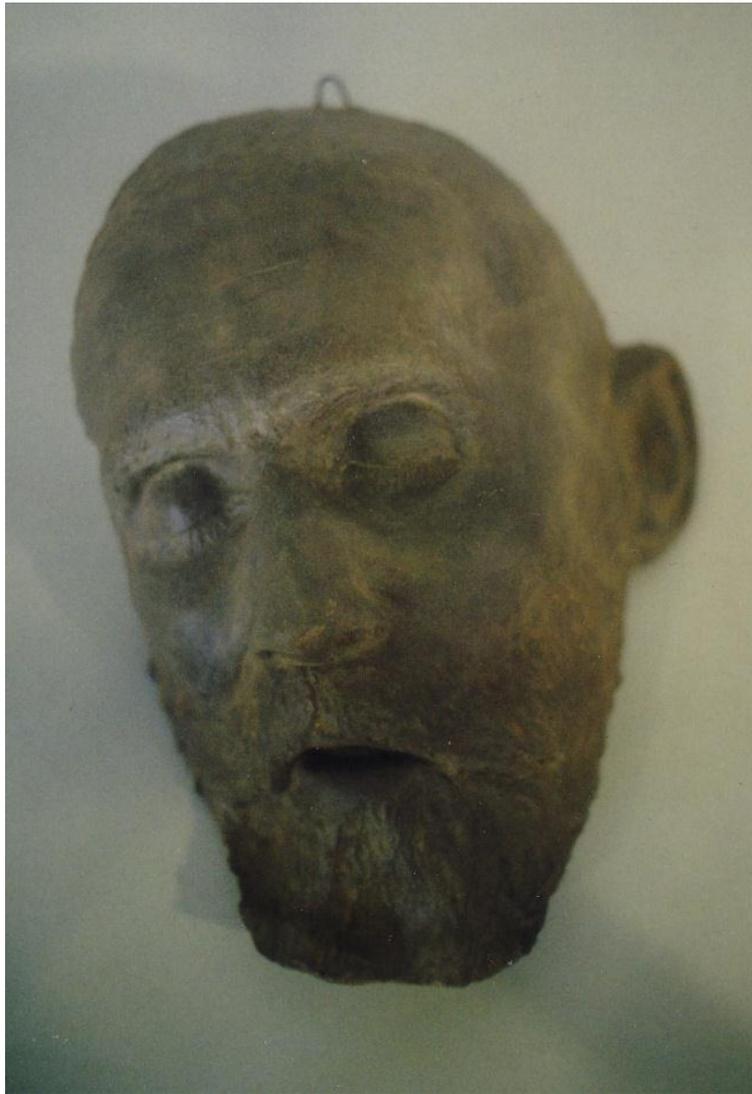


Figure 1-16 - Death mask of Paul Ehrlich

Upon the declaration of war, Dr. Bertheim was summoned to the Cavalry corps in the German army, after working with Ehrlich until the start of the war. On the 14th of August, 1914 Dr. Bertheim was killed when he caught his spurs in the carpet and fell, fracturing his skull.²⁷

Hata returned to Japan in 1910 where he continued his research. He helped to found the Kitasato institute and later became its director.

Hata died in November 1938.

Ehrlich's life is remembered in two biographies, the first written by his former secretary after the Second World War.

This book paints an unusual picture of Ehrlich – part mad scientist and part heroic leader and as has already been mentioned, this may not be as reliable as it could be. The second biography has not been available for this study and was published in 1984. As most of Ehrlich's papers are still extant, it would be an interesting task for someone to study these and clear up some of the unknowns in the Salvarsan story.

By the time the war was over in 1918, Germany's previously thriving chemical industry had been destroyed, partially due to the involvement of some of the major companies in making weaponry and war gasses and also because worldwide patents had been lost.

1.6 Literature

Ehrlich's discovery started a cascade of interest in arsenical compounds but because of the First World War these seem to flow in with a delayed reaction. The pre-war papers were mostly debates about the side effects and administration of Salvarsan while the post-war papers are more interested in chemical composition and toxicity of the different Salvarsan preparations.

Ehrlich's proposed formula (Figure 1-17) for Salvarsan in particular caused much debate as did the levels of arsenic he reported.

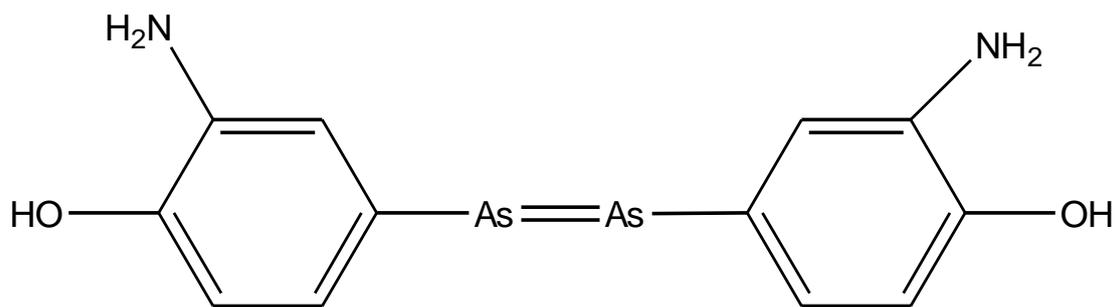


Figure 1-17 - Ehrlich's proposed structure of Salvarsan

Initially each batch had to be tested for toxicity as this was known to vary between batches because of some unknown impurities.⁷⁵ Much of the later debate was centred on the nature of these impurities, as well as variations in the preparation method and analytical details. When the First World War started and German patents were abolished in many countries Salvarsan began to be produced on a worldwide scale. This meant that Ehrlich's controls were unable to be enforced and Salvarsan seems to have gained a reputation for differing markedly between the different sites of production.⁷⁶

It was also known by several names – Salvarsan continued to be used in many European countries and sometimes it was known simply as 606. American manufacturers started marketing the drug as Arsphenamine – the origins of this name are unknown but Lewis mentions that

*“the obligation has been imposed on all licensees to use the new official names of these drugs – The new official names for Salvarsan, Neo-Salvarsan and sodium Salvarsan are Arsphenamine, Neo-Arsphenamine and Sodium Arsphenamine”.*⁵⁰

Lewis's paper⁵⁰ also provided an interesting review of the patent literature on Salvarsan and related arsenicals in America at the time.

It is very hard to work out if the variable toxicity was caused by differences in preparation, handling or raw materials as during the period 1911 – 1920 it is hard to track what was going on in the scientific literature as most of the information was limited to company and patent literature.

In 1918 more publications started to surface about Salvarsan – roughly these could be divided into two groups – people working with Salvarsan in an effort to understand its chemical nature and composition and people working with other phenyl arsonic acids with a view to introducing a more effective and less toxic compound.

1.6.1 The Salvarsan investigations

The Bart reaction by which arsonic acids could be prepared by diazotizing an amine and coupling it with a solution of sodium arsenite was patented in 1912⁷⁷ and this allowed for many arsonic acids to be synthesized and studied - this reaction allowed for much easier synthesis of arsonic acids than was previously available via the Bechamp reaction. Bertheim's book,⁵² published in 1913 provided a summary of much of the work done on therapeutic arsenicals at that time and by 1919 there were groups in the USA and Britain doing extensive work on the subject.

All of these groups assumed Ehrlich's formula with an As=As bond.

The first problem for researchers in this area was the synthesis of sufficiently pure starting materials and much research was done into improving the Bart reaction to improve yield and improving the methods of altering functional groups on arsonic acids while leaving the arsonic acid group unchanged. Jacobs *et al*⁷⁸ published one of the earliest papers with in-depth preparations of nitroarsonic acids using the Bart reaction and selective reductions to aminoarylarsonic acids using ferrous hydroxide as a

reducing agent. They went on to investigate the direct arsonation of phenol and the various products formed in this reaction.⁷⁹

Kober, working for the New York State Department of Health, started work on Salvarsan from the other end of the chain⁷⁵ – looking at Salvarsan itself. He queried the claims that Salvarsan had two molecules of water in the end product – despite the fact that the original preparation involved precipitating the Salvarsan from a methanol solution with ether. He also pointed out some of the inconsistencies in the literature on this point.

He makes it quite clear that Salvarsan was known to have variable toxicity and that up to 50% of all batches made had to be discarded as they did not meet specifications.

His conclusion was that the toxicity of the Salvarsan had something to do with the final precipitation with methanol and hydrochloric acid – and proposed that Salvarsan had a molecule of methanol in its structure.

The formula proposed by Ehrlich was initially $C_{12}H_{12}O_2N_2As_2 \cdot 2HCl$ which theoretically has 34.2% arsenic. Analysis of the commercial product showed that Salvarsan only contained 31.6% arsenic. To account for this, two molecules of water were incorporated into the formula bringing down the arsenic content to 31.6%. Kober suggested that maybe Salvarsan contained a molecule of methanol which added to its toxicity.

Kober went on to describe a method for making Salvarsan without the need for organic solvents. Particularly interesting are some of the observations and comments he quotes such as

“Some chemists lay great stress in using only the purest nitro-oxyphenyl arsonic acid and only to treat it with commercial hydro-sulfite, which itself, in many cases gives, if

*not a muddy solution, at least a dark colored one; all this in spite of Ehrlich and Bertheim pointing out that the reduction, even with the purest nitro-oxyphenyl arsonic acid produces its own by-products.”*⁷⁵

English chemists were also working on Salvarsan. Fargher working in the Wellcome laboratories published a paper on substituted phenylarsinic acids⁸⁰ where he attempted to make arsenic acids with the same substitution as Salvarsan. This group then went on to investigate Salvarsan.

In 1920 they published a paper “The composition of Salvarsan”⁸¹ in which they investigated commercial Salvarsan and some of the other patented methods of preparation. They disagreed with Kober’s⁷⁵ suggestion of methyl alcohol being found in Salvarsan and they measured the amount of methyl alcohol in Salvarsan solutions – finding values between nil and 1.4%. They then went on to measure the concentrations of sulfur and chlorine in different Salvarsan samples and described several methods for the production of Salvarsan with no sulfur content. These included a method previously described by Ehrlich and Bertheim involving reduction of 3-amino-4-hydroxyphenyl arsonic acid with sodium amalgam, as well as reduction with hypophosphorous acid to get pure samples of Salvarsan.

This paper was one of the earliest papers to mention that the sulfur containing impurities might be contributing to the toxicity of commercial Salvarsan.

Soon after this, another American group working in the public health service published a paper on the oxidation of Salvarsan.⁸² This was the first paper published in English which confirmed some earlier observations – namely that the toxicity and trypanocidal activity of Salvarsan and Neosalvarsan increased as it was allowed to oxidize and also

that the R-As=O (now known to be R-As(OH)₂) compounds were the only compounds to exhibit trypanocidal activity.

In September 1920, Christiansen published a paper detailing the hypophosphorous acid preparation of Salvarsan⁸³ - this was the first in a series of papers by Christiansen investigating the toxicity of Salvarsan. This paper provides the clearest English preparation of Salvarsan by reduction with hypophosphorous acid and also provides useful detail on purifying 3-amino-4-hydroxyphenylarsonic acid. It is interesting to note that Christiansen used Salvarsan which had been deemed too toxic to use by oxidizing it back to the amino acid, purifying this and then reducing it back to Salvarsan which was reliably less toxic.

In 1921 King published the first⁸⁴ in a series of papers on sulfur contaminants in commercial Salvarsan – he seems to have noted Fargher's observations about sulfur compounds⁸¹ and attempts to prepare some of the possible sulfur compounds which might theoretically exist in commercial Salvarsan.

King also reiterates that the problem with Salvarsan was its variable toxicity – attributing this to the amorphous (or barely crystalline) nature of Salvarsan which makes purification difficult and to the use of sodium dithionite in the process.

He goes on to suggest some formulae of the possible contaminants and provides preparations for these compounds which he then tested for toxicity and trypanocidal activity. Part two⁸⁵ of this study was published later in the same year and carries on attempting to synthesize and identify some of these sulfur compounds. These papers also hint at the existence of unsymmetrical or mixed arsenobenzene compounds.

The toxicity of Salvarsan was also being investigated by Christiansen who was comparing the methods of preparation and the toxicity of Salvarsan.⁸⁶ In this paper he

quotes Hunt who had observed several different toxicities in Salvarsan – those arising from oxidation prior to injection, those arising from incorrect administration and patient sensitivity and that arising from unidentified toxic compounds in the Salvarsan. He also discusses the preparation of unsymmetrical arsenobenzene compounds as at this stage the toxic compounds were believed to be asymmetrical sulfur compounds which were produced at the stage of the reduction of NO_2 to NH_2 . The conclusions of this paper suggest that the toxicity of Salvarsan arises at the point at which the nitro group is reduced – suggesting that using pure 3-amino-4-hydroxyphenylarsonic acid will give a pure Salvarsan of low toxicity which confirmed his earlier observations.⁸³

Christiansen continued his studies and published a series of papers titled “The sulfur content of Arsphenamine and its relation to the mode of synthesis and the toxicity”.⁸⁷

These studies showed a weak correlation between sulfur content and toxicity and although they agreed with his previous conclusions, they seemed to fall short of finding and isolating the compounds responsible for the toxicity of Salvarsan.

Christiansen went on to prepare some interesting polyarsenide compounds with similar toxicity to Salvarsan.⁸⁸

In 1925 Christiansen published a short article titled “Future Research in the Field of Organic Arsenicals”⁸⁹ which suggests topics still to be addressed in the field of therapeutic arsenic chemistry and gives a useful snap-shot of the problems and questions in the field to that date.

Raiziss was also researching the toxic components of Salvarsan and suggested that 3,5,3',5'-tetraamino-4,4'-dihydroxy arsenobenzene might be the toxic component – this compound was prepared but no mention made to its toxicity.⁹⁰ He then went on to make some derivatives of Salvarsan with aldehydes.⁹¹ Raiziss and Gavron published a monograph on organic arsenic compounds,⁷⁷ which remains a useful source of

information and although some of the methods are dubious it provides a summary of much of the work done on organoarsenic compounds to that date including many obscure compounds with equally obscure references.

The papers on the chemical nature of Salvarsan waned, without really conclusively identifying the more toxic contaminants, or more surprisingly coming up with other proposals for its structure. A good review of Salvarsan and related compounds was published by Voegtlin⁹² in 1925 which reviews Salvarsan and its use from chemical and pharmacological points of view.

Many of the papers mentioning Salvarsan in this period also mention other aspects of organoarsenic chemistry and Salvarsan was discussed from a chemical point of view rather than simply from a pharmacological standpoint. Organoarsenic chemistry had also featured in chemical warfare in World War One, with several dichloroarsines being used as blister agents (2-chloroethenyldichloroarsine - Lewisite – developed by the Americans and used in WW2, phenyl, methyl and ethyl dichloroarsines were made by the Germans and used in WW1) – so it was a fertile field for research. There was therefore some overlap in the chemistry of these compounds and organoarsenic chemistry became a research field in its own right detached from possible medicinal use of the new compounds.

One of the first papers to hint at the true structure of Salvarsan was published in 1928⁹³ and didn't mention Salvarsan at all, however the authors noted the difference between arsenobenzene compounds and the azobenzene compounds that they were assumed to be analogous to. At the time the structure of these compounds was assumed to consist of compounds with As=As bonds and so the authors investigated the simplest - arsenobenzene and arsenomethane and found that representing arseno compounds as R-

As=As-R was unjustified as the molecular weights they found using different solvents varied widely.⁹³

Blicke and Smith investigated a range of arsenical compounds including some arsenobenzenes which they also found to be stable to oxygen unless the compounds were contaminated in which case they reacted with oxygen quickly. They also found that arsenobenzene compounds were associated in some solvents.⁹⁴

1.6.2 Neosalvarsan

Neosalvarsan had been introduced by Ehrlich in an attempt to improve Salvarsan.

It was reported to be more soluble and easier to use as well as being better tolerated with fewer toxic side effects. Neosalvarsan was first mentioned by Ehrlich in 1912⁷¹ as a condensation product of Salvarsan with sodium formaldehyde sulfoxalate. It was trademarked in 1912⁵² and had been assigned number 914 in Ehrlich's series.⁷⁷

Bertheim's book mentions Neosalvarsan in passing and seems to indicate that the product trademarked was the mono substituted version.⁵²

In early 1921 some of the first papers on Neosalvarsan (Neoarsphenamine in America) began to appear.

A paper by Macallum⁹⁵ in 1921 starts with

*“During the last few years many articles have appeared on the chemistry of Arsphenamine, but practically nothing on that of Neoarsphenamine. This is doubtless owing to the generally admitted obscure nature of the product. The only references to the preparation of Neoarsphenamine are confined to the patent literature.”*⁹⁵

This paper went on to discuss the degree of substitution of sulfoxylate in various Neoarsphenamine preparations and opened the way for further papers on the subject,

which would remain slightly more obscure and contentious than Salvarsan. Macallum published a second paper⁹⁶ in 1922 on the composition of the French produced Neoarsphenamine which differed slightly from the American product and consisted mainly of double substituted product. He also notes the presence in the French preparations of bisulfite substituted products - $\text{CH}_2\text{O}\cdot\text{SO}_2\text{Na}$ instead of the $\text{CH}_2\text{O}\cdot\text{SONa}$ substitution which Bertheim called Neosalvarsan.

Raiziss *et al*⁹⁷ published in 1921 with more detail on the substance and examined samples for sulfur, arsenic and nitrogen content. He noted several points, the most important of which were that the lower arsenic content was due to the presence of non-arsenical compounds such as uncombined sodium formaldehyde sulfoxylate, sodium sulfate and sodium chloride. The arsenic-nitrogen ratio was a good indicator of the purity of Neoarsphenamine and variation in substitution could account for irregularities in toxicity and therapeutic effects.

Voegtlin and Smith found Neoarsphenamine to show slightly more variable trypanocidal activity than Salvarsan.⁷⁶

In 1922 Voegtlin *et al*⁹⁸ demonstrated the preparation of a “disubstituted” ester formed by reacting formaldehyde, sodium bisulfite and Salvarsan (Arsphenamine) and called it “sulfarsphenamine” further adding to the confusion about Neosalvarsan. At the time of publication, the new drug was in clinical trials after proving effective and stable in animal studies. This paper designates Neoarsphenamine as the mono and di-substituted $\text{CH}_2\text{O}\cdot\text{SONa}$ condensation products of Salvarsan and Sulfarsphenamine as the disubstituted $\text{CH}_2\text{O}\cdot\text{SO}_2\text{Na}$ condensation product with Salvarsan. It is not known if sulfarsphenamine ever entered clinical usage as a product apart from Neoarsphenamine. A preparation of Neoarsphenamine was discussed in a 1922 paper by Heyl *et al*, as none of the previous papers had included a preparative method.⁹⁹ This paper reports that

Neoarsphenamine is unusually sensitive to oxidation and shaking a solution of it was reported to increase the toxicity markedly. The method described in this paper included diagrams of apparatus designed to exclude oxygen.

Raiziss's monograph⁷⁷ briefly mentions Neosalvarsan and discusses several methods of preparation.

Christiansen¹⁰⁰ investigated the sulfur content and chemistry of Neoarsphenamine and found that there was sulfur in Neoarsphenamine which was no longer present as sulfoxylate and that there were probably two types of combination between the Arspenamine base and the sodium formaldehyde sulfoxylate.

Similar research was being carried out in Europe by Dyke and King who published a series of papers starting in 1933 with an examination of Sulpharsphenamine¹⁰¹ – the $\text{CH}_2\text{O}\cdot\text{SO}_2\text{Na}$ condensation product of Salvarsan, which they gave a preparation for and much analysis of commercial products and their product. This seems to indicate that Voegtlin's Sulpharsphenamine did enter commercial production as it was available to Dyke and King to investigate in 1933. They conclude that commercial Sulpharsphenamine is a tri-substituted OO'N-methylenesulphurous acid derivative of Salvarsan base.

Dyke and King examined Neosalvarsan¹⁰² (as Neoarsphenamine, which was the name adopted by the British Pharmacopoeia in 1932) in 1934. They saw this as a substance distinct from Sulpharsphenamine and seem to suggest that it was probably N-substituted. They published an in depth analysis and give a method for preparing Neoarsphenamine using special apparatus designed to exclude oxygen.

Dyke and King revisited their Sulpharsphenamine findings in 1935¹⁰³ and suggested that their suggestion of O-substitution was probably in error as no O-methylenesulphites were known at that time and that the di-NN substituted product was more likely.

They published an improved preparation of Sulpharsphenamine later in that year which was purported to give a more consistent product.¹⁰⁴

Despite all this research Neosalvarsan still remained somewhat of a mystery substance.

1.6.3 Mapharsen

The last arsenic compound to enter the market for the treatment of syphilis was Mapharsen – 4-hydroxy-3-aminophenylarsineoxide. Mapharsen had been investigated by Ehrlich and Bertheim initially as #599 in 1909 and then again as #644. It could be that the first investigation was against trypanosomes and the second against syphilis but why it would have two such numbers is unknown. It seems to have been effective against both trypanosomes and *Treponema* but it was abandoned by Ehrlich because of its high toxicity.

“It may be further mentioned that the arsenoxide develops a remarkably strong spirillocide power.....the toxicity is much higher than that of the arseno compound (Salvarsan), so that for treatment of syphilis and allied spirillooses, it certainly cannot come into consideration as a direct drug, but instead at most as a combination drug with the arseno compound.”¹⁰⁵

It is not known if any combination therapy with this substance was ever tried. Ehrlich seems to have been unaware of the relationship between the arsenobenzene compound and the arsenoxide – namely that the latter was the oxidation product of the former - with the arsenobenzene compound acting as a slow release form of the arsenoxide *in vivo*; and that the arsenoxide was the active compound.⁷⁶ It is not certain how much Ehrlich was aware that the arsenic(III) compounds were the active agents in the

treatment of trypanosomes and *Treponema* and that *in vivo*, use of arsenic(V) and arsenic(I) compounds were dependent on their reduction or oxidation to arsenic(III) in the organism being treated in order to be effective. Some of the things Ehrlich said

*“It may be taken as certain that the final destructive action is attributable to the threefold value arsenical derivative contained in the arseno group”*¹⁰⁵

seem to indicate that Ehrlich didn't understand that Salvarsan contained As(I) and Mapharsen contained As(III) but it is hard to understand what was meant chemically by his above statement.

It should also be noted that the structures assigned to these compounds (commonly called arsenoxides – RAs=O) were also probably incorrect and that these substances are more probably R-As(OH)₂ or (R-As-O)_n polymers or cyclic compounds.

Mapharsen seems to have been forgotten by Ehrlich and although the compounds were known^{52, 77} very little attention seems to have been paid to them other than that they were the toxic oxidation products of Salvarsan.

A collaborative group consisting of scientists from Parke Davis together with Hamilton and Whitmore – chemists from Northwestern University Chemistry Department - and Arthur Loevenhart from the department of pharmacology and toxicology at the University of Wisconsin school of medicine came together with an agreement in 1927 with the goal of doing

*“certain research work, covering organic arsenical compounds, designed for the treatment of syphilis and tyranosomiasis.”*¹⁰⁵

This group was in many ways similar to Ehrlich's group – a chemist, a pharmacologist and a drug company for funding. Hamilton had published many papers on aryl arsenic compounds after initially becoming involved with arsenical compounds during the war, Parke Davis had started their own arsenic research soon after the discovery of Salvarsan and Loevenhart had much experience with antisyphilitic drugs. Initially the progress for the group was slow and Loevenhart died in 1929 and was replaced by Tatum. In 1930, Tatum started working with Mapharsen, initially as a tool to understand the involvement of the host in arsenotherapy. These earlier experiments indicated that it had similar effectiveness to Salvarsan in experimental syphilis with accidental oxidation not increasing the toxicity of the compound. By the mid-thirties Mapharsen was in full clinical trials and was seen to have numerous advantages over Salvarsan and Neosalvarsan – namely that it was soluble, didn't need alkalisation, didn't decompose to more toxic products and allowed for smaller doses to be administered. Tatum criticized Ehrlich many years later for using impure Mapharsen in his studies. It was patented in September 1937. When released it quickly became a popular drug and it was much used in the Second World War. By the end of the war penicillin had come into production and the demand for arsenical drugs dropped off.

Arsenical drugs were very quickly forgotten with the advent of penicillin, which had been observed by Fleming in 1928, but had remained a curiosity until the Second World War when antibiotic research increased and by the fifties, penicillin had replaced arsenical compounds for the treatment of syphilis.

The composition of Salvarsan and some other arseno- therapeutic compounds was to remain a mystery as most research into arsenical compounds was abandoned with the discovery of penicillin.

1.6.4 Cyclopolyarsines

During the time in which research into arsenical drugs was being carried out (1920-1950) much attention was focused on substituted phenylarsonic compounds but very few new or useful drugs seemed to come from this research. The As=As structure proposed by Ehrlich seems to have remained largely unchallenged, despite the lack of evidence to support this structure.

Similar compounds had been created using other phenyl arsonic acids – the earliest dating back to 1881 when Michaelis had published the synthesis of arsenobenzene using phosphorous acid.⁷⁷ This was probably the source for Ehrlich's erroneous structure of Salvarsan as the two compounds could be prepared in the same way and were thought to be structurally analogous to azobenzenes.

Several papers were published in between 1928 and 1931 which attempted to work out the molecular weight of arsenobenzene and related compounds using ebullioscopic and cryoscopic methods⁹³⁻⁹⁴ – the results obtained varied between the method and the solvent used, indicating something more complex.

Arsenobenzene was investigated by X-ray diffraction in 1960 by a Russian group¹⁰⁶ and found to be a cyclic hexamer. This was confirmed in 1961 by an American group¹⁰⁷ Arsenomethane had also been examined by X-ray diffraction¹⁰⁸ and found to consist of cyclic pentamers.

These compounds came to be known as cyclopolyarsines and a review in 1975¹⁰⁹ covered all the known compounds to date. The similarity between the cyclopolyarsines which had been structurally elucidated by X-Ray crystallography and Salvarsan was noted but Salvarsan and related compounds were excluded from the review because

*“All of the derivatives of arsenobenzene and Salvarsan, with the exception of (*p*-CH₃C₄H₄As)₆, (*m*-CH₃C₆H₄As)₆ and (*p*-CH₃OC₆H₄As)₆ appear to be polymers, not cyclopolyarsines.”¹⁰⁹*

Comparisons were also made to cyclopolyphosphines which were also known.

Mass spectral studies were carried out using electron impact mass spectrometry¹¹⁰ and these examined arsenomethanes and ethanes and hexaphenylcyclohexaarsine as well as mixed compounds (the so called “unsymmetric arsenobenzenes”) of the two for which they observed a complex mixture and noted that in related cyclic phosphines redistribution reactions can occur.

At this stage, Salvarsan and related compounds were considered to be polymers of unknown size, however the As=As double bond continued to feature in textbooks and teaching material. Levinson¹¹¹ discussed this error and ended his paper with

“But it is clear that the simple arsenic-arsenic double bond is not known at this time”.

Less than ten years later the first compound containing an As=As double bond was prepared¹¹² by using bulky ligands to prevent the formation of cyclic structures and force the formation of the double bond. Several of these compounds have been reported to date¹¹³ including symmetrical structures of the type R-As=As-R. where R = a very bulky group.

Recently a four membered aryl arsenic ring compound has been reported with mesityl groups – the smallest aryl arsenic ring known to be elucidated by X-Ray crystallography.¹¹⁴

To date the only aryl cyclopolyarsines to have been structurally elucidated have included non-polar aryl groups. This is possibly because of the problems of solubility of some of the compounds related to Salvarsan which are difficult if not impossible to obtain in a crystalline state mainly due to their lack of solubility in standard solvents and tendency to oxidize quickly.

1.6.5 More Salvarsan related chemistry

Arsenic chemistry continued to advance long after Salvarsan had faded from mainstream interest but Salvarsan related chemistry became more obscure as Salvarsan and arsenicals were superseded by penicillin which became widely available after World War Two.

Doak published a modified Bart Reaction¹¹⁵ in 1940, which allowed arsonic acids with virtually any substitution pattern to be made – even those which had previously been difficult to produce via the standard method.

In 1948 a paper was published which attempted to make fluorine derivatives of some chemotherapeutic agents including Salvarsan. This was in response to the interest in fluorine chemistry at the time. This paper outlined the synthesis of 4-fluoronitroaniline, 4-fluoro-3-nitrophenyl arsonic acid, 3,3'-diamino-4,4'-difluoroarsenobenzene and 3-amino-4-fluorophenyl arsonic acid via standard methods.¹¹⁶ The latter two compounds were found to be extremely sensitive to oxidation and were not characterized in detail. It is unknown if they were successful in making 4-fluoro-3-nitrophenyl arsonic acid by this method as we were not able to replicate these results with the methods described (see 2.3.2.5).

Salvarsan was investigated for anti-tumour activity in 1974¹¹⁷ and found to be effective when combined with antibody-glucose oxidase conjugates and a peroxidase enzyme and

tested *in vitro* in cell lines. It is not known if this research was ever followed up in animal models but it seems that Salvarsan in this case was acting as a prodrug and being oxidized to the more toxic arsenoxide to bring about cytotoxicity.

1.7 The end of syphilis? or of arsenic?

Salvarsan and related compounds were the mainstays in the treatment of syphilis from the time when they were discovered until the time when they were replaced by penicillin, which came even closer to Ehrlich's goal of "*therapia sterilisans magna*" than Salvarsan had.

A minor point of interest which is malarial treatment for syphilis which was "discovered" in 1917 by Julius Wagner-Jauregg when he observed that patients with general paresis of the insane (tertiary neurosyphilis) seemed to improve after bouts with fever.¹¹⁸ A soldier arriving at his institution with malaria gave him the opportunity to test his theory by infecting patients with neurosyphilis with malaria. Some of these patients improved and the new treatment came into vogue. The patient would be infected with malaria, which would be allowed to go through 7-10 fever cycles before being treated. It was a desperate remedy for a desperate condition and often combined with arsenotherapy in the form of Salvarsan or Neosalvarsan.¹¹⁹

The fevers caused by the malaria apparently killed the spirochetes and this method seemed to succeed in some cases where chemotherapeutic means had failed.

Wagner-Jauregg received the Nobel prize in Medicine in 1927 for this work.¹²⁰

Syphilis remains a relatively common disease to this day with antibiotic resistant strains being reported,¹²¹ however it remains treatable with penicillin.¹²²

Arsenic remains in use for some trypanosomal diseases – the drug melarsoprol being used in treatment for human African trypanosomiasis, or sleeping sickness.

1.8 Prelibation

Building on the history of Salvarsan and related compounds it was apparent that no conclusive structural evidence has ever been put forward for the structure of Salvarsan. People have hypothesized various other structures based on similar compounds and although Ehrlich's As=As structure is accepted to be unlikely there exists no historical evidence to suggest a realistic structure for Salvarsan.

Hence the present project was designed to investigate Salvarsan and related compounds using modern techniques to try and solve the long standing problem.

Chapter Two details the synthesis of variously substituted arsonic acids by several methods and fully characterises them using modern techniques including full mass spectrometry data and X-ray crystallographic structures and are discussed in detail.

These arsonic acids were required for the synthesis of Salvarsan and related compounds.

Chapter Three reports a detailed re-investigation of the various syntheses of Salvarsan. Related compounds were prepared and were all subjected to a detailed mass spectrometric investigation to determine molecular structure. As well as characterising the main components an analysis of minor impurities is reported.

Chapter Four details the synthesis of various arsonous acids which are believed to be the biologically active compounds formed *in vivo* by Salvarsan. Several examples were prepared and analysed. Comparison is made between a sample of commercially prepared Mapharsen and the compound we synthesised. This chapter also includes some preliminary protein-binding experimental results.

2 Chapter Two - Arsonic Acids

2.1 Introduction to arsonic acids

The starting materials for the arsenic(III) and arsenic(I) compounds that are the subject of this thesis are the aryl arsonic acids, RAsO_3H_2 .

Only a few of these [phenylarsonic acid, 3-nitro-4-hydroxyphenylarsonic acid (“Roxarson”) and *p*-aminophenylarsonic acid (*p*-arsanillic acid)] are commercially available.

The other examples required needed to be synthesized. Because much of the published data for these compounds is incomplete and historical, the opportunity was taken to characterize them with modern spectroscopic and structural methods. These results form the basis for this chapter.

Figure 2-1 shows the number of papers published with the keywords “arsonic acids” which appear in the SciFinder database. It can be seen that the interest in arsonic acids started in about 1910 and peaked between 1926 and 1930 before tailing off after 1940, when penicillin was becoming commonly available. There was a brief resurgence of interest during the 1970’s and interest has been increasing recently – with the majority of modern publications in the sphere of biochemistry, as opposed to pure chemistry.

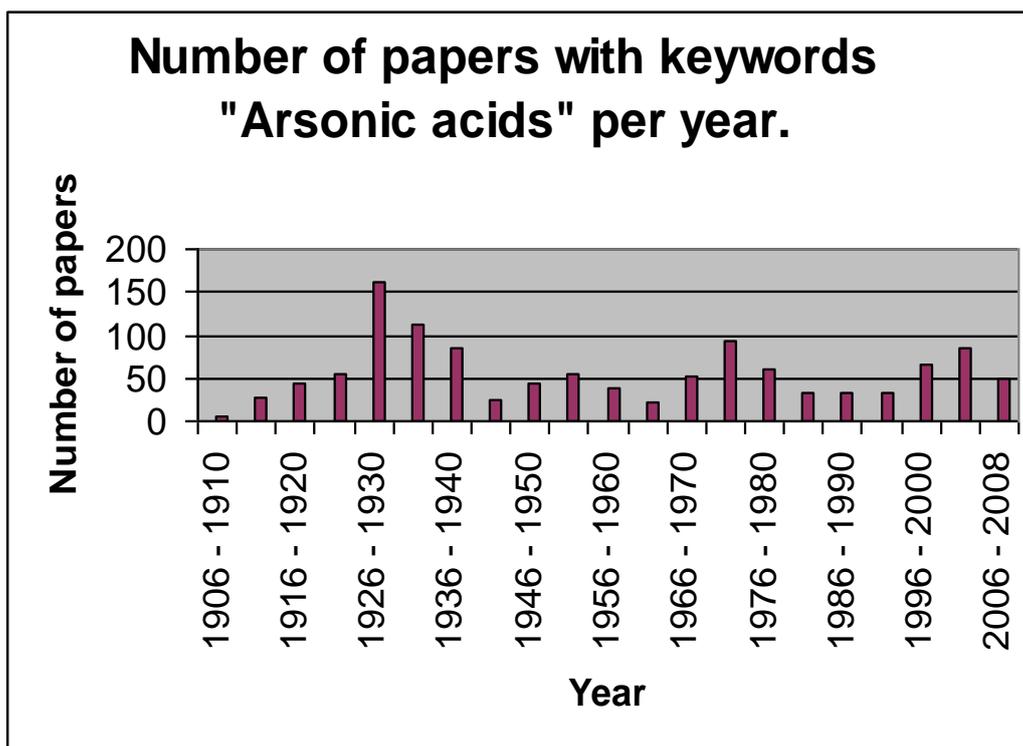


Figure 2-1 - Plot showing number of papers with keywords "Arsonic acids" since 1900

Arsonic acids are still used commercially – with large quantities of 3-nitro-4-hydroxyphenylarsonic acid being used as a poultry feed supplement.¹²³ As of June 2011, the FDA has stopped sale of 3-nitro-4-hydroxyphenylarsonic acid in the United States of America as inorganic arsenic was detected in chickens treated with 3-nitro-4-hydroxyphenylarsonic acid.¹²⁴

Smaller quantities of some specific arsonic acids are available as colorimetric indicators for some inorganic ions.

2.2 Background.

Doak notes three main reactions for preparing arsonic acids however they are not all applicable to the preparation of aryl compounds.

2.2.1 The Béchamp reaction

Historically the methods for preparation of arsonic acids evolved from the initial preparation of *p*-aminophenylarsonic acid by Béchamp in 1860.

As has already been noted in section 1.4.6, Béchamp had concluded that this compound was a monobasic acid anilide of arsonic acid.⁴³ Béchamp's "anilide de l'acide" remained obscure for the next forty two years when Ehrlich took an interest in the compound and elucidated the correct structure.⁴⁹

The Béchamp reaction (as it is now known) is useful for preparing arylarsonic acids from aniline and some phenols by direct arsonation. Doak¹²⁵ reports that the yield for this reaction is rarely over 25% (33% when phenol is used).

It involves heating aniline (also aromatic amines, phenols and phenyl ethers) with syrupy arsenic acid under reflux with no additional solvent for several hours (Figure 2-2).

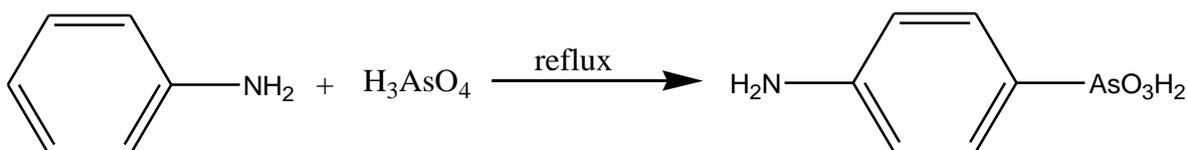


Figure 2-2 - The Béchamp reaction

No mechanistic studies have been carried out but it is assumed by Doak¹²⁵ the mechanism involves the electrophilic attack of the arsenic on the activated *para* position of the ring in a similar way to the "somewhat analogous" sulfonation reaction.¹²⁵

Bertheim⁵² investigated many arsonic acids and published several preparations of different arylarsonic acids using *p*-aminophenylarsonic acid as a starting point.

2.2.2 The Bart reaction.

The Bart reaction was patented in 1910¹²⁵ and is the most commonly used reaction for preparing arsonic acids as it allowed for a full range of substitution patterns to be prepared and investigated as almost any substituted aniline can be used to produce an arsonic acid.

The Bart reaction involves the addition of an aqueous alkaline solution of sodium arsenite to the diazo salt of the desired amine. The reaction requires a catalyst (usually a copper salt) and gives the maximum yields when buffered with sodium carbonate.

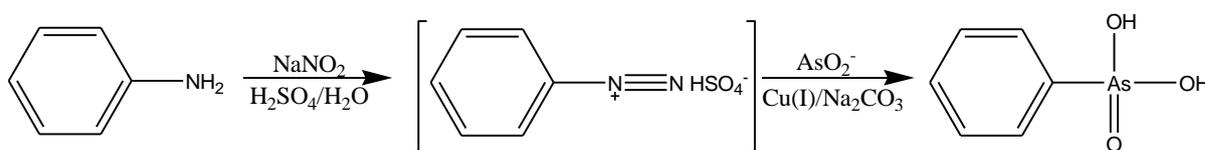


Figure 2-3 - The Bart reaction

There are several variations of this reaction, the most useful being the Scheller variation,¹¹⁵ which can be adapted into a one pot synthesis, where the diazo compound is made and decomposed with a cuprous bromide catalyst in the presence of arsenic trichloride in an alcoholic solution. This variation also does away with the excessive foaming sometimes encountered in the Bart reaction.

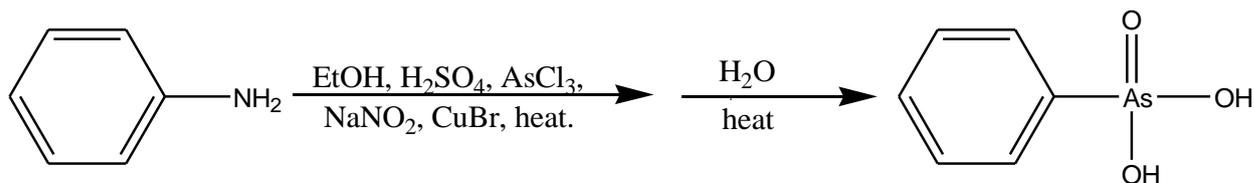


Figure 2-4 - The Scheller variation of the Bart reaction

Other variations exist, including the use of dry diazonium fluoroborate salts and various metallic salts as catalysts but Doak¹²⁵ notes that no systematic study of these variations has been made and that any increase in yield is minimal.

2.2.3 The Rosenmund reaction.

In 1883 Meyer published the first method for the production of alkylarsonic acids from sodium arsenite and alkyl iodides,¹²⁶ however this proved to be unsuccessful when adapted for the preparation of aryl compounds.¹²⁵ Rosenmund studied the reaction with aryl halides and in 1921 published a preparation of phenylarsonic acid by heating bromobenzene and potassium arsenite in a sealed tube at 180-200°C with poor yields.¹²⁷ Doak notes that the Rosenmund reaction is of “little synthetic importance”.¹²⁵

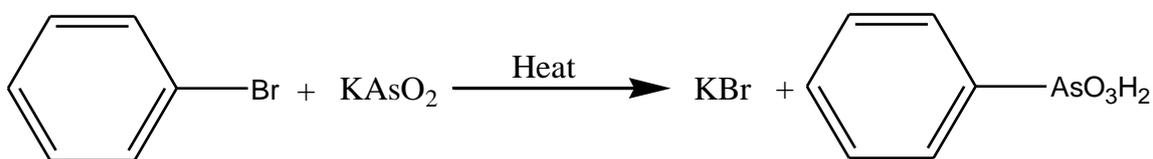


Figure 2-5 - The Rosenmund reaction

It is a poor variation of the Meyer reaction and is rarely used or mentioned in the literature.

2.2.4 Other reactions.

Doak¹²⁵ goes on to list several other minor methods including oxidation of As(III) species to the As(V) arsonic acids which is useful in the case of those compounds which can be prepared easily from reacting the aniline with arsenic trichloride (Figure 2-6)

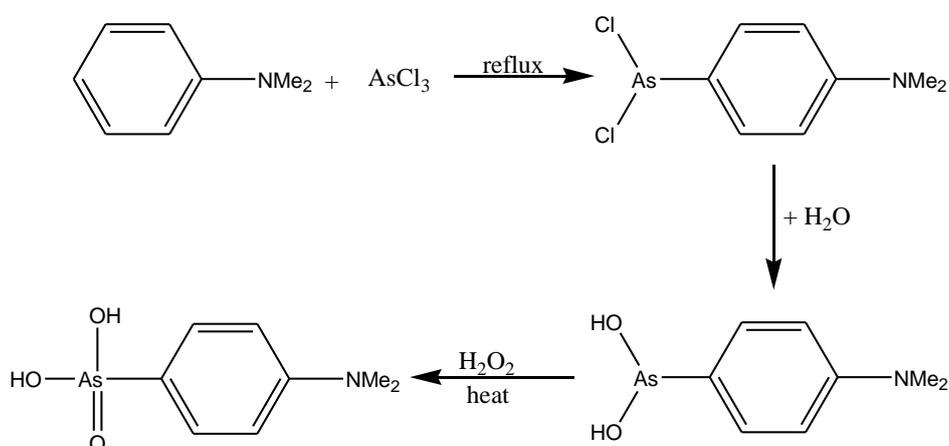
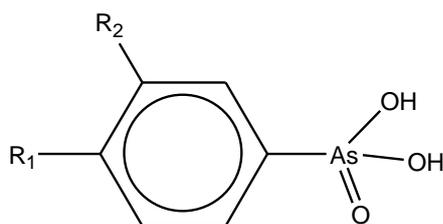


Figure 2-6 – Preparation of *N,N*-dimethylphenylarsonic acid

There is also one reaction mentioned in Doak¹²⁵ which involves the replacement of a sulfonic acid group in which *p*-hydroxybenzene sulfonic acid is heated with arsenic acid (H_3AsO_4) at 135°C for four hours to give a 90% yield of *p*-hydroxyphenylarsonic acid.

2.3 Preparation of Arsonic acids.

Since much of the literature on arylarsonic acids is quite old, we have taken the opportunity to prepare and re-examine the series **1-16** (Figure 2-7) with modern spectroscopic techniques and to structurally define key examples, **1**, **10**, **13-16**, by single crystal X-ray diffraction.



1	R ₁ = H	R ₂ = H	9	R ₁ = EtO	R ₂ = NO ₂
2	R ₁ = OH	R ₂ = H	10	R ₁ = F	R ₂ = H
3	R ₁ = OH	R ₂ = NO ₂	11	R ₁ = H	R ₂ = NO ₂
4	R ₁ = NH ₂	R ₂ = H	12	R ₁ = MeO	R ₂ = NO ₂
5	R ₁ = NO ₂	R ₂ = H	13	R ₁ = F	R ₂ = NO ₂
6	R ₁ = MeO	R ₂ = H	14	R ₁ = OH	R ₂ = NH ₂
7	R ₁ = Me	R ₂ = H	15	R ₁ = MeO	R ₂ = NH ₂
8	R ₁ = EtO	R ₂ = H	16	R ₁ = F	R ₂ = NH ₂

Figure 2-7 - Arsonic acids investigated in this study

Compounds **1-4** were commercially available and were used as purchased; Compound **1** was also synthesized using the Bart reaction. Compounds **5-9** were synthesized using the Scheller variation of the Bart reaction.

Compound **10** was synthesised using a further variation of the Scheller reaction when this compound proved impossible to make by the normal method. Compounds **11-13** were prepared by nitration of compounds **8**, **1**, **6** and **10** respectively. Compounds **14-16** were prepared by reduction of **3**, **12** and **13** respectively.

2.3.1 General

PhAsO₃H₂ (**1**) (BDH), 4-HOC₆H₄AsO₃H₂ (**2**) (TCI), 3-NO₂-4-HOC₆H₃AsO₃H₂ (**3**) (Aldrich) and 4-H₂NC₆H₄AsO₃H₂ (**4**) (TCI), were commercially available.

Arsenic trichloride was both from commercial sources (BDH) and made as required.¹²⁸ All substituted anilines were used as purchased, except for 4-fluoro-3-nitroaniline which was synthesized using standard methods.

Arsenic trioxide was used as purchased from BDH.

NMR spectra were recorded on Bruker AC300 or AC400 spectrometers in *d*⁶-DMSO as solvent and internal standard. Assignments were by standard proton-carbon HSQC and HMBC experiments. ESI-MS were measured on a Bruker MicrOTOF spectrometer, using MeOH or H₂O as solvent. DSC data were obtained on a Perkin-Elmer DSC6 machine.

2.3.2 Syntheses

The detailed syntheses of arylarsonic acids presented here using the modified Bart reaction¹¹⁵ were adapted from older literature which generally gave procedures for making compounds on large scales. These modified methods are reproduced here for convenience.

2.3.2.1 Preparation of phenylarsonic acid (**1**) using the Bart reaction¹²⁹

Anhydrous Na₂CO₃ (400 g, 3.77 mol), As₂O₃ (200 g, 1.01 mol) and CuSO₄·5H₂O (10 g, 0.06 mol) were suspended in water (800 mL) in a 5 L beaker and warmed with stirring. When most of the solid had dissolved the solution was allowed to cool.

In a separate 5 L beaker, aniline (150 g, 1.6 mol) was carefully added to a solution of concentrated HCl (320 mL), water (800 mL) and enough ice to make approximately 2.4 L. To this a saturated aqueous solution of NaNO₂ (112 g) was slowly added.

The resulting diazonium salt solution was slowly added over 1 h to the arsenite mixture, with cooling to keep the temperature below 15°C. Small amounts of acetone were added to minimize foaming, caused by the effervescing N₂ gas.

The resulting slurry was stirred overnight, to allow complete evolution of nitrogen. The mixture was filtered and the volume reduced to 1 L on a hot plate. Activated carbon was added and the mixture filtered to give a brown solution. This was acidified by adding conc. HCl until a brown oil precipitated. The mixture was filtered through Celite which absorbed the oil. The filtrate was treated again with carbon and re-filtered. More conc. HCl was slowly added, precipitating off-white crystals which were collected by filtration and dried under vacuum. On standing overnight the mother liquor precipitated a further crop of crystals. Total crude yield was 89.3 g (44%). Recrystallisation from hot water with further activated carbon treatment gave pure phenylarsonic acid (66.3 g, 32%) as white crystals. ESI-MS: *m/z* [M-H]⁻ 200.954 (Calc. 200.953).

2.3.2.2 Preparation of 4-nitrophenylarsonic acid (5) using the Scheller variation of the Bart reaction¹¹⁵

Concentrated H₂SO₄ (10 g) was added to 4-nitroaniline (13.8 g, 0.1 mol) in absolute ethanol (250 mL). AsCl₃ (28 g, 0.16 mol) was added and the mixture cooled to < 5°C in an ice bath. A solution of sodium nitrite (8.28 g, 0.12 mol) in water (12 mL) was added slowly to the cooled mixture with thorough stirring.

CuBr (1 g) was added to the mixture and it was warmed to 60°C for 6 hours before cooling overnight. The solution was transferred to a round-bottomed flask and steam

distilled to remove solvent and unreacted aniline. The liquid residue in the still-pot was transferred to a beaker while still hot. Activated carbon (10 g) was added and the mixture boiled for 10 min and filtered. The green solution was cooled at 4°C and allowed to crystallise overnight. Yellow crystals were collected and recrystallised from the minimum quantity of boiling water to give 4-nitrophenylarsonic acid as fine yellow crystals, 8.13 g, (33%). Found: C 29.40; H 2.44; N 5.38%. C₆H₆NO₅As requires C 29.17; H 2.45; N 5.67%. ESI-MS: m/z [M-H]⁻ 245.941 (Calc. 245.938).

2.3.2.3 Preparation of 4-methoxyphenylarsonic acid (6)

This was prepared by the Scheller variation of the Bart reaction,¹¹⁵ as described for compound **5**, from *p*-anisidine (12.3 g, 0.10 mmol). Recrystallisation from boiling water gave 4-methoxyphenylarsonic acid as white crystals (14.3 g, 61%). Found: C 36.91; H 3.92%. C₇H₉O₄As requires C 36.23; H 3.91%. ESI-MS: m/z [M-H]⁻ 230.9651 (Calc. 230.9639).

2.3.2.4 Preparation of 4-methylphenylarsonic acid (7)

This was prepared by the Scheller variation of the Bart reaction,¹¹⁵ as described for compound **5**, from 4-methylaniline (10.7 g, 0.10 mmol), 4-methylphenylarsonic acid was obtained as white crystals. ESI-MS: m/z [M-H]⁻ 214.9697 (Calc. 214.9689).

2.3.2.5 Preparation of 4-ethoxyphenylarsonic acid (8)

This was prepared by the Scheller variation of the Bart reaction,¹¹⁵ as described for compound **5**, in an attempt to make 4-fluorophenylarsonic acid, from 4-fluoroaniline (11.1 g, 0.10 mmol) fine yellow crystals of 4-ethoxyphenylarsonic acid were obtained.

Found: C 38.94; H 4.36%. $C_8H_{11}O_4As$ requires C 39.04; H 4.51%. ESI-MS: m/z $[M-H]^-$ 244.9777 (Calc. 244.9795).

2.3.2.6 Preparation of 4-ethoxy-3-nitro-phenylarsonic acid (9)

This was prepared by the Scheller variation of the Bart reaction,¹¹⁵ as described for compound **5**, in an attempt to make 4-fluoro-3-nitrophenylarsonic acid from, 4-fluoro-3-nitroaniline (15.6 g, 0.10 mmol) gave 4-ethoxy-3-nitro-phenylarsonic acid as fine yellow crystals of the monohydrate. Found: C 30.42; H 3.06; N 4.73%. $C_8H_{10}NO_6As.H_2O$ requires C 31.09; H 3.91; N 4.53%. ESI-MS: m/z $[M-H]^-$ 289.9669 (Calc. 289.9646).

2.3.2.7 Preparation of 4-fluorophenylarsonic acid (10)

This was prepared by the Scheller variation of the Bart reaction,¹¹⁵ as described for compound **5**, except THF (220 mL) was used as the solvent instead of EtOH, 4-fluoroaniline (11.1 g, 0.10 mmol) gave 4-fluorophenylarsonic acid as fine white crystals (11.3 g, 51%). Found: C 33.01; H 2.77%. $C_6H_6O_3FAs$ requires C 32.75; H 2.75%. ESI-MS: m/z $[M-H]^-$ 218.9475 (Calc. 218.9439).

2.3.2.8 Preparation of 3-nitrophenylarsonic acid (11)⁷⁷

Phenylarsonic acid (8.08 g, 0.04 mol) was dissolved in concentrated H_2SO_4 (30 mL) and cooled to $-8\text{ }^\circ\text{C}$ in an ice/salt bath. A mixture of concentrated HNO_3 and H_2SO_4 acids (1:1, 5.6 mL) was added slowly ensuring that the temperature was $<0\text{ }^\circ\text{C}$. The mixture was left to return to room temperature overnight with stirring, then poured over 200 g ice and left at $4\text{ }^\circ\text{C}$ for a further 24 hours. Yellow crystals were collected and

recrystallised from boiling water to give 3-nitrophenylarsonic acid as pale yellow crystals. Found C 29.54; H 2.44; N 5.40%. $C_6H_8AsNO_4$ requires C 29.17; H 2.45; N 5.67%. ESI-MS: m/z $[M-H]^-$ 245.9399 (Calc. 245.9384).

2.3.2.9 Preparation of 4-methoxy-3-nitrophenylarsonic acid (12)^{87c, 130}

This was prepared by nitration as described for compound **5**, from 4-methoxyphenylarsonic acid (9.2 g, 0.04 mol), nitration with concentrated H_2SO_4/HNO_3 gave pale yellow crystals from water of 4-methoxy-3-nitrophenylarsonic acid (7.21 g, 73%). Found C 30.60; H 2.90; N 4.83%. $C_7H_8AsNO_6$ requires C 30.35; H 2.91; N 5.06%. ESI-MS: m/z $[M-H]^-$ 275.9505 (Calc. 275.9489).

2.3.2.10 Preparation of 4-fluoro-3-nitro-phenylarsonic acid (13)

4-Fluorophenylarsonic acid (13.2 g, 0.06 mol) was dissolved in conc. H_2SO_4 (45 mL). To this solution fuming nitric acid (6 mL) was added. The mixture was heated with stirring in a water bath for approximately 6 hours. The heat was then turned off and the mixture left overnight to cool. The mixture was poured over ice (300 g) and stored at 4°C overnight. The precipitate was filtered and dried.

Found C 27.42; H 1.85; N 5.06%. $C_6H_5FAsNO_5$ requires C 27.19; H 1.90; N 5.28%. ESI-MS: m/z $[M-H]^-$ 263.9284 (Calc. 263.9289).

2.3.2.11 Preparation of 3-amino-4-hydroxyphenylarsonic acid (14)^{80, 131}

3-Nitro-4-hydroxyphenylarsonic acid (13.1g, 0.05 mol) was dissolved in a solution of aqueous NaOH (100 mL, 1 mol L^{-1}) and cooled to 0°C in an ice/salt slush bath with stirring. $Na_2S_2O_4$ (30.25 g) was added in one portion with vigorous stirring. The

solution effervesced vigorously. As soon as the colour changed from orange to pale yellow, concentrated HCl (12 mL) was added. This mixture was held at <0°C until the frothing ceased and the product had precipitated from the solution. This was filtered and washed twice with ice-cold water to give crude 3-amino-4-hydroxyphenylarsonic acid (6.50 g, 56%) as a cream coloured solid which was dried under vacuum. The crude product (6 g) was dissolved in a mixture of H₂O (25 mL) and conc. HCl (2 mL) and stirred with decolourising carbon for 15 minutes before filtering. To the filtrate, sodium acetate solution (25%) was added until the solution was no longer acidic to Congo Red. The solution was cooled at 4°C for 20 min. and the precipitated crystals were collected by filtration and dried under vacuum. Yield was 4.7 g, 78%, of pure **14** as off-white microcrystals. Found C 30.76; H 3.41; N 6.32%. C₆H₈AsNO₄ requires C 30.90; H 3.43; N 6.01%. ESI-MS: *m/z* [M-H]⁻ 231.9604 (Calc. 231.9591).

2.3.2.12 Preparation of 3-amino-4-methoxyphenylarsonic acid (15)

4-Methoxy-3-nitrophenylarsonic acid (5 g) was reduced with ferrous sulfate following the method of Jacobs *et al.*⁷⁸ Found C 34.14; H 4.05; N 5.40%. C₇H₁₁AsNO₄ requires C 34.03; H 4.08; N 5.67%. ESI-MS: *m/z* [M-H]⁻ 245.9747 (Calc. 245.9748).

2.3.2.13 Preparation of 3-amino-4-fluorophenylarsonic acid (16)

4-fluoro-3-nitrophenylarsonic acid (2.65 g, 0.01 mol) was reduced with ferrous sulfate following the method of Jacobs *et al.*⁷⁸ The compound was isolated in very low yields and recrystallised from hot water for the crystal structure.

ESI-MS: *m/z* [M-H]⁻ 233.9556 (Calc. 233.9542).

2.3.3 Discussion of the attempted preparations of the fluorinated compounds.

All these reactions proceeded as described in the literature except for the attempted preparation of 4-fluorophenylarsonic acid and 4-fluoro-3-nitro-phenylarsonic acid using the Scheller variation of the Bart reaction.¹¹⁵ When the preparation of the fluorinated compounds was attempted by this method it was impossible to get the reactions to work as planned as at some stage in the reaction the fluorine group would be substituted by an ethyl group and the ethyl compounds would be produced instead.

In order to get around this problem, THF was used as a solvent and this gave the expected product.

It is not known if the Bradlow¹¹⁶ paper successfully prepared the fluorinated compound, or if they had the same results as this work and made the same ethyl substituted compounds and misidentified them as the fluoro compounds. The arsonic compounds mentioned in the paper are only characterised by melting point and elemental analysis and so it is difficult to ascertain what products were made. It remains a possibility that variations in pH might still allow the correct product to be isolated from the reaction using ethanol as a solvent.

2.4 X-ray crystallography

2.4.1 Previous X-Ray Crystallography

Several crystal structures of arylarsonic acids are currently known, dating back from 1960. These earlier determinations are listed in Table 2.1, along with CCDC codes, R_1 values, dates and some important bond lengths. The data highlighted has R_1 values > 0.08 and has been excluded from the calculations.

Salts have also been excluded from the averages.

This table does not include all the compounds with the RAsO_3H_2 group but only the arylarsonic acids which are not part of a larger complex structure.

These arsonic acids can exist in solid state as zwitterions, salts or neutral molecules.

The As-C bond exists within a relatively narrow range from 1.866\AA to 1.930\AA .

Variation in this bond length is linked to substituents on the ring, with NO_2 in the 4 position having the greatest effect on the bond length. Variation in the other bond lengths is reasonably consistent and is probably linked to the bonding between molecules.

Table 2.1 - Selected X-ray data for known arylarsonic acids - ArAsO₃H₂

Aryl group	CCDC	R	Date	As-C	As=O	As-OH
4-NO ₂ C ₆ H ₄	AHILAE	0.03	2002	1.930	1.664	1.732
3-NH ₂ C ₆ H ₄	AMBARS	0.10	1962	1.908	1.728	1.779
C ₆ H ₅	ARSACP	0.14	1960	1.906	1.624	1.726
C ₆ H ₅	ARSACP01	0.096	1960	1.970	1.651	1.748
[4-OH-3-NH ₃] ⁺ C ₆ H ₃	BUTDEP	0.017	1983	1.879	1.652	1.719
4-NH ₂ C ₆ H ₄	CUDSEZ	0.13	1961	1.951	1.669	1.861
4-NHC ₆ H ₄	CUDSEZ01	0.044	1996	1.930	1.663	1.737
C ₆ F ₅	GORKAZ	0.048	1999	1.902	1.652	1.696
2-C=OPhC ₆ H ₄	JACFUO	0.018	1998	1.928	1.652	1.708
2-(NHCH ₂ COOH)C ₆ H ₄	LACJII	0.046	1992	1.891	1.651	1.712
2-(NH(CH ₂) ₂ COOH)C ₆ H ₄	LACJOO	0.040	1992	1.880	1.650	1.709
4-OH-3-NO ₂ C ₆ H ₃	NHXPAS	0.12	1977	1.909	1.673	1.720
4-OH-3-NO ₂ C ₆ H ₃	NHXPAS01	n/a	1963	1.886	-	-
4-OH-3-NO ₂ C ₆ H ₃	NHXPAS02	0.058	1995	1.886	1.653	1.697
2-NH ₂ C ₆ H ₄	OAPASA	0.086	1977	1.923	1.637	1.725
2-NH ₂ C ₆ H ₄	OAPASA01	0.056	2002	1.905	1.644	1.694
4-OH C ₆ H ₄	POVMUI	0.035	1996	1.885	1.643	1.713
4-OMe-3-NO ₂ C ₆ H ₃	POVNAP	0.047	1996	1.888	1.660	1.699
2-(NHC=OCHCH ₂)C ₆ H ₄	VAZFOS	0.038	2005	1.892	1.642	1.704
4-NO ₂ C ₆ H ₄	WAMFUM	0.059	2005	1.866	1.604	1.721
4-(NHC=OCCH ₃ CH ₂)C ₆ H ₄	WUKKOC	0.037	2001	1.893	1.649	1.706
4-NHC=OCCH ₃ CH ₂ C ₆ H ₄	WUKKOC01	0.042	2001	1.899	1.644	1.702
2-NHRC ₆ H ₄	XUTFAT	0.048	2002	1.902	1.643	1.704
4-NH-triazineC ₆ H ₄	ZAYWOL	0.075	1995	1.901	1.560	1.705
3-NH ₂ -4-OHC ₆ H ₃	This work	0.0286	2005	1.904	1.677	1.728
3-NH ₂ -4-OMeC ₆ H ₃	This work	0.0243	2006	1.895	1.664	1.719
3-NO ₂ -4-FC ₆ H ₃	This work	0.0180	2006	1.894	1.658	1.710
3-NH ₂ -4-FC ₆ H ₃	This work	0.0255	2008	1.905	1.659	1.717
4-FC ₆ H ₄	This work	0.0173	2006	1.897	1.657	1.713
C ₆ H ₅	This work	0.0118	2007	1.888	1.662	1.707
Average				1.893	1.649	1.707

2.4.2 X-ray analysis

X-ray intensity data for compounds **1**, **10**, **13-16** were obtained from a Bruker SMART CCD diffractometer, and were processed using standard software. Crystal data and refinement details are summarized in Table 2.2.

Corrections for absorption were carried out using SADABS.¹³² The structures were solved and refined using the SHELX programs¹³³, operating under WinGX.¹³⁴

All H atoms were located from penultimate difference maps and refined, except for **14** and **15** where only the NH₂ and OH hydrogen atoms were refined, and the others were placed in calculated positions.

Analyses were straightforward, except for PhAsO₃H₂ which was refined as a racemic twin, with the twinning parameter converging to a value of 0.44.

Table 2.2 - Crystal data and refinement details for aryl arsonic acids 1, 10, and 13- 16

	PhAsO₃H₂ (1)	4-FC₆H₄AsO₃H₂ (10)	3-NO₂-4-F-C₆H₃AsO₃H₂ (13)	3-NH₂-4-MeO-C₆H₃AsO₃H₂ (15)	3-NH₂-4-HO-C₆H₃AsO₃H₂ (14)	3-NH₂-4-F-C₆H₃AsO₃H₂ (16)
Formula	C ₆ H ₇ AsO ₃	C ₆ H ₆ AsFO ₃	C ₆ H ₅ AsFNO ₅	C ₇ H ₁₀ AsNO ₄	C ₆ H ₈ AsNO ₄	C ₆ H ₇ AsFNO ₃
M _r	202.04	220.03	265.03	247.08	233.05	235.05
T(K)	93(2)	84(2)	84(2)	84(2)	84(2)	93(2)
crystal system	orthorhombic	orthorhombic	orthorhombic	monoclinic	monoclinic	monoclinic
space group	P2 ₁ 2 ₁ 2 ₁	Pbca	Pca2 ₁	P2 ₁ /n	P2 ₁ /c	P2 ₁ /c
a (Å)	4.6854(1)	8.4575(3)	10.4661(2)	4.6581(1)	7.1511(2)	7.200(4)
b(Å)	10.3540(3)	10.7817(3)	7.5937(1)	28.2798(4)	15.8277(4)	15.363(8)
c(Å)	14.8614(4)	16.5150(5)	21.3387(3)	20.4669(3)	7.3779(2)	7.483(4)
α(deg)	90	90	90	90	90	90
β(deg)	90	90	90	90.33(1)	116.25(1)	115.05(3)
γ(deg)	90	90	90	90	90	90
V(Å ³)	720.97(3)	1505.94(8)	1695.92(5)	2696.06(8)	748.93(3)	749(7)
Z	4	8	8	12	4	4
ρ(g cm ⁻³)	1.861	1.941	2.076	1.826	2.067	2.082
μ(mm ⁻¹)	4.66	4.48	4.02	3.76	4.51	4.514
Size (mm ³)	0.80x0.58x0.40	0.34x0.26x0.22	0.32x0.28x0.18	0.46x0.12x0.12	0.26x0.11x0.05	0.48x0.29x0.02
F(000)	400	864	1040	1488	464	464
θ _{max} (deg)	29.6	26.4	26.5	26.3	26.3	31.7
Reflns collected	9541	10803	9640	15644	4408	13310
Tmax, min	0.257, 0.118	0.440, 0.333	0.451, 0.295	0.719, 0.537	0.696, 0.416	1.000, 0.538
Unique reflns	2028(R _{int} 0.025)	1544 (R _{int} 0.020)	3257 (R _{int} 0.0269)	5435 (R _{int} 0.0245)	1526 (R _{int} 0.0302)	2426 (R _{int} 0.0407)
Parameters	121	331	293	392	129	137
R ₁ [I > 2σ(I)]	0.0117	0.0173	0.0173	0.0243	0.0286	0.0255
wR ₂ (all data)	0.0332	0.0465	0.0420	0.0523	0.0745	0.0717
GOF on F ²	1.004	1.151	1.046	1.107	1.034	1.025
Flack x parameter	0.441(8)		0.010(6)			

2.4.2.1 *PhAsO₃H₂* (1)

This compound has been analysed twice before,¹³⁵ but neither determination was precise so the structure was repeated to provide an accurate benchmark for the substituted species. *PhAsO₃H₂* crystallizes as a racemic twin (not apparent in the earlier determinations¹³⁵), but despite this it refined cleanly, with all hydrogen atoms located (Figure 2-8). The As=O bond length is 1.6617(10) Å, the two As-O lengths are equal at 1.7070(10) Å and the As-C(1) bond is 1.8882(12) Å. The geometry around the As atom shows small deviations from tetrahedral, with angles in the range 106-112°. These more reliable parameters give shorter As-C and As-O, and longer As=O, bonds than those previously reported for this compound.

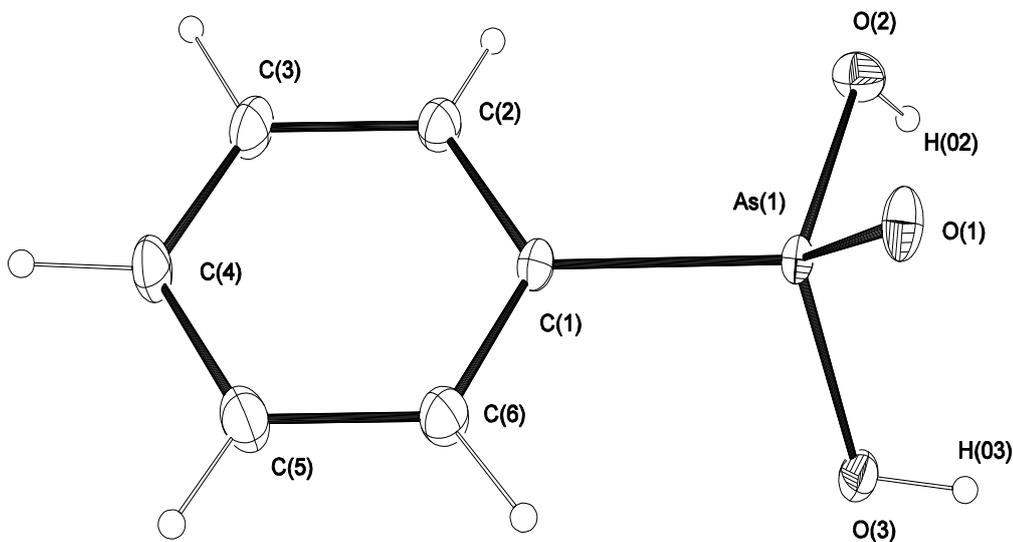


Figure 2-8 - The structure of *PhAsO₃H₂* (1) (50% ellipsoids) showing numbering scheme

2.4.2.2 4-FC₆H₄AsO₃H₂ (10)

This crystallises with one molecule comprising the asymmetric unit. The structure (Figure 2-9) is similar to that of the unsubstituted example with the electronegative 4-F substituent having the expected effect of shortening the aryl C-C bonds adjacent to the 4-position, but having no significant effect on the bond lengths around the As atom, where the As=O [1.6567(11) Å], As-OH [1.7121(12) Å], and As-C [1.8974(16) Å] bonds are essentially the same as in PhAsO₃H₂. There is a wider variation in the angles around the As atom (105-115°) but this is presumably to accommodate the different H-bonding network in the crystal.

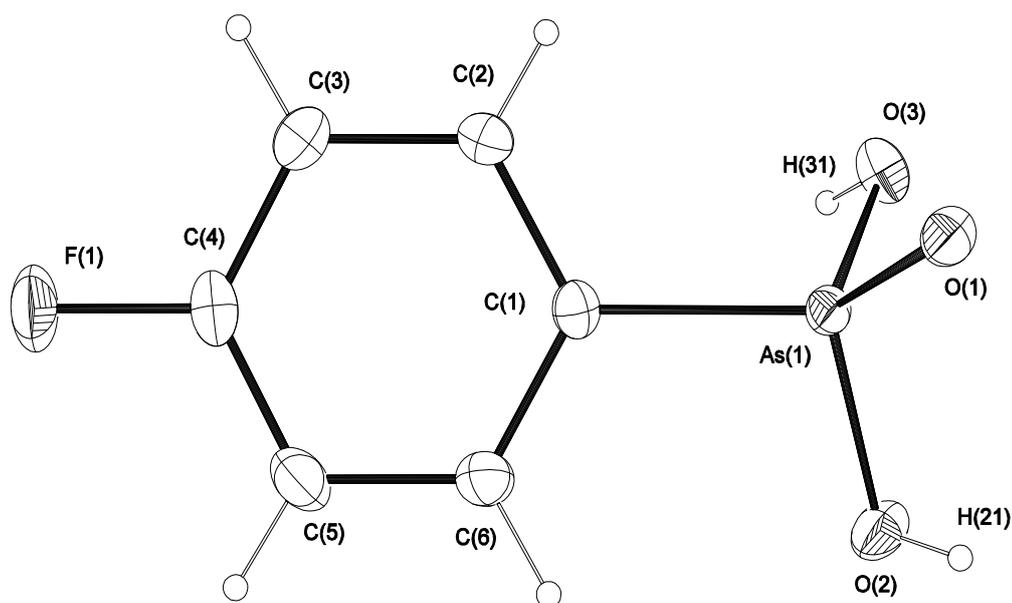


Figure 2-9 - The structure of 4-FC₆H₄AsO₃H₂ (10) (50% ellipsoids) showing numbering scheme

2.4.2.3 3-NO₂-4-F-C₆H₃AsO₃H₂ (13)

This crystallises with two independent molecules in the asymmetric unit, but they differ only in small details (Figure 2-10). The most apparent are the degree of twisting of the plane of the –NO₂ group from the plane of the phenyl ring (35° and 30°) and the displacement of the As atom from the phenyl plane (0.18 Å and 0.83 Å) respectively. Otherwise the individual bond parameters are indistinguishable from the corresponding ones in the 4-F example **10**.

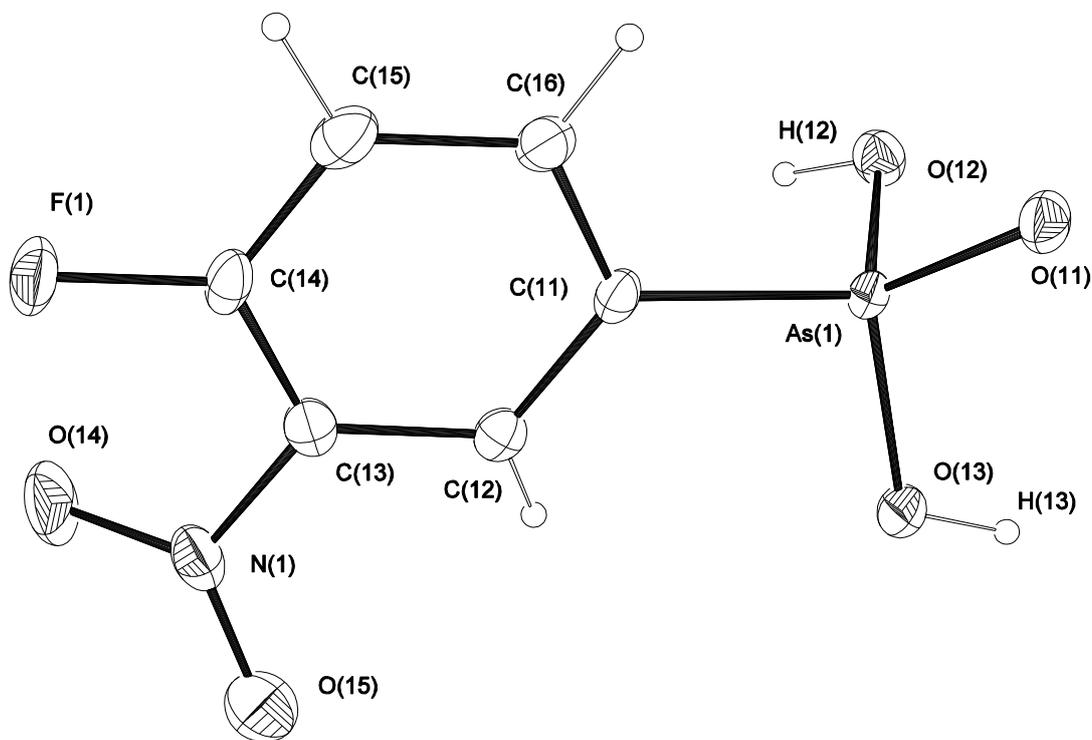


Figure 2-10 - The structure of one of the two independent molecules of 3-O₂N-4-FC₆H₃AsO₃H₂ (13)

2.4.2.4 3-NH₂-4-MeO-C₆H₃AsO₃H₂ (15)

This has a complicated structure with three independent molecules in the asymmetric unit. Interestingly they are all molecular species (Figure 2-11), in contrast to the closely related 3-NH₂-4-HO-C₆H₃AsO₃H₂ which packs in the zwitterionic form (see below). The three independent molecules show only small variations in bond parameters, so the discussion here is based on average values. The As=O [1.664(2) Å] and As-OH [1.719(2) Å] bonds are essentially the same as in the simpler examples, as is the As-C [1.899(2) Å]. The most noticeable effect of the substituents is bond alternation within the aryl ring – the C(3)-C(4) bond between the two adjacent groups is the longest (1.419 Å), presumably because of steric crowding, and this has induced significantly longer C(1)-C(2) (1.405 Å) and C(5)-C(6) (1.399 Å) bonds than the C(1)-C(6) (1.392 Å), C(2)-C(3) (1.390 Å) and C(4)-C(5) (1.394 Å) ones. This effect is much more noticeable here than for the 3-NO₂-4-F example **13** discussed above. The angles around the As atoms are again within the 103-113° range.

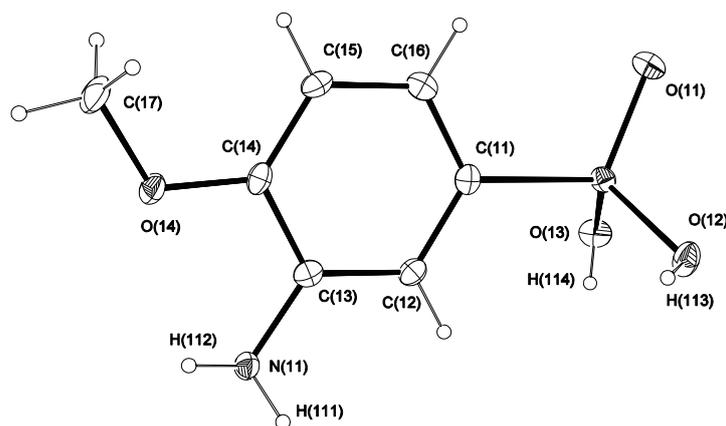


Figure 2-11 - The structure of one of the three independent molecules of 3-NH₂-4-MeOC₆H₃AsO₃H₂ (15)

2.4.2.5 3-NH₂-4-HO-C₆H₃AsO₃H₂ (14)

This structure is quite different from the others discussed, in that it packs in the crystal as zwitterions, 3-⁺H₃N-4-HO-C₆H₃AsO₃H⁻ (Figure 2-12). This is readily seen from the pattern of two shorter As=O bonds [1.677(2) Å] and one long As-OH [1.728(2) Å], and from the location in the penultimate difference map of the three H atoms on the nitrogen. There is no immediately apparent reason why this compound should pack in this form, while the analogous methoxy compound does not, since the substitution of an -OH group for a -OMe is not expected to change the pK_a values of the acid nor the pK_b value for the -NH₂ group. Presumably it is simply a consequence of accommodating the optimum H-bonding interactions in the crystal. It may be significant that the -OH group in the zwitterion is involved in H-bonding, whereas the -OMe in the previous example has no intermolecular interactions. One consequence of the zwitterion packing is a relatively high density for this compound (2.07 g cm⁻³) compared with the methoxy analogue (1.83 g cm⁻³) which indicates a tighter packing for the ionic arrangement.

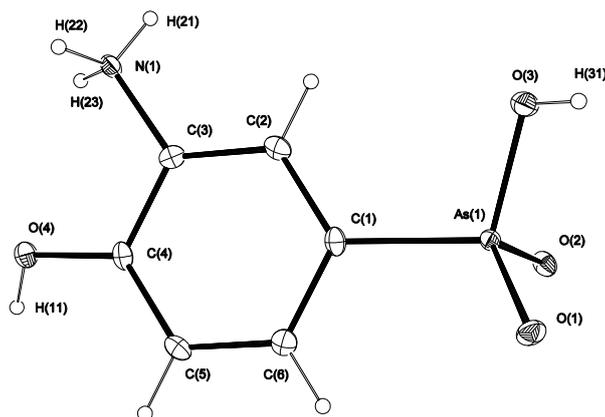


Figure 2-12 - The zwitterionic structure of 3-NH₂-4-HOC₆H₃AsO₃H₂ (14)

2.4.2.6 3-NH₂-4-F-C₆H₃AsO₃H₂ (16)

The structure of this compound is isomorphous and nearly isostructural with the preceding compound (**14**) in that it packs as zwitterions in the same space group. There is the same pattern of two shorter As=O bonds [1.659 (2) Å] and one longer As-O bond [1.717(2) Å]. These bonds are significantly shorter than in the 4-OH compound – in the case of the As=O bonds by 0.018(3) Å and in the case of the As-O bond by 0.011(3). Presumably this is caused by the effects of the hydrogen bonding differences in the fluorinated compound, this is discussed later. The density is 2.082 g cm⁻³ which is slightly higher than in **14**.

The only other arsonic acid reported to be a zwitterion in the solid state is 4-H₂NC₆H₄AsO₃H₂,¹³⁶ while the corresponding 2- and 3-H₂NC₆H₄AsO₃H₂ pack in their molecular forms,¹³⁷ so there is no obvious predictability.

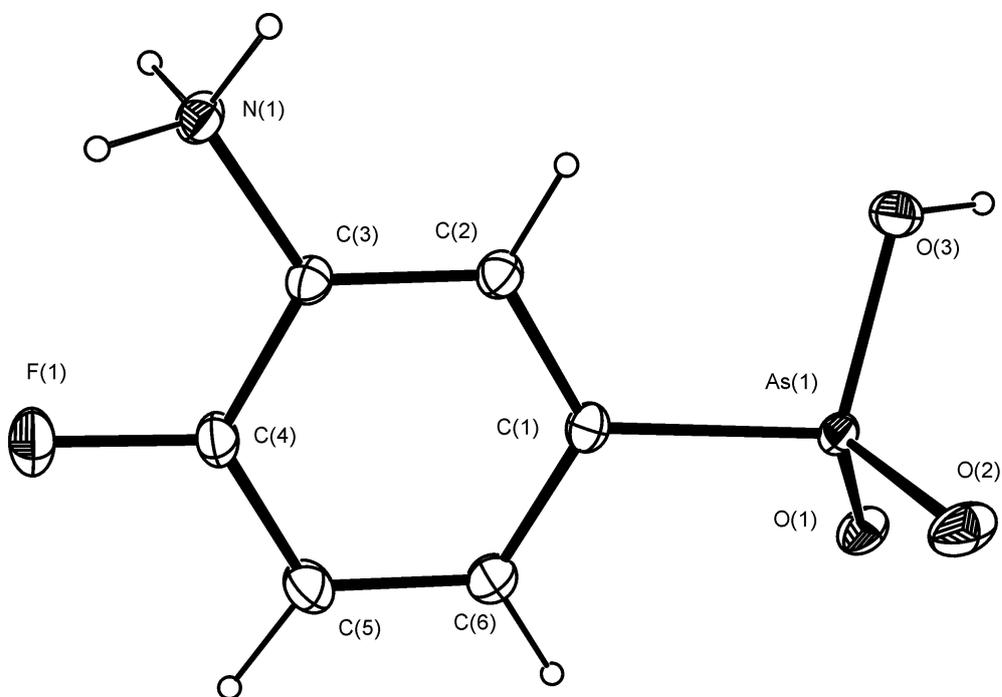


Figure 2-13 - The zwitterionic structure of 3-NH₂-4-F-C₆H₃AsO₃H₂ (16)

2.4.3 Summary.

Overall, these arsonic acid structures show very little variation with substitution patterns. The As-C (1.888-1.895 Å), As=O (1.657-1.664 Å) and As-O (1.707-1.719 Å) bonds cover a narrow range for the compounds with molecular packing, and these conform to the other examples in the literature. For the zwitterionic compound **14** the As-C (1.904 Å), As=O (1.677 Å) and As-O (1.728 Å) are all longer than the equivalent bonds in the molecular examples, possibly because of the stronger participation of hydrogen bonding in the charged species. The tetrahedral geometry around the As atoms in all of the examples shows some flexibility with angles ranging between 103 and 115°, presumably to accommodate the hydrogen bonding, as discussed below.

2.4.4 Hydrogen bonding networks.

The arsonic acids described here have many possibilities for H-bonding interactions in the crystals. In addition to those involving the AsO₃H₂ moieties, the -NH₂, -OH or -NO₂ groups on the phenyl rings are also likely to act as donors or acceptors.

For PhAsO₃H₂, molecules pack in the crystal so they form single chains parallel to the *a* axis. Each of the two As-O-H groups acts as a donor to the As=O of two adjacent molecules, with each As=O acting as an acceptor to two separate O-H donors, forming 10-membered rings as shown in **1A** (Figure 2.14). The O...O distances between donor and acceptor are all about 2.57 Å, making them on the strong/moderate borderline using Jeffrey's classification.¹³⁸ This arrangement is also further stabilised by allowing the phenyl rings to π -stack parallel to each other at 3.4 Å apart. There are only Van der Waals interactions cross-linking these chains. This particular H-bonding arrangement was also found for 2-H₂NC₆H₄AsO₃H₂.^{137a}

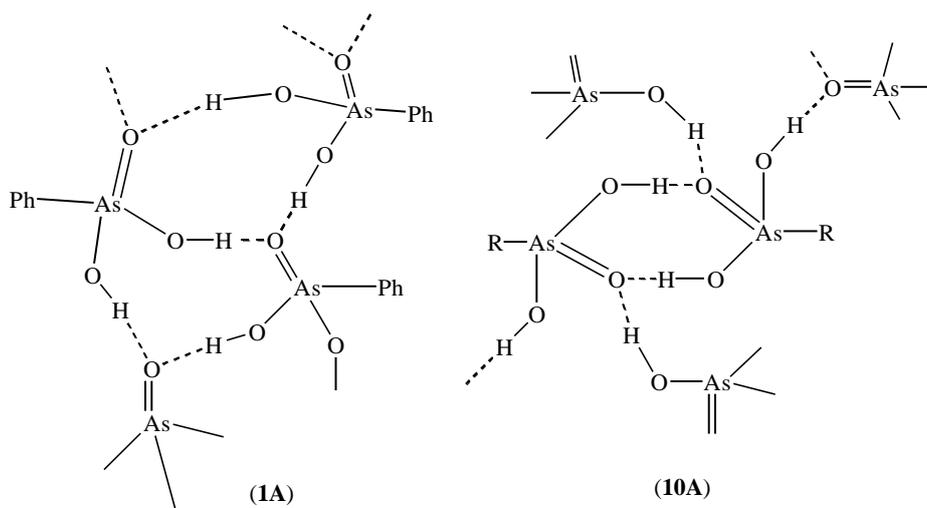


Figure 2-14 - Hydrogen bonding networks for 1A and 10A

For 4-FC₆H₄AsO₃H₂, (**10**), there are no significant interactions involving the fluoride atom, which is not unexpected since C-F groups are known to be weak H-bond acceptors.¹³⁹ The basic motif is a dimer, about a crystallographic inversion centre, reminiscent of those commonly formed by carboxylic acids¹³⁸ with As-O-H...O=As interactions characterised by an O...O distance of 2.58 Å. These generate nearly planar 8-membered rings, as shown in **10A** (Figure 2-14). These dimers are further linked into 2-dimensional sheets perpendicular to the c axis, by the remaining As-O-H bonding to an As=O of an adjacent molecule, so that each As=O acts as an acceptor to As-O-H from two separate molecules.

This same basic arrangement is also seen for 3-NO₂-4-FC₆H₃AsO₃H₂, where the two independent molecules in the lattice form a dimer with each other. This gives an eight-membered ring with 2.60 Å O...O separations, but this is distinctly chair-shaped. The remaining As-O-H...O=As linkages again generate a two dimensional sheet in the *ab* plane, as in **10A** (Figure 2-14). There is no H-bonding involvement of either the F or

NO₂ substituents, although there is a relatively close O...O (2.98 Å) contact between one of the oxygen atoms of the NO₂ group and an As=O group. This contrasts with the reported structure of 3-NO₂-4-MeOC₆H₃AsO₃H₂ where strong H-bonding between an As-O-H and an adjacent NO₂ group links molecules nose-to-tail, with additional As-O-H...O=As interactions completing the network.¹³⁰ The basic eight-membered ring dimer motif is common for arylarsonic acids, with at least seven other examples known,^{137a, 140} however, the way these dimers stack varies in each case. For example, 4-HOC₆H₃AsO₃H₂ also has the chair-shaped dimer unit, further linked into tetrameric units held by alternating H-bonds between the C-O-H group acting as both a donor and acceptor, and As=O and As-O-H groups acting as acceptors and donors respectively, giving 12-membered rings.¹³⁰

For 3-NH₂-4-MeOC₆H₃AsO₃H₂ (**15**) the packing is very complicated. There are three independent molecules in the asymmetric unit, and each of these is involved in different H-bonding arrangements to generate three cross-linked strands, each parallel to the short *a* axis.

The As(1) molecules form a simple strand based on strong As-O-H...O=As links, with the remaining As-O-H donating to the NH₂ group of As(2) molecules as in **15A** (Figure 2-15).

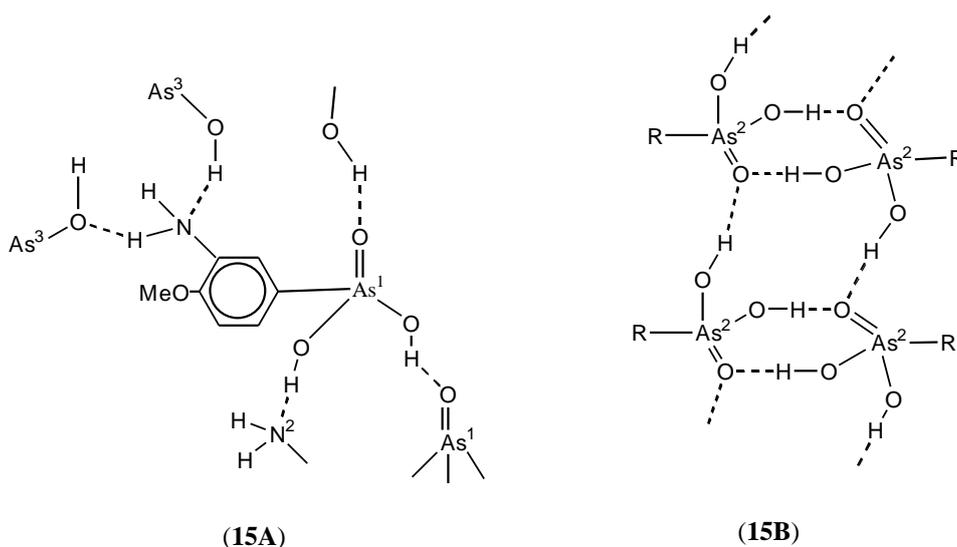


Figure 2-15 - Hydrogen bonding networks for 15A and 15B

The As(2) molecules form a double chain based on slightly chair-shaped eight-membered dimers, formed about an inversion centre, similar to the arrangement **10A** found for compounds **10** and **13**, with O...O distances of 2.57 Å. These stack up the *a* axis, with further 2.52 Å O...O links between the remaining As-O-H and the As=O of adjacent molecules to give 12-membered rings, with each As=O acting as acceptor to two As-O-H groups, as shown in **(15B)** (figure 2-15)). The -NH₂ group acts as an acceptor to an As-OH from As(1) molecules, and as an N-H donor to the O of an As-O-H of As(3) molecules. The 8/12-membered alternating ring motif was also found for 4-NO₂-C₆H₄AsO₃H₂, though with a small variation in the 12-membered ring.^{140e}

The As(3) strand links the others together through a variety of interactions. The single chain is based on As-O-H groups H-bonding as a donor to the As=O group of the adjacent molecules (O...O 2.52 Å), as also found for the As(1) molecules. This -OH further acts as an acceptor from an -NH₂ group of an As(1) molecule (N...O 2.94 Å). The As=O also accepts a H-bond from the -NH₂ group of an As(2) molecule (N...O 2.97 Å), while the remaining As(3)-O-H acts as an H-bond donor to the -NH₂ group of

an As(1) molecule. This is summarized in **15C** (figure 2.16). For this As(3) molecule the -NH_2 group does not participate in any H-bonding interactions.

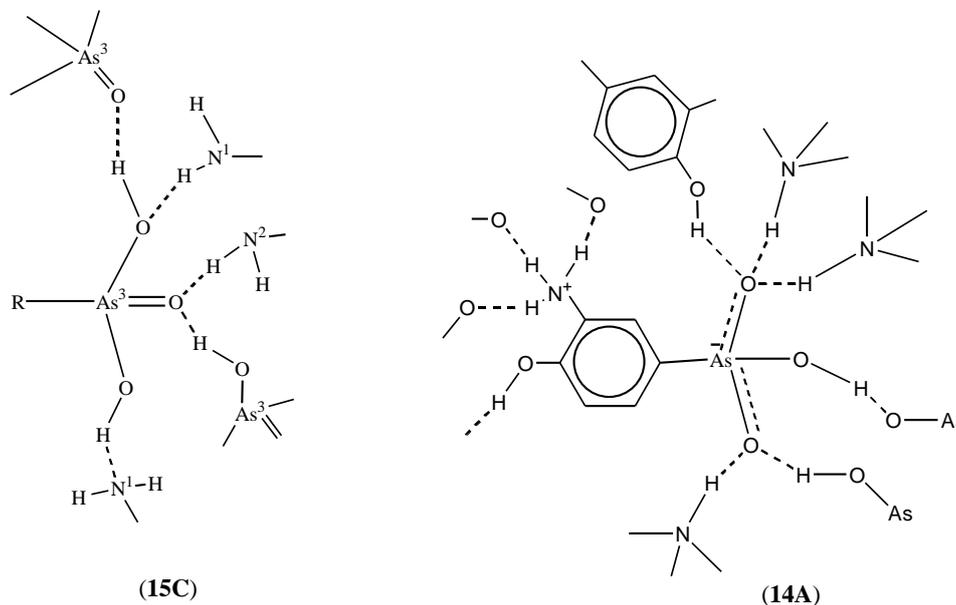


Figure 2-16 - Hydrogen bonding networks for 15C and 14A

For the zwitterionic $3\text{-}^+\text{H}_3\text{N-4-HOC}_6\text{H}_3\text{AsO}_3\text{H}^-$ compound **14**, there is a strong H-bonding network, as shown in **14A** (figure 2-16). Each of the three N-H hydrogens is linked to the As-O groups of three different molecules ($\text{N}\dots\text{O}$ 2.77-2.82 Å), with the phenolic -OH also bonded to an As-O of another one ($\text{O}\dots\text{O}$ 2.60 Å). At the other end of the molecule, the As-O-H is donating to an As-O with $\text{O}\dots\text{O}$ 2.64 Å, one of the As-O groups accepts an H-bond from both an N-H and an As-O-H, while the other As-O is involved as an acceptor in three hydrogen bonds; one from the phenolic -O-H ($\text{O}\dots\text{O}$ 2.60 Å), and two from two different N-Hs ($\text{N}\dots\text{O}$ 2.78 and 2.82 Å). This arrangement accommodates an offset π -stacking arrangement of the phenyl rings, 3.57 Å apart.

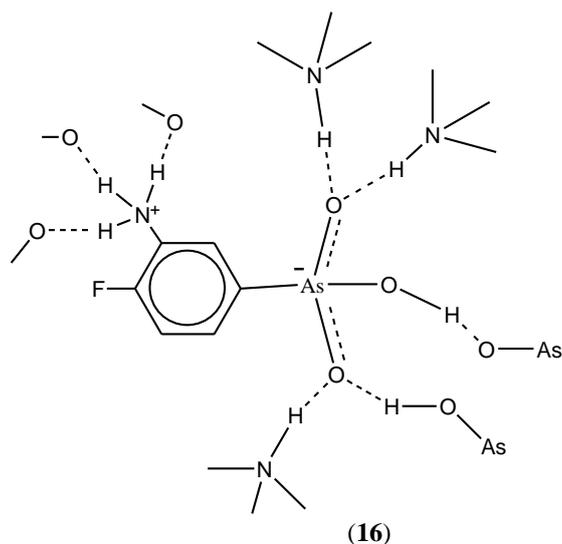


Figure 2-17 - Hydrogen bonding networks for 16

The fluorinated derivative 3-NH₂-4-FC₆H₃AsO₃H₂ (**16**) has a similar hydrogen bonding network to **14**, except the F is not involved in hydrogen bonding in any way (also seen in compound **10**). Each of the 3 N-H hydrogens is linked to the As-O groups of three different molecules (N...O 2.70-2.75 Å) with the closest contacts to the F being to the NH₂ group on the adjacent molecule ((F...N 2.81 Å, F...H 2.61 and 2.58 Å) and to one of the As oxygens (F...O 2.99 Å).

This arrangement accommodates an offset π -stacking arrangement of the phenyl rings, 3.46 Å apart, which is slightly closer than for compound **14**.

2.5 NMR

¹H and ¹³C NMR data are listed in Tables 2.4 and 2.5 together with their assignments, which were reasonably straightforward using standard proton-carbon HSQC and HMBC techniques.

Table 2.4 - ^1H NMR data (δ) for arylarsonic acids 1-15

Compound	H-2	H-3	H-4	H-5	H-6	CH ₂	CH ₃	OH
1	7.78(d)	7.59(m)	7.65(m)	7.59(m)	7.78(d)			5.45
2	7.59(d)	6.97(d)	-	6.97(d)	7.59(d)			4.79
3	8.19	-	-	7.31(d)	7.84(d)			4.35
4	7.40	6.71	-	6.71	7.40			
5	8.04(d)	8.40(d)	-	8.40(d)	8.04(d)			4.37
6	7.70(d)	7.13(d)	-	7.13(d)	7.70(d)		3.79	5.06
7	7.64(d)	7.41(d)	-	7.41(d)	7.64(d)		2.37	3.93
8	7.67(d)	7.11(d)	-	7.11(d)	7.67(d)	4.08(q)	1.32(t)	3.95
9	8.17(d)	-	-	7.54(d)	7.96(d)	4.27(q)	1.33(t)	4.84
10	7.83(dd)	7.42(t)	-	7.42(t)	7.83(dd)			4.52
11	8.48(s)	-	8.17(d)	7.90(t)	8.46(d)			4.56
12	8.18(s)	-	-	7.57(d)	7.99(d)		3.99	4.43
13	8.41(d)	-	-	7.78(m)	8.14(m)			4.51
14	6.98(s)			6.84(d)	6.84(d)			
15	7.01(s)			6.96(d)	6.94(d)		3.80	

Table 2.5 - ^{13}C NMR data (δ) for arylarsonic acids 1-15.

Compound	C-1	C-2	C-3	C-4	C-5	C-6	CH ₂	CH ₃
1	133.2	130.4	129.9	133.5	129.9	130.4		
2	122.3	132.7	117.0	162.2	117.0	132.7		
3	123.2	128.3	137.7	156.2	121.0	136.6		
4	116.8	131.5	113.8	153.2	113.8	131.5		
5	140.3	132.1	124.5	150.5	124.5	132.1		
6	124.2	132.2	115.3	163.1	115.3	132.3		55.8
7	130.4	130.2	130.2	143.5	130.2	130.2		21.4
8	124.4	132.2	115.5	162.2	115.5	132.2	63.8	14.6
9	124.9	127.2	139.7	154.8	116.5	136.4	66.2	14.4
10^a	129.5	133.5	117.3	165.4	117.3	133.5		
11	135.5	125.0	148.2	136.6	131.7	127.9		
12	124.9	127.2	139.5	155.5	115.9	136.5		57.5
13^b	130.7	128.7	137.7	157.7	120.8	138.5		
14	122.7	115.4	137.9	148.8	114.9	119.6		
15	124.3	114.1	138.9	149.9	110.9	118.8		55.8

^a $J_{\text{F-C}}$ (Hz): C-1 3.1; C-2,6 9.2; C-3,5 22.0; C-4 251.6.

^b $J_{\text{F-C}}$ (Hz): C-1 4.2; C-2 1.8; C-3 7.1; C-4 268.6; C-5 21.6; C-6 10.3.

2.6 Mass Spectrometry

ESI-MS was a useful technique for characterising the acids, with clean $[M-H]^-$ ions for each showing in negative ion mode when run in H_2O as solvent. If MeOH was used then ions of the type $[M-OH+OMe-H]^-$ were seen through formation of methyl esters of the acid, as has been reported earlier.¹⁴¹

An example of a typical negative ion ESI spectrum is shown in figure 2-18 for compound **16** showing the $[M-H]^-$ ion at 233.9556. The calculated mass for $[FNH_2C_6H_3AsO_3H]^-$ is 233.9542.

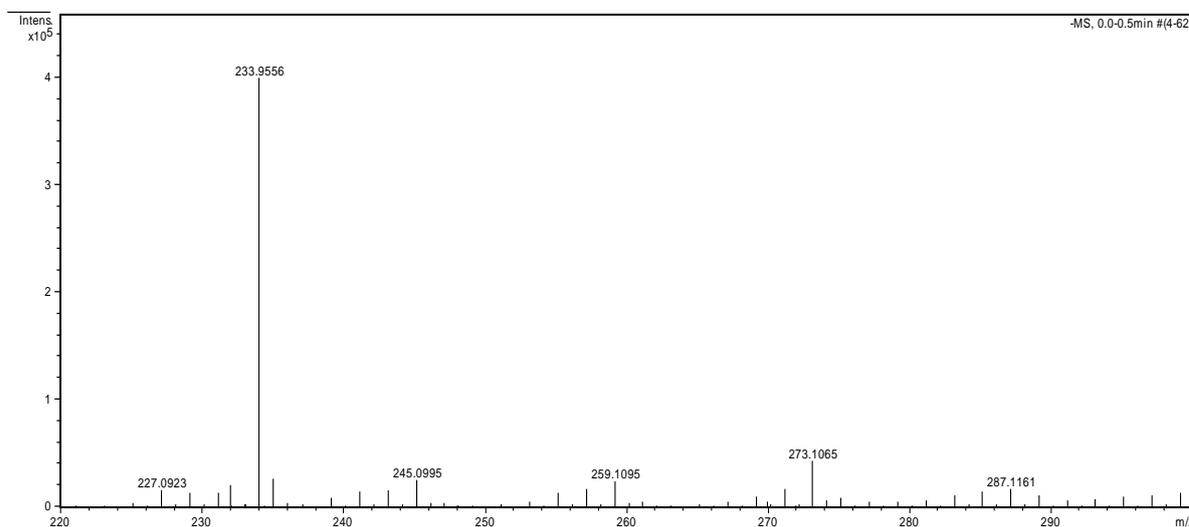


Figure 2-18 - Negative ESI mass spectrum for $[FNH_2C_6H_3AsO_3H]^-$ (16)

2.7 Differential Scanning Calorimetry

Arylarsonic acids often decompose before melting, so were surveyed using DSC. As summarized in Table 2.6, these generally showed one or two endothermic features in the range 140-180°C, presumably lattice water loss (if present) and/or water-elimination giving condensed acids, followed by an exothermic process just above 300°C which will be an oxidative degradation. A typical example is shown in figure 2-19.

Table 2.6 - DSC Data for arsonic acids 1-15

Compound	1 st endo (°C)	2 nd endo (°C)	final exo (°C)
1	160		392
2	175	188	332
3	184		320
4	165		250
5	162	245	332
6	165	180	352
7	160		380
8	170		355
9	148	225	303
10	175	182	none <400
11	160	175	330
12	170	245	315
13	145		310
14	200	240	-----
15	180		-----

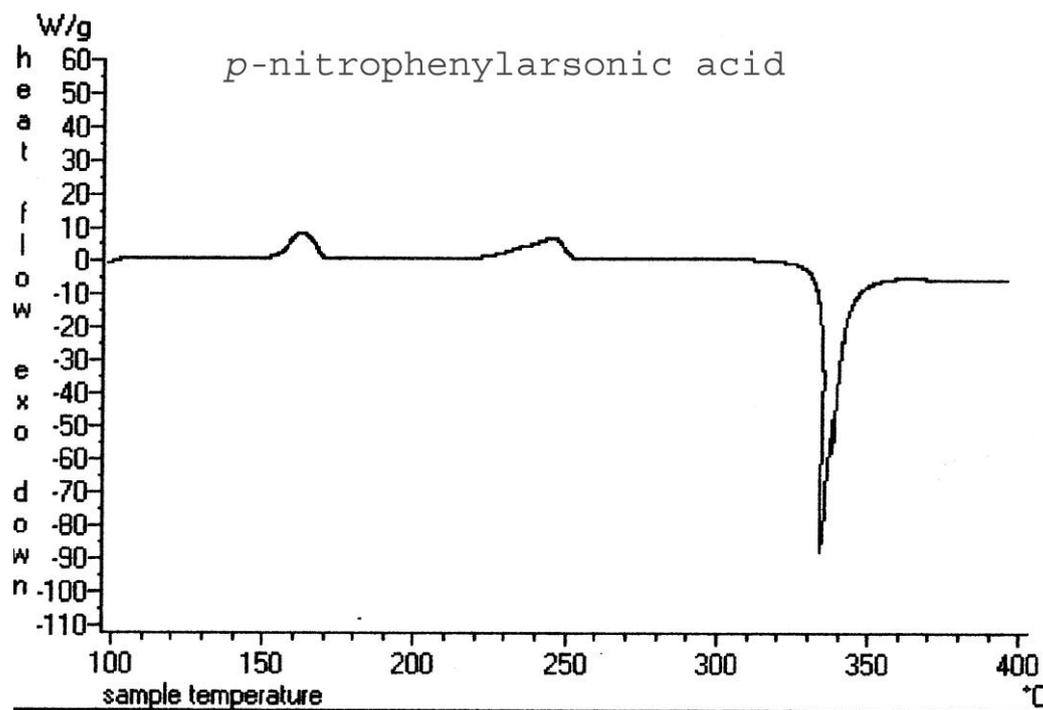


Figure 2-19 - The DSC trace for 4-O₂NC₆H₄AsO₃H₂

2.8 Conclusions

16 different arsonic acids were prepared and fully characterised using modern techniques in this chapter.

The preparations of these compounds proved straight forward, except for the examples containing fluorine which needed slight adaptations to the standard method.

X ray crystallography showed that the structures vary little with different substitution.

Two compounds crystallized as zwitterions and this appears to be influenced by how the other substituents on the ring affect the hydrogen bonding networks.

3 Chapter Three - The Structure of Salvarsan

3.1 Salvarsan background

Salvarsan (3-amino-4-hydroxyphenylarsenic(I), arsphenamine, Ehrlich 606,) was introduced by Ehrlich in 1910 as a remedy for syphilis (see 1.5). Despite its long history and importance, the actual structure of Salvarsan is still debated.^{111, 142} The compound was synthesized by Ehrlich by reaction of 3-nitro-4-hydroxyphenylarsonic acid with sodium dithionite.¹³¹ This simultaneously reduced both the $-\text{NO}_2$ group to $-\text{NH}_2$, and the As(V) to As(I) to give a material of stoichiometry $[\text{3-H}_2\text{N-4-HO-C}_6\text{H}_3\text{As}]$ (Figure 3-1).

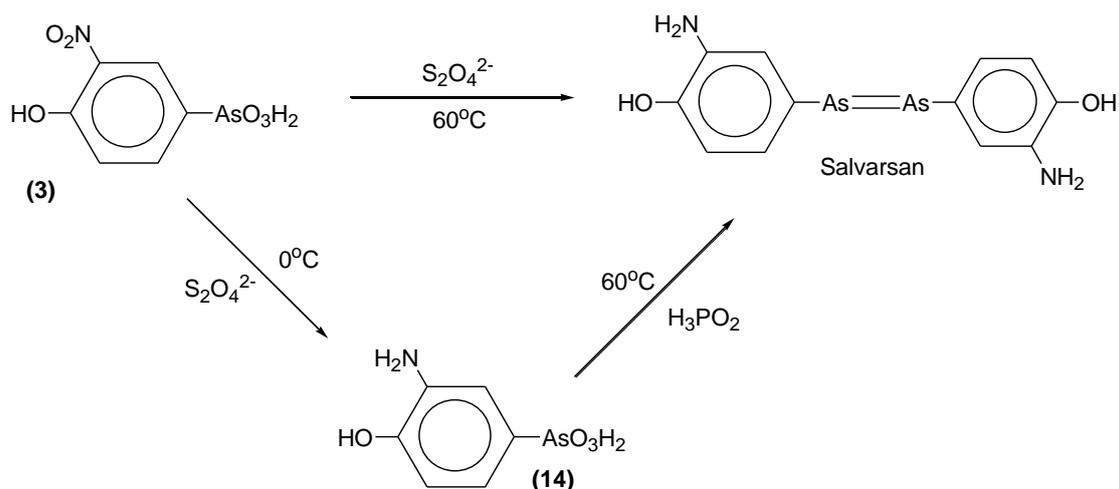


Figure 3-1 - Scheme showing methods of making Salvarsan

The product was isolated as the hydrated hydrochloride salt of empirical formula $(\text{3-H}_2\text{N-4-HOC}_6\text{H}_3\text{As} \cdot \text{HCl} \cdot \text{H}_2\text{O})$. This synthesis was not always reproducible, and inevitably gave rise to sulfur-containing impurities which may have accounted for the variable toxicity of different batches of Salvarsan⁸³ (see Chapter One for a detailed review). A two-step process involving initial reduction of the NO_2 group by sodium

dithionite, followed by reduction of the As(V) with hypophosphorous acid was subsequently shown by Christiansen to lead to sulfur-free material⁸³ (Figure 3-1).

By analogy with azo-compounds, Ehrlich assigned structure **1** to the free base of Salvarsan. Although it is now recognised that As=As bonds are only found in sterically crowded molecules,¹¹³ the As=As form is repeatedly cited, with text-books and reviews still giving structure **1**.¹¹¹ Various suggestions for the true structure of Salvarsan have been proposed, including large polycyclic molecules¹⁰⁹ and polymeric versions,⁸ but without strong supporting data.

3.2 Cyclopolyarsines

Cyclopolyarsines are arsenic (I) compounds with the general formula (RAs)_n where R is any alkyl or aryl group and n has been reported to be 4,¹¹⁴ 5 or 6,¹⁰⁹ although larger examples are probable. Cyclopolyarsines have been a somewhat mysterious group of compounds and as they have low solubility in most common solvents, analysis with most techniques is difficult.

The only crystal structures known of aryl cyclopolyarsines are those where the compounds have non-polar R groups and are therefore soluble in non-polar solvents.¹⁰⁹

The cyclic nature of these compounds was only revealed when X-ray crystallography began to be used as an analytical technique. Prior to this they were assumed to be either dimeric with As=As double bonds (see 1.6.4) or polymeric in nature.

These compounds also have unusual physical properties with substituted examples often being highly coloured and insoluble in common solvents - Salvarsan itself is a pale yellow powder when dry and forms a bright yellow solution which exhibits gel like properties at higher concentrations.

Earlier reviews¹⁰⁹ have excluded Salvarsan from discussion of cyclopolyarsines due to its unknown structure but this research places it firmly in this category.

The only aryl cyclopolyarsines which have been structurally elucidated are presented in Table 3.1. These compounds are all non-polar variations on arsenobenzene.

Compounds where n=5 and n=6 make up the bulk of the reported structures and a recent example establishes n=4 as a possibility. Compounds in which n=3 are known for phosphorus and antimony but have never been elucidated for arsenic.

Very little work has been done on polar substituted cyclopolyarsines since the 1930's.

Both the Bertheim and Raiziss books mention these compounds, but they consider them to be arseno compounds, with the structure R-As=As-R. These compounds have been largely ignored since those books were published.

Table 3.1 - Known cyclopolyarsines in the CCDC files

Compound	Date	CCDC code	Reference
(As-Mesityl) ₄	2008	DOGGAI	114
(AsPh) ₅	1997	PIFPUP	143
(As- <i>p</i> -tolyl) ₅	1997	PIFQAW	143
(AsPh) ₆	1961	ASBENZ	107
(AsPh) ₆	1983	ASBENZ01	144
(As- <i>p</i> -tolyl) ₆	1997	PIFQEA	143
(AsC ₆ H ₄ OMe) ₆	1997	TIDHIX	145

3.3 Synthesis of cyclopolyarsines

Cyclopolyarsines can be most easily synthesized by reducing aryl arsonic acids but reduction of arsonous acids and dihaloarsines can also be used. Hypophosphorous acid is a common reducing agent as it will specifically reduce the arsenic containing group and leave other organic groups unchanged.

Sodium amalgam is another reducing agent which has historically been used.

For the investigation reported in this thesis Salvarsan was synthesized by both the original Ehrlich and Bertheim method, as reported by Kober,⁷⁵ and by the two step method reported by Christiansen.⁸³

The methods used here were both adapted from the originals for small scale preparations.

Other cyclopolyarsines in this investigation were produced using hypophosphorous acid

3.3.1 General

Arsonic acids were synthesized or purchased as specified in Chapter Two.

Hypophosphorous acid was used as purchased from BDH.

Sodium dithionite was purchased from Merck and was stored under vacuum in the refrigerator.

Methanolic HCl was prepared by bubbling HCl gas through methanol for two minutes.

This solution was stored and used as required.

Distilled water was used throughout this work.

NMR data were recorded on a Bruker Avance 300 spectrometer.

3.3.2 Preparation of Salvarsan [3-amino-4-hydroxyphenylarsonic(I)] (adapted from Christiansen)⁸³

All solvents and solutions were degassed and the reaction was carried out under nitrogen.

3-Amino-4-hydroxyphenylarsonic acid (2.3 g, 0.01 mol) was dissolved in a solution of hypophosphorous acid (14 mL, 50%) in water (73 mL). Aqueous KI solution (1 mL of 3%) was added and the solution was heated gradually to 55°C and held between 55 and

60° for 90 minutes. The mixture was cooled to 10°C and poured with vigorous stirring into cold HCl / H₂O (1:1 mixture, 164 mL). Salvarsan precipitated as a pale yellow gelatinous solid which was collected and dried under vacuum as the hydrochloride. Elemental analysis: found C 30.66, H 3.70, N 5.62%; calcd for C₆H₆AsNO.HCl.H₂O: C 30.39, H 3.82, N 5.91%.

Yield - 2.12g (89%)

Alternatively, ethanol was added to the cooled reaction mixture after the heating step with vigorous stirring, until a precipitate formed. The solid was collected and dried as above, and was characterised as a mixed hypophosphite/phosphite salt of 3-amino-4-hydroxyphenylarsenic(I). Elemental analysis: found C 27.50, H 3.92, N 5.14%; calcd for C₆H₆AsNO.H₂PO₂.H₂O. C 26.97, H 4.12, N 5.24%; ³¹P NMR (D₂O) δ 9.7 (t, ¹J_{H-P} 536 Hz) H₂PO₂⁻, δ 4.1 (d, ¹J_{H-P} 648 Hz) HPO₃²⁻ (ca 5:1).

Yield 2.03 g (81%)

3.3.3 Preparation of Salvarsan [3-amino-4-hydroxyphenylarsenic(I)] (Ehrlich's method, adapted from Kober)⁷⁵

Anhydrous magnesium chloride (22 g, 0.23 mol) was dissolved in water (550 mL) and sodium dithionite (110 g) was added quickly. This solution was then connected to a Schlenk line and degassed with the remainder of the reaction being carried out under nitrogen.

3-Nitro-4-hydroxyphenylarsonic acid (8.50 g, 0.0323 mol) was dissolved in sodium hydroxide solution (2 mol L⁻¹, 30 ml) and diluted to 200 mL with water. This was added to the dithionite solution and filtered while still cold under nitrogen using a filter stick.

The filtered mixture was degassed and slowly heated to 50°C and held at 50-60°C for 2-2.5 hours with stirring.

Salvarsan base began to precipitate out as the temperature increased.

After 2.5 hours the contents of the flask were filtered using a filter stick under nitrogen and the Salvarsan base was dried under vacuum overnight.

Yield 4.84 g (82%)

The dried Salvarsan base was dissolved in methanolic hydrochloric acid (70 mL) and filtered under nitrogen.

Dry diethyl ether (450 mL) was added to precipitate the Salvarsan as the hydrochloride.

This was filtered and dried under vacuum.

Yield 5.61g (79%)

3.3.4 Preparation of 3-amino-4-methoxyphenylarsenobenzene

All solvents and solutions were degassed and the reaction was carried out under nitrogen.

3-Amino-4-methoxyphenylarsonic acid (1.15 g 0.005 mol) was dissolved in a solution of hypophosphorous acid (7 mL 50%) in water (36.5 mL). Aqueous KI solution (0.5 mL of 3%) was added and the solution was heated gradually to 55°C and held between 55 and 60° for 90 minutes.

The yellow solution was poured into cold ethanol and gave no precipitate.

Concentrated HCl was added and an extremely fine precipitate was formed which was very difficult to filter.

No yield was recorded due to the difficulties filtering the product. The yellow product seemed to be more readily oxidised than Salvarsan itself – turning brown very quickly on exposure to air. Mass spectra were recorded and are discussed later.

3.3.5 Preparation of arsenobenzene¹⁴⁶

Phenylarsonic acid (20 g, 0.1 mol) was dissolved in absolute ethanol. The solution was heated to 50-60°C with stirring in a water bath. Hypophosphorous acid (40 g, 50% aq. 0.3 mol) was added. The mixture was stirred with heating for 5-6 hours during which time arsenobenzene precipitates out as a pale yellow solid. This was filtered and dried under vacuum. Yield 10.29 g (68%).

3.3.6 Preparation of p-aminoarsenobenzene

p-Aminophenylarsonic acid (2.17 g, 0.01 mol) was dissolved in a solution of hypophosphorous acid (14 mL 50%), water (73 mL) and KI solution (1 mL, 3%). This solution was heated gradually to 55°C and held between 55 and 60° for 90 minutes.

The resultant mixture was then cooled to 10°C and poured with vigorous stirring, into cold HCl solution (164 mL, 50%)

The precipitate was filtered and washed with HCl (10 mL, 50%)

Yield 1.52 g (75%) as an insoluble red powder.

Several other arsonic acids were reduced using the same method, but none of these yielded anything soluble enough to be investigated further.

3.3.7 Preparation of mixed compounds

p-Aminophenylarsonic acid (1.15 g, 0.005 mol) and 3-amino-4-hydroxyphenylarsonic acid (1.085 g 0.005 mol) were dissolved in a solution of hypophosphorous acid (14 mL

50%), water (50 mL) and KI solution (1 mL, 3%). This solution was heated gradually to 55°C and held between 55 and 60° for 90 minutes.

The resultant mixture was then cooled to 10°C and precipitated with cold ethanol before being filtered and dried under vacuum.

3.3.8 Preparation of Salvarsan polyarsenide⁸⁸

Anhydrous magnesium chloride (10.6 g 0.18mol) was dissolved in water (260 mL) and sodium dithionite (50 g) was added quickly. This solution was then connected to a Schlenk line and degassed with the remainder of the reaction being carried out under nitrogen.

3-Nitro-4-hydroxyphenylarsonic acid (2 g, 0.008 mol) was dissolved in sodium hydroxide solution (0.66 g in 46 mL) and diluted to 200 mL with water. This was cooled and added to the dithionite solution.

A solution of sodium arsenite (0.009 mol, 1.17 g in 45 mL water) was then added to the solution. The mixture was then slowly heated to 50°C and held at 50-60°C for 90 minutes with stirring.

The resultant precipitate was centrifuged out, washed with cold water and dried under vacuum.

Once dry, the red precipitate was dissolved in methanolic HCl and filtered to give a bright red solution. Excess cold ether was added to precipitate out the hydrochloride.

Yield 0.64 g

3.4 Electrospray mass spectra of Salvarsan

Electrospray mass spectra of Salvarsan were obtained on three different instruments, all of which used different desolvation and detection methods. Initially the Fisons VG Platform II was used – this desolvates the sample with a stream of hot gas and uses a quadrupole detector. The ThermoFinnigan LCQ Advantage desolvates using a heated metal capillary and has an ion trap, which allows for MS-MS experiments to be carried out. The Bruker MicrOTOF was also used and this desolvates the sample with a heated glass capillary and has a time of flight detector, which allows for high resolution spectra to be obtained.

The high precision analysis discussed here was all carried out on the Bruker MicrOTOF while the induced fragmentation experiments were carried out on the LCQ Advantage.

Initial investigations were carried out on the Fisons Platform II machine.

For the Platform II, the compounds were dissolved in the appropriate solvent and injected into the spectrometer via a Rheodyne injector with a 10 mL sample loop. A flow rate of 0.02 mL min⁻¹ and a source temperature of 60°C was used, and nitrogen was used as both a nebulising and drying gas. For the LCQ the sample was directly injected into the spectrometer at 5 µL min⁻¹ via a syringe pump. The capillary temperature was set at 100°C; nitrogen was used as drying gas. In the MicrOTOF, the sample was introduced into the machine via a syringe pump at 180 µL min⁻¹. Nitrogen was used as the nebulising gas.

Analyzed in detail were samples of Salvarsan prepared in the course of this research by dithionite reduction of 3-nitro-4-hydroxyphenylarsonic acid (Kober's⁷⁵ adaption of Ehrlich and Bertheim's original method)¹³¹ and by the hypophosphorous acid reduction of 3-amino-4-hydroxyphenylarsonic acid (Christiansen's method).⁸³ We were also

fortunate to obtain a sample of original commercial Salvarsan (presumable from the Hoechst factory), from the Paul Ehrlich Institute thanks to Professor Steve Reithmiller (see Figure 3-2).



Figure 3-2 - Ampoules of Hoechst Salvarsan and Neosalvarsan from the archives of the Paul Ehrlich Institute, Frankfurt

For the high precision analyses, samples of the different preparations of Salvarsan were made up to approximately 2 mg mL^{-1} in distilled water and centrifuged carefully to remove any small particles of insoluble material. The exact concentration of the Salvarsan solutions was hard to gauge due to the formation of gels when the Salvarsan was added to water – the Salvarsan produced with hypophosphorous acid was more prone to gel formation than the other samples possibly because of the presence of remaining hypophosphorous ions in the sample.

The settings of the MicroTOF spectrometer were set to allow a wide window of observation and the same settings were used to analyse all samples on the same day to ensure that everything was consistent.

All calculation of molecular masses was carried out on the Bruker Daltonics DataAnalysis program.

Data are tabulated in Table 3.2.

Table 3.2 - Mass spectrum data for Salvarsan produced by Christiansen's method (3.3.2), Kober's method (3.3.3) and the Hoechst sample.

Waikato 3.3.2		Waikato 3.3.3		Hoechst		Assignment	Calculated
		257.8864	vw	257.8856	w	$[\text{As}_2\text{R}]^+$	257.8875
		273.8940	vw	273.8940	w	$[\text{As}_2\text{RO}]^+$	273.8825
		288.2883	w	288.2883	w	-	
		288.9930	vw	288.9943	w	-	
291.0106	s	291.0106	vs	291.0106	vs	$[\text{R}_2\text{As}]^+$	291.0109
		305.0244	vw	305.0242	vw	$[\text{R}_2\text{As}+\text{CH}_2]^+$	305.0266
307.8449	vw					-	
				309.0202	w	-	
		316.3200	m			-	
		331.8008	vw	331.8009	w	$[\text{R}^{\text{ox}}\text{As}_3\text{H}]^+$	331.8014
				333.8161	w	$[\text{RAs}_3+\text{H}]^+$	333.8170
351.0465	m	351.0464	vw	351.0462	w	-	
		353.2648	w			-	
		363.7749	w	363.7759	w	-	
		364.9244	vw	364.9243	w	$[\text{RR}^{\text{ox}}\text{As}_2\text{H}]^+$	364.9247
		366.9397	w	366.9395	m	$[\text{R}_2\text{As}_2\text{H}]^+$	366.9403
		368.9557	vw	368.8553	w	$[\text{R}_2\text{As}_2\text{H}_2+\text{H}^+]^+$	368.9560
				370.9668	w	$[(\text{RSO}_3\text{H})\text{RAs}]^+$	370.9677
		378.9387	vw	378.9379	vw	-	
380.9182	m	380.9238	w	380.9239	m	$[\text{RR}^{\text{ox}}\text{As}_2\text{OH}]^+$	380.9196
		381.2970	w			-	
		382.9701	vw	382.9623	vw	$[\text{R}_2\text{As}_2\text{OH}]^+$	382.9353
398.8973	w	398.9108	w	398.9073	w	$[\text{R}_2\text{As}_2\text{SH}]^+$	398.9124
		400.0640	w	400.0639	m	$[\text{R}_3\text{AsH}]^+$	400.0637
		407.7326	w	407.7325	s	$[\text{RAs}_4]^+$	407.7308
		414.9269	vw	414.9222	vw	-	
416.8975	m	416.9002	vw	416.9000	w	$[\text{R}_2\text{As}_2\text{ClO}]^+$	316.8963
		420.9090	vw			-	
				427.2858	vw	-	
438.9214	w	438.9200	m	438.8426	w	$[\text{RR}^{\text{ox}}\text{As}_3]^+$	438.8385
440.8552	w	440.8555	s	440.8552	s	$[\text{R}_2\text{As}_3]^+$	440.8541

454.8906	w	454.8927	vw			-	
456.8907	m	456.8907	m	456.8817	vw	[R ₂ As ₃ O] ⁺	456.8490
458.8871	vw	458.8876	w			-	
471.9626	m	471.9625	m	471.9627	s	[R ₂ R ^{ox} As ₂ H] ⁺	471.9618
472.8647	m	472.8407	w	472.9629	w	-	472.9696
473.9777	w	473.9794	s	473.9795	vs	[R ₃ As ₂] ⁺	473.9775
		487.9899	vw	487.9918	vw	-	
500.8555	vw					-	
502.8855	vw					-	
		514.7726	w	514.7712	m	[RR ^{ox} As ₄ H] ⁺	514.7679
		516.7918	w	516.7887	m	[R ₂ As ₄ H] ⁺	516.7835
				547.8922	w	[R ^{ox} R ₂ As ₃ +H ⁺] ⁺	547.8912
				548.8993	w	[R ₃ As ₃] ⁺	548.8991
549.9081	s	549.9081	vs	549.9081	vs	[R₃As₃+H⁺]⁺	549.9069
		554.5524	w			-	
		563.9227	s	563.9224	s	[R ₃ As ₃ CH ₃] ⁺	563.9225
		571.8877	m	571.8863	vw	[R ₃ As ₃ Na] ⁺	571.8888
				577.9351	vw	-	
		581.8807	m	581.8817	w	[R ₃ As ₃ SH] ⁺	581.8790
				587.8617	vw	-	
		595.8944	vw			-	
597.8605	vw	597.8881	vw	597.8815	vw	-	
		613.8546	m	613.8665	wv	-	
615.8533	vw					-	
		621.8055	w	621.8051	m	[R ₂ R ^{ox} As ₄] ⁺	621.8050
623.8203	m	623.8211	vs	623.8211	vs	[R ₃ As ₄] ⁺	623.8207
		629.8632	vw	629.8635	w	[(RSO ₃ H)R ₂ As ₃ H] ⁺	629.8637
		637.8635	w	637.8358	vw	[R ₃ As ₄ +CH ₂] ⁺	637.8363
				639.8143	vw	-	
				652.9099	vw	-	
				654.9280	vw	-	
		655.8061	m	655.7941	m	[R ₃ As ₄ S] ⁺	655.7927
655.8352	vw					-	
				656.9419	s	[R ₄ As ₃] ⁺	656.9440
671.8191	vw					[R ₃ As ₄ POH] ⁺	671.7972
				690.0650	vw	-	
				697.7340	w	[R ₂ R ^{ox} As ₅ H] ⁺	697.7344
				699.7497	vw	[R ₃ As ₅ H] ⁺	699.7501
				729.8464	vw	-	
730.8551	w	730.8562	m	730.8560	s	[R ^{ox} R ₃ As ₄ +H ⁺] ⁺	730.8577
731.8613	vw	731.8624	m	731.8619	m	[R ₄ As ₄] ⁺	
732.8732	vs	732.8732	vs	732.8731	vs	[R₄As₄+H]⁺	732.8734
		746.8877	vs	746.8875	vs	[R ₄ As ₄ CH ₃] ⁺	746.8891
748.8651	vw					[R ₄ As ₄ OH] ⁺	748.8683
		754.8513	w			[R ₄ As ₄ Na] ⁺	754.8554
				760.8976	vw	-	
		764.8452	vs	764.8451	vs	[R ₄ As ₄ SH] ⁺	764.8455
				773.6645	vw	-	

		778.8599	m	778.8567	vw	-	
		786.8270	w			-	
				804.7730	vw	$[\text{R}^{\text{ox}}\text{R}_3\text{As}_5]^+$	804.7715
806.7856	w	806.7862	vs	806.7862	vs	$[\text{R}_4\text{As}_5]^+$	806.7872
		812.8276	vw	812.8276	w	$[(\text{RSO}_3\text{H})\text{R}_3\text{As}_4\text{H}]^+$	812.8302
				820.7968	vw	$[\text{R}_4\text{As}_5\text{CH}_2]^+$	820.8028
				822.7859	vw	$[\text{R}_4\text{As}_5\text{O}]^+$	822.7821
		838.7593	w	838.7597	w	$[\text{R}_4\text{As}_5\text{S}]^+$	838.7593
839.9085	vw	839.9089	m	839.9094	s	$[\text{R}_5\text{As}_4]^+$	839.9105
		844.7994	vw	844.7969	vw	-	
				880.6993	w	$[\text{R}^{\text{ox}}\text{R}_3\text{As}_6+\text{H}]^+$	880.7010
				882.7140	w	$[\text{R}_4\text{As}_6\text{H}]^+$	882.7166
				911.8051	vw	$[\text{R}^{\text{ox}}_2\text{R}_3\text{As}_5+\text{H}]^+$	911.8087
		913.8212	w	913.8218	m	$[\text{R}^{\text{ox}}\text{R}_4\text{As}_5+\text{H}]^+$	913.8243
914.8280	vw	914.8283	w	914.8272	m	$[\text{R}_5\text{As}_5]^+$	914.8321
915.8398	s	915.8397	vs	915.8379	vs	$[\text{R}_5\text{As}_5\text{H}]^+$	915.8400
		929.8511	s	929.8509	s	$[\text{R}_5\text{As}_5\text{CH}_3]^+$	929.8556
		937.8011	w			$[\text{R}_5\text{As}_5\text{Na}]^+$	837.8219
		945.8011	vw			-	
		947.8098	m	947.8096	w	-	
				956.6299	w	-	
		961.8270	vw			$[(\text{R-H})\text{R}_4\text{As}_5\text{SO}]^+$	961.7913
989.7510	vw	989.7515	m	989.7518	vs	$[\text{R}_5\text{As}_6]^+$	989.7537
		995.7905	w	995.7930	w	$[(\text{RSO}_3\text{H})\text{R}_4\text{As}_5\text{H}]^+$	995.7968
				1003.7655	vw	$[\text{R}_5\text{As}_6\text{CH}_2]^+$	1003.7694
				1005.7432	w	$[\text{R}_5\text{As}_6\text{O}]^+$	1005.7486
		1006.3173	vw	1006.3191	vw	$[\text{R}_{11}\text{As}_{11}]^{2+}$	1006.3154
		1021.7253	w	1021.7270	m	$[\text{R}_5\text{As}_6\text{S}]^+$	1021.7257
1022.8624	vw	1022.8720	m	1022.8733	s	$[\text{R}_6\text{As}_5]^+$	1022.8771
				1063.6643	w	$[\text{R}^{\text{ox}}\text{R}_4\text{As}_7+\text{H}]^+$	1063.6675
				1065.6809	w	$[\text{R}_5\text{As}_7+\text{H}]^+$	1065.6831
				1069.7092	vw	-	
		1096.7875	w	1096.7868	m	$[\text{R}^{\text{ox}}\text{R}_5\text{As}_6+\text{H}]^+$	1096.7908
		1097.7979	m	1097.7961	m	$[\text{R}_{12}\text{As}_{12}]^{2+}$	1097.7987
1098.8041	m	1098.8042	vs	1098.8043	vs	$[\text{R}_6\text{As}_6\text{H}]^+$	1098.8065
		1112.8178	m	1112.8174	m	$[\text{R}_6\text{As}_6\text{CH}_3]^+$	1112.8221
		1120.7860	vw			$[\text{R}_6\text{As}_6\text{Na}]^+$	1120.7884
		1130.7799	w	1130.7804	vw	$[\text{R}_6\text{As}_6\text{SH}]^+$	1130.7786
		1132.7722	vw	1132.7682	vw	-	
				1139.5978	w	-	
1172.7161	w	1172.7176	m	1172.7189	vs	$[\text{R}_6\text{As}_7]^+$	1172.7203
		1178.7572	vw	1178.7585	w	$[(\text{RSO}_3\text{H})\text{R}_5\text{As}_6\text{H}]^+$	1178.7633
1189.2774	vw	1189.2858	w	1189.2856	w	$[\text{R}_{13}\text{As}_{13}]^{2+}$	1189.2819
		1204.6933	w	1204.6941	w	$[\text{R}_6\text{As}_7\text{S}]^+$	1204.6923
		1205.8270	w	1205.8301	m	$[\text{R}_7\text{As}_6]^+$	1205.8436
				1246.6343	vw	$[\text{R}^{\text{ox}}\text{R}_5\text{As}_8\text{H}]^+$	1246.6340
				1248.6501	vw	$[\text{R}_6\text{As}_8\text{H}]^+$	1248.6497
				1252.6759	vw	-	

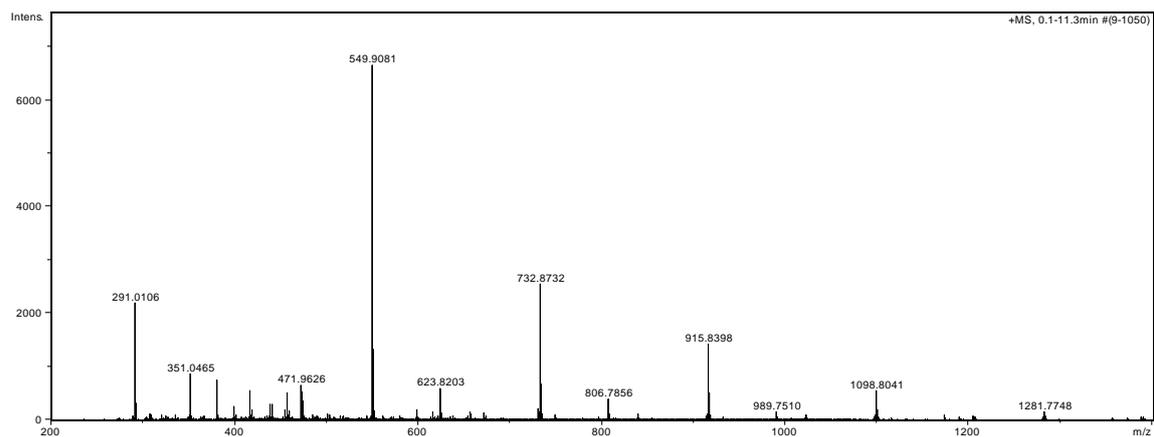
				1279.7577	w	$[\text{R}^{\text{ox}}\text{R}_6\text{As}_7\text{H}^+]^+$	1279.7574
				1280.7635	w	$[\text{R}_7\text{As}_7]^+$	1280.7652
1281.7748		1281.7748	s	1281.7747	vs	$[\text{R}_7\text{As}_7\text{H}]^+$	1281.7730
		1464.7412	m	1464.7407	m	$[\text{R}_8\text{As}_8\text{H}]^+$	1464.7396

3.4.1 Notes on the assignment of peaks

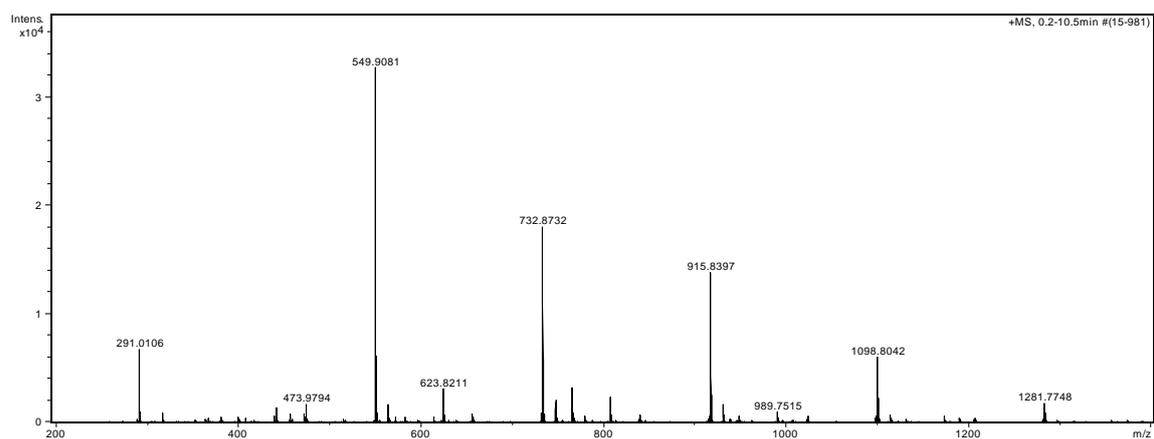
Peaks are assigned as logically as they can be. In areas of the spectrum where peaks overlap or are close together this seems to decrease the accuracy of the peaks - presumably by broadening the peaks and so the observed values can differ a little from the predicted values.

The mass spectra of all the different Salvarsan preparations were very similar with differences occurring mainly in the minor peaks. Several different series can be observed in these spectra.

Figures 3-3, 3-4 and 3-5 show the full spectra for the three compounds for overall comparison and show the patterns observed. These spectra are broken up in the following discussion.



**Figure 3-3 – ESI spectrum of Salvarsan produced by Christiansen's method (3.3.2)
obtained on the MicrOTOF**



**Figure 3-4 – ESI spectrum of Salvarsan produced by Ehrlich's method (3.3.3)
obtained on the MicrOTOF**

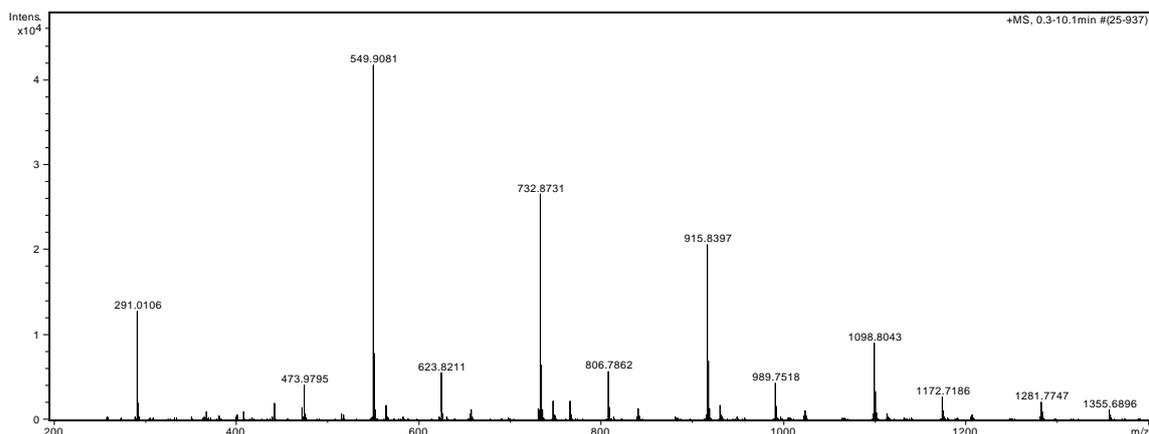


Figure 3-5 - ESI spectrum of Hoechst Salvarsan obtained on the MicrOTOF

3.4.2 Major peaks

Each spectrum is dominated by the $[(RAs)_n+H]^+$ peaks, where $R = 3-NH_2-4-HO-C_6H_3-$ and $n = 3$ (m/z 549.908), 4 (m/z 732.873), 5 (m/z 915.840) and 6 (m/z 1098.804). Higher peaks in the same series are also observed up to $n = 8$ at m/z 1464.7396. Along with the R_2As^+ peak at m/z 291.011, these were the peaks that were used as an internal standard to calibrate the spectra.

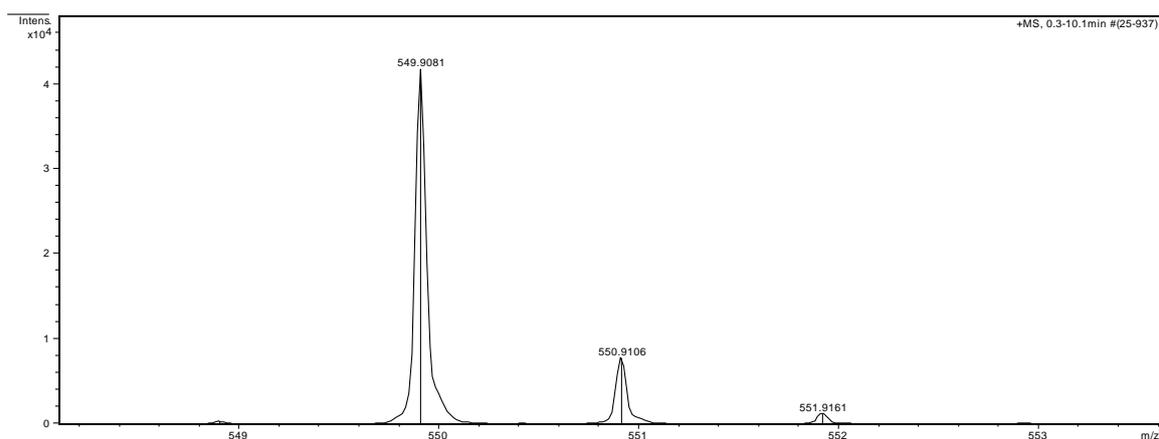


Figure 3-6 - Blowup of the $n = 3$ (m/z 549.908) peak

Higher numbers of n can be observed with diminishing intensity.

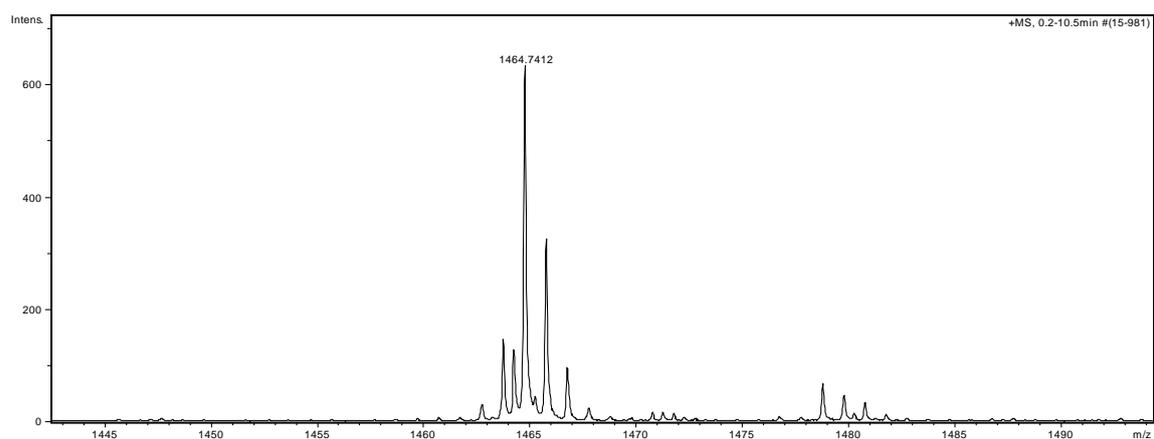


Figure 3-7 - Blowup of $n = 8$ at m/z 1464.7396

These major peaks are a consistent feature of all the samples of Salvarsan analysed on several different instruments and therefore strongly indicate the state that Salvarsan exists in aqueous solution.

NL01 #89 RT: 1.98 AV: 1 NL: 3.30E5
T: + p sid=15.00 Full ms [150.00-1200.00]

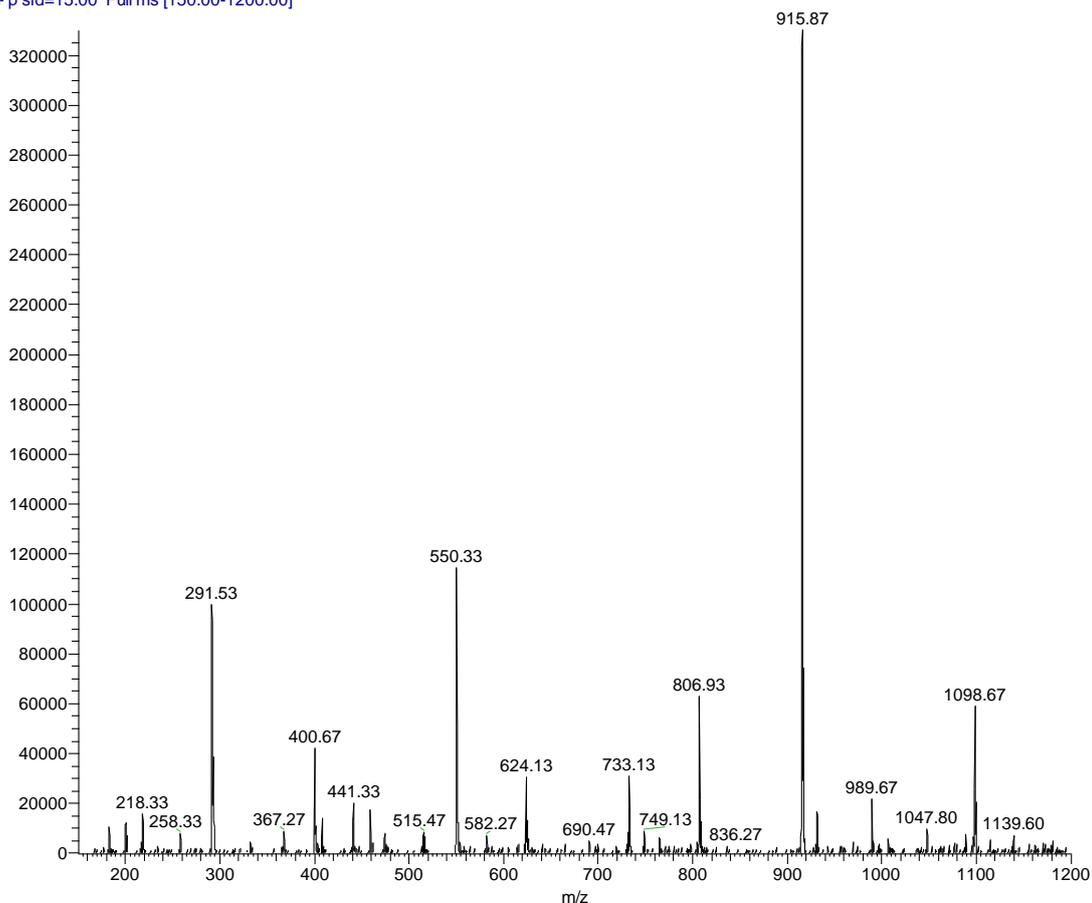


Figure 3-8 - Salvarsan spectrum obtained on the LCQ instrument.

Hence the first conclusion that can be drawn is that Salvarsan is mainly a mixture of $(RAs)_n$ rings in aqueous solution. Although structures are not assignable in ESMS these species are clearly rings as linear polymers do not fit the observed masses. Precedence for As rings is well established for $n=4, 5,$ and $6,$ as discussed earlier (see Table 3.1). For $n=3$ there are no structurally characterized As species, though both $(RP)_3^{147}$ and $(RSb)_3^{148}$ examples are known.

The spectra obtained on the Platform MS (Figure 3-10) is most likely to be a reflection of the abundance of these species in solution as the detection window for this machine approaches linear, although relative abundance of the different ring sizes is hard to

gauge. It can be assumed that they are all forming positive ions in the same way (by protonation of the NH_2 groups) and therefore that a correlation between signal intensity and concentration in solution must be present. Given these assumptions, it can be seen that the main peaks represent the $[(\text{RAs})_3+\text{H}]^+$ (2) and $[(\text{RAs})_5+\text{H}]^+$ (3) rings which appear to be the dominant species in solution.

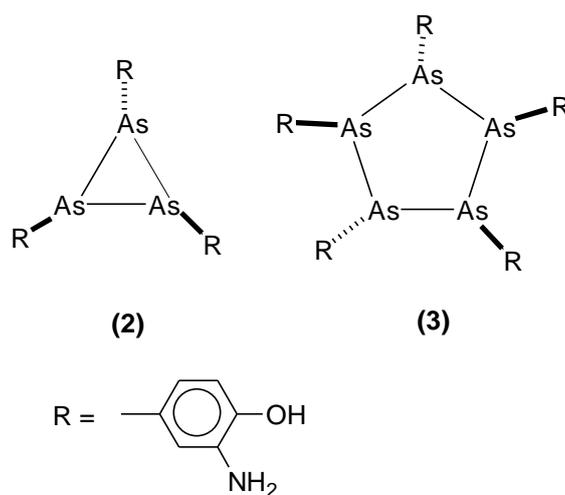


Figure 3-9 - The structures of various Salvarsan rings

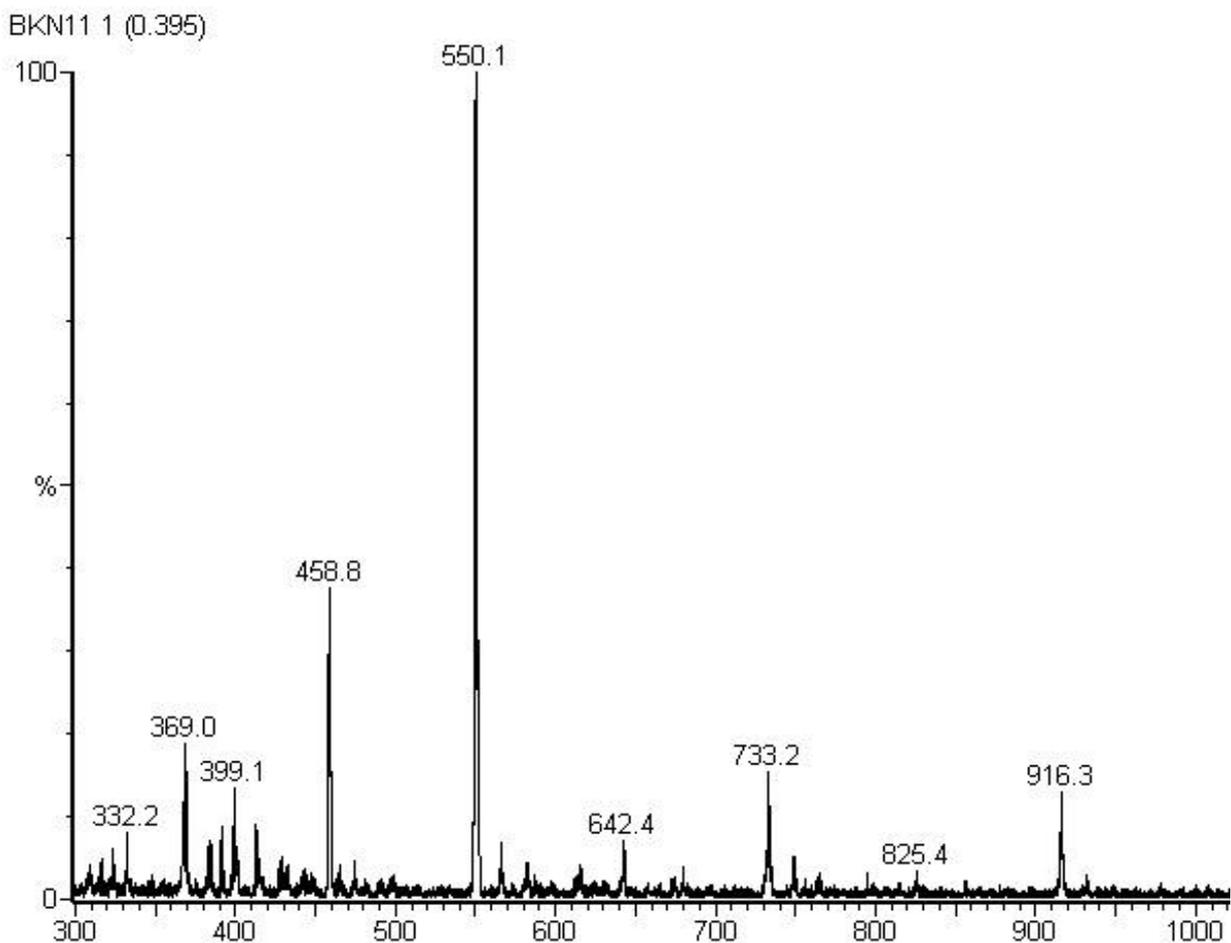


Figure 3-10 – ESI spectrum of Salvarsan obtained on the platform instrument

Doubly protonated peaks representing some of the major rings have also been observed in some spectra. These represent $[(RAs)_n+2H]^{2+}$. The appearance of these peaks is largely determined by the settings of the mass spectrometer and the even numbered peaks overlap the major peaks. For example, the $[(RAs)_6+2H]^{2+}$ has the same m/z as $[(RAs)_3+H]^+$ but is apparent from the spacing of the adjacent peaks (Figure 3-11).

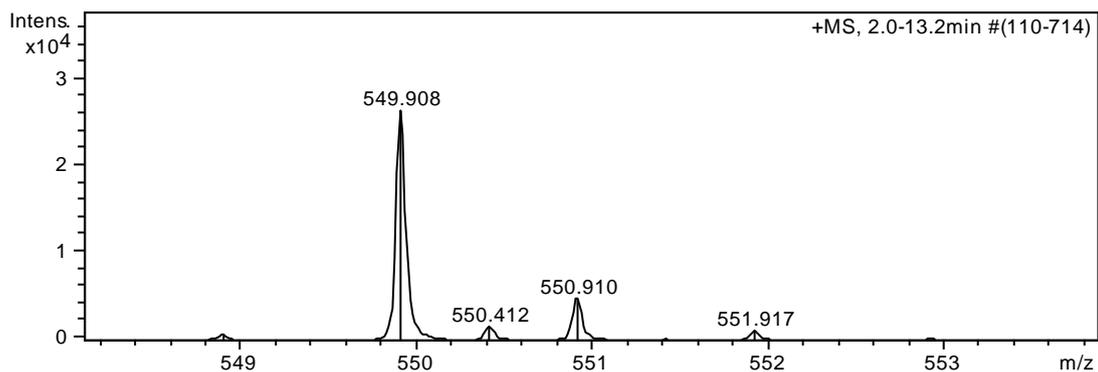


Figure 3-11 -Overlap of $[(RAs)_6+2H]^{2+}$ and $[(RAs)_3+H]^+$ peaks

The clearest example of a 2+ peak observed was at 458.427 and represents $[(RAs)_5+2H]^{2+}$ but equivalent species can be seen for all values of n in some spectra.

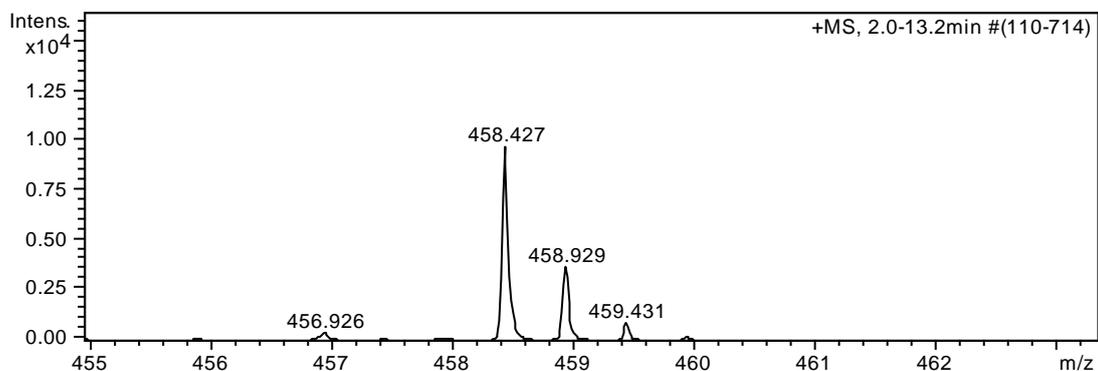


Figure 3-12 - Blowup of the $[(RAs)_5+2H]^{2+}$ peak

Sodium adducts $[(RAs)_n+Na]^+$ were also observed – either from residual sodium ions in the mass spectrometer, or from sodium in the Salvarsan base left over from the sodium dithionite used for the reduction.

During the course of this study attempts were made to separate the different cyclic molecules which make up Salvarsan. This was attempted using size exclusion chromatography, but separation of the mixture could not be achieved by this means.

3.4.2.1 Selective fragmentation

Selective fragmentation of the major peaks showed elimination of R, R₂As and R₃As fragments.

For example fragmentation of the m/z 550.00 [(RAs)₃+H]⁺ peak gave rise to peaks at m/z 474.0 [R₃As₂]⁺, m/z 440.7 [R₂As₃]⁺, m/z 400.2 [R₃AsH]⁺, m/z 367.00 [R₂As₂H]⁺ and m/z 291.3 [R₂As]⁺.

Fragmentation of the m/z 915.00 [(RAs)₅+H]⁺ peak gives rise to peaks at m/z 764.9 (R₅As₃⁺), m/z 623.8 [R₃As₄]⁺, m/z 515.2 [R₂As₄]⁺, m/z 473.8 [R₃As₂]⁺ and m/z 400.3 [R₃AsH]⁺

All of these fragmentation peaks represent the loss of R groups, RAs units and migration of R groups within the mass spectrometer.

Clearly fragmentation of larger rings does not generate smaller rings in the mass spectrometer and so the various rings are present in the sample and are not artefacts.

It is interesting to note that when Elmes *et al*¹¹⁰ examined a sample of hexaphenylcyclopolyarsine by electron impact mass spectrometry in 1970 they observed peaks corresponding to smaller rings (PhAs)₃, (PhAs)₄, and (PhAs)₅ in their spectra but assumed that they were fragments. It is possible that these were actually present in the sample being analysed as their spectra show many of the corresponding peaks that we see in ESMS for Salvarsan.

3.4.2.2 Minor peaks related to the major peaks

Many peaks representing adducts of the major peaks were seen in the spectra.

Amongst these minor peaks, sulfur containing adducts were seen in the Salvarsan which had been produced using dithionite – these peaks were observed at m/z 581.882 [R₃As₃SH]⁺, m/z 764.845 [R₄As₄SH]⁺, m/z 947.810 [R₅As₅SH]⁺ and m/z 1130.780

$[\text{R}_6\text{As}_6\text{SH}]^+$ and match more closely to these assignments for sulfur containing species, rather than species containing two oxygen atoms instead of one S which would give a similar mass.

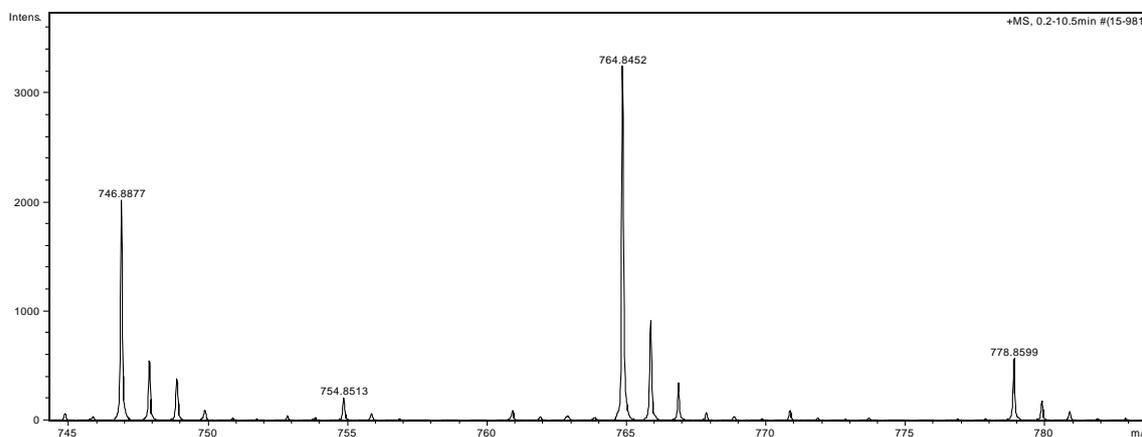


Figure 3-13 - Blowup of the m/z 764.845 $[\text{R}_4\text{As}_4\text{SH}]^+$ peak. Also visible is $[\text{R}_4\text{As}_4+\text{Na}]^+$ at m/z 754.851 and $[\text{R}_4\text{As}_4+\text{CH}_3]^+$ at m/z 746.888

It is not known how the sulfur fits into the ring, but induced fragmentation of the m/z 674.7 peak $[\text{R}_4\text{As}_4\text{SH}]^+$ gives rise to m/z 655.7 $[\text{R}_3\text{As}_4\text{S}]^+$ which suggest that the sulfur has been incorporated into the core structure in some way, possibly into the ring and not in a way where it can be easily dislodged by fragmentation.

Compounds with sulfur incorporated into arsenic rings are also known, so this is not unlikely.

The equivalent oxygen containing entities were also sometimes seen, but not as often as expected – the peak at m/z 748.865 in the hypophosphorous acid sample of Salvarsan appears to be one of these adducts - $[\text{R}_4\text{As}_4\text{O}+\text{H}]^+$

Another series could be assigned as $[(\text{RAs})_n+\text{CH}_2+\text{H}]^+$, most likely involving an OCH_3 group in place of the phenolic OH. This could have been formed during the treatment of the Salvarsan base with methanolic HCl during workup and is interesting given the earlier findings that Salvarsan could contain some methanol as a contaminant as the

methylated derivatives of Salvarsan would probably hydrolyse in basic solution to give free methanol. These peaks were not observed in the sample made with hypophosphorous acid as methanol was not used in the workup.

An unusual series of peaks are seen at just lower masses from the main $[(RAs)_n+H]^+$ peaks.

For example, before the main peak at m/z 732.873 $[(RAs)_4+H]^+$ there are peaks at m/z 731.862, m/z 730.856 and m/z 729.846.

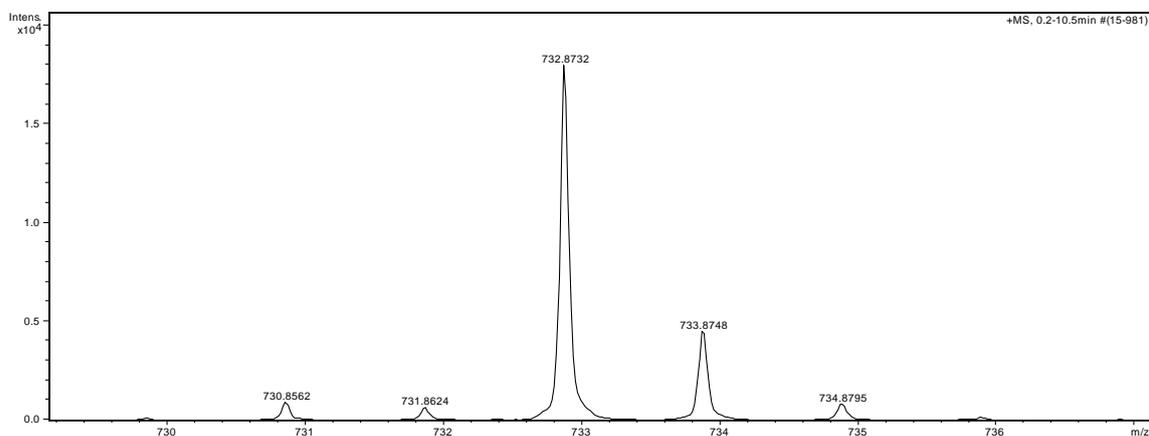


Figure 3-14 -Blowup showing peaks at m/z 731.862, m/z 730.856 and m/z 729.846

The peaks which are one mass unit down from the main peak seem to correspond to $[(RAs)_n]^+$ and are probably caused by *in situ* oxidation in the mass spectrometer – something which is commonly observed for electron rich species.¹⁴⁹

The peaks which are two mass units lower than the $[(RAs)_n+H]^+$ peaks probably represent ions formed by oxidation of the R groups to a favourable *o*-quinoimine compound (designated as R^{OX}).

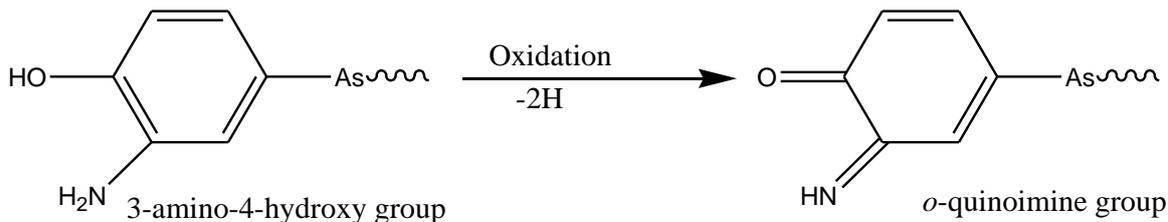


Figure 3-15 -Scheme showing the oxidation to an *o*-quinonimine compound

It is known that amino phenols are readily oxidized to such species.¹⁵⁰ These peaks are much weaker than the main peaks but are observed in all the different preparations of Salvarsan. It is not known whether these are present in the original samples, or whether they are formed on exposure to air, or if they are generated as artefacts in the mass spectrometer.

Many of the peaks involving RAs groups have the corresponding R^{ox}As species as satellites two mass units lower.

3.4.2.3 Minor peaks from breakdown of the major peaks

The next series of peaks observed are of the type $[\text{R}_{n-1}\text{As}_n]^+$ and include m/z 440.856 $[\text{R}_2\text{As}_3]^+$, m/z 623.820 $[\text{R}_3\text{As}_4]^+$, m/z 806.786 $[\text{R}_4\text{As}_5]^+$, m/z 989.751 $[\text{R}_5\text{As}_6]^+$ and m/z 1172.719 $[\text{R}_6\text{As}_7]^+$. These are presumably generated in the mass spectrometer by loss of R groups from the appropriate rings. These peaks were also observed in the induced fragmentation experiments. Methyl and sulfur adducts and derivatives with oxidized R groups are also seen in these peaks.

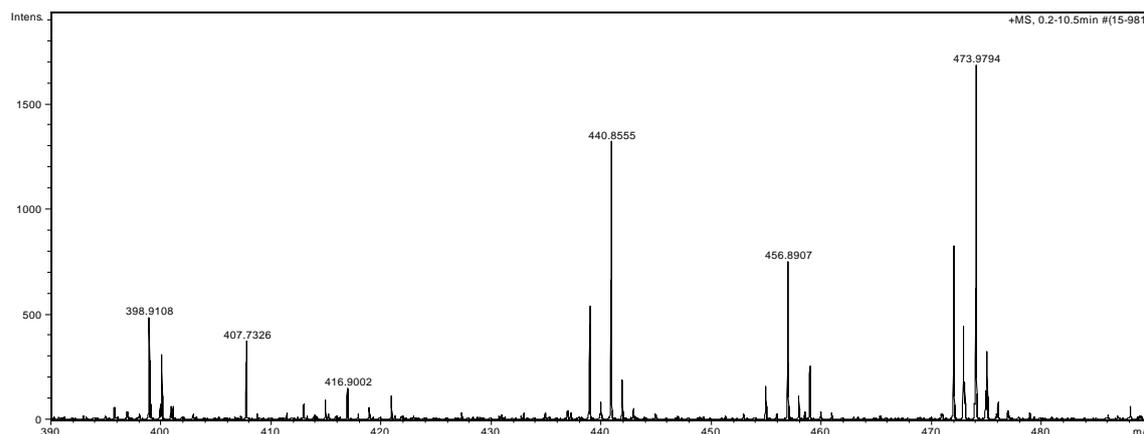


Figure 3-16 - Blowup showing the peak at m/z 440.856 $[R_2As_3]^+$

The series $[R_nAs_{n-1}]^+$ also occurs starting with the simplest fragments $[R_2As]^+$ at m/z 291.011 and $[R_3As_2]^+$ at m/z 473.979. These first two members of this series are considerably stronger in the spectra than the higher members as they will be the breakdown and rearrangement products of many of the higher molecules. They should also be more stable – being simple arsine and diarsine ions.

Other members of this series include m/z 656.942 $[R_4As_3]^+$, m/z 839.909 $[R_5As_4]^+$, m/z 1022.872 $[R_6As_5]^+$ and m/z 1205.827 $[R_7As_6]^+$.

3.4.2.4 Peaks corresponding to arsines and diarsines

The smaller peaks in this series can also be thought of as simple arsine and diarsine ions and peaks corresponding to other arsine and diarsine compounds are also seen as well as some of their methylated and oxidized derivatives of these ions.

The peak corresponding to $[R_3As+H]^+$ is seen at m/z 400.064. It is not known if this species is formed in the mass spectrometer, by rearrangement in solution or if it is present in the original samples.

The related di-arsine peaks also occur – m/z 516.792 $[\text{R}_4\text{As}_2+\text{H}]^+$, m/z 473.980 $[\text{R}_3\text{As}_2]^+$, m/z 368.855 $[\text{R}_2\text{As}_2\text{H}_2+\text{H}]^+$, m/z 366.940 $[\text{R}_2\text{As}_2\text{H}]^+$ and its oxidized derivative at m/z 364.924 $[\text{RR}^{\text{ox}}\text{As}_2\text{H}]^+$.

Peaks related to these species incorporating oxygen and sulfur are also observed.

3.4.2.5 Cluster peaks

Minor peaks which appear to represent clusters of As atoms and R groups are also seen. Some of these are very hard to reconcile as having a positive charge but the matches with the calculated values are generally very good. The first of these is the peak at m/z 257.886 which corresponds well to RAs_2^+ but the exact formulation of this unit is unknown. There is also a peak at m/z 273.894 which appears to be a related oxidation product $[\text{RAs}_2\text{O}]^+$ and this species is easier to justify as having a positive charge. These peaks are however only tentatively assigned.

Other “cluster” peaks observed include those forming a series $[\text{R}_{n-2}\text{As}_n+\text{H}^+]^+$ including m/z 333.816 $[\text{RAs}_3\text{H}]^+$, m/z 516.789 $[\text{R}_2\text{As}_4\text{H}]^+$, m/z 699.750 $[\text{R}_3\text{As}_5\text{H}]^+$, m/z 882.714 $[\text{R}_4\text{As}_6\text{H}]^+$, m/z 1065.681 $[\text{R}_5\text{As}_7\text{H}]^+$ and m/z 1248.650 $[\text{R}_6\text{As}_8\text{H}]^+$. Peaks with oxidized R groups are also observed in this series.

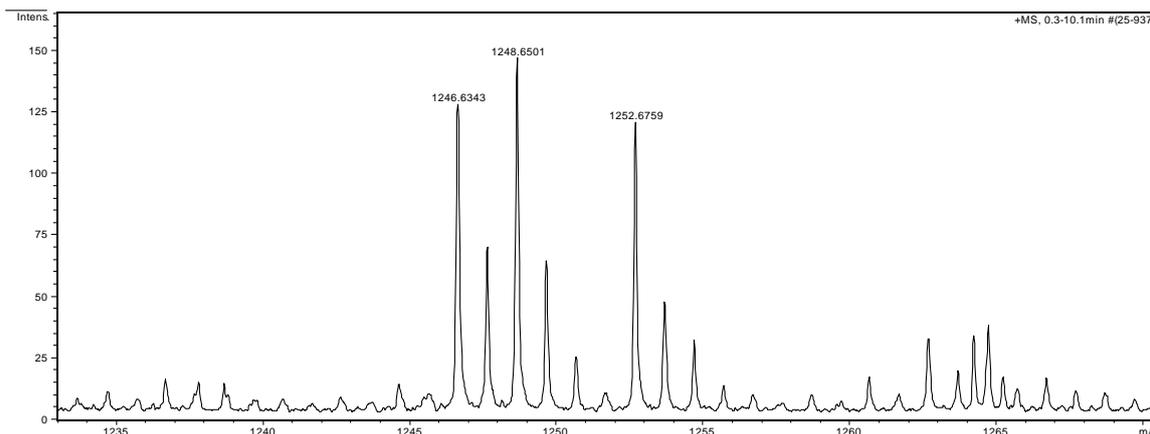


Figure 3-17 - Blowup showing the peak at m/z 1248.650 [R_6As_8H]⁺ and oxidised version two mass units lower

3.4.2.6 Confirmation of sulfur containing impurities

Some other sulfur compounds are also observed – these correspond to the substitution pattern on the structures proposed by King⁸⁴⁻⁸⁵ for a possible toxic sulfur-containing contaminant in commercially available Salvarsan.

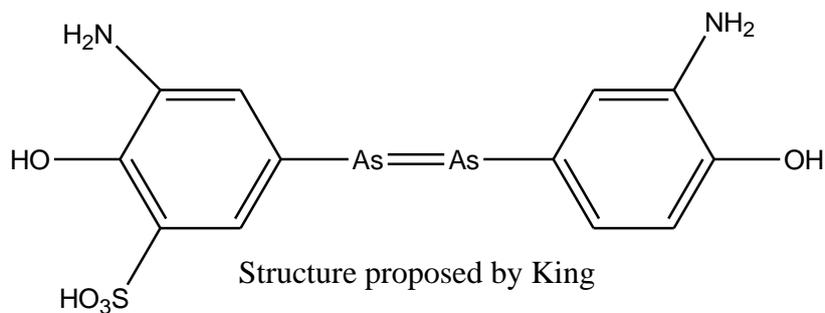


Figure 3-18 - Sulfur containing impurity proposed by King

These are present in the spectra of both the Waikato-prepared Salvarsan using dithionite and the sample from Hoechst. Peaks corresponding to this contaminant occur at m/z 629.863 [(R-SO₃H)R₂As₃+H]⁺, m/z 812.828 [(R-SO₃H)R₃As₄+H]⁺, m/z 995.791 [(R-SO₃H)R₄As₃+H]⁺ and m/z 1178.757 [(R-SO₃H)R₅As₆+H]⁺.

The fragment $[(R-SO_3H)RAs^+]$ is also observed at m/z 370.967 in the Hoechst sample. The apparent presence of these species in Salvarsan suggests that King might have been correct that these compounds were impurities in Salvarsan but determining their toxicity requires more research.

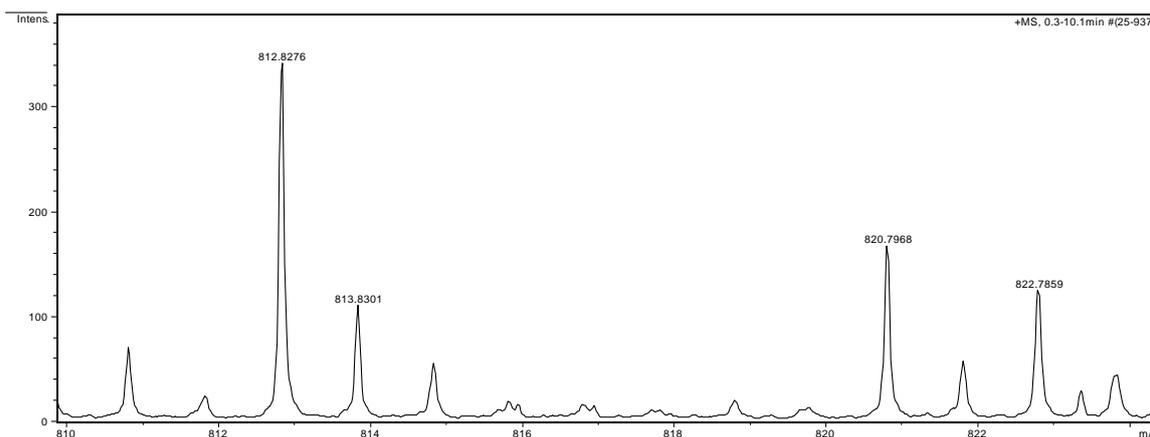


Figure 3-19 - Blowup showing peak at m/z 812.828 $[(R-SO_3H)R_3As_4+H]^+$

3.4.2.7 Possible polymers

In the higher range of the spectra, a series of doubly charged peaks was observed.

These correspond to $[R_nAs_n]^{2+}$ and are observed weakly where $n = 9-16$.

The origin of these peaks is unknown and they could represent breakdown products from larger rings, or alternatively, larger polymeric molecules. For example, a species such as $HOAsR-(AsR)_n-AsROH$ would readily give $[(RAs)_{n+2}]^{2+}$ by loss of the OH groups.

3.4.2.8 Other peaks

A peak was observed in all spectra which appears to contain chlorine at m/z 416.900. This matches closely what would be expected for $[\text{R}_2\text{As}_2\text{ClO}]^+$ calculated at m/z 416.896. The related $[\text{R}_2\text{As}_2\text{OH}]^+$ peak is also seen.

Likely structures for these species are shown below in Figure 3-20.



Figure 3-20 – Proposed structures for m/z 382.970 and m/z 416.896

One peak which appears to match up to a phosphorus containing species is also seen in the Christiansen Salvarsan. This peak is at m/z 671.819 and is close to what can be calculated for a $[\text{R}_3\text{As}_4\text{POH}]^+$ species calculated for m/z 671.797. The structure of this species is unknown.

3.4.3 Differences between the three Salvarsan samples

Salvarsan made by all three methods is essentially the same, the major differences being in the minor peaks. The sulfur compounds in the Salvarsan made with dithionite would be the major differences between the two products, as was suggested in earlier papers.

It can probably be concluded that these compounds were responsible for the toxicity of batches of Salvarsan as was suggested as they are the only major differences between the methods of preparation. There is a small amount of the methylated compound in the dithionite samples which had methanol included in the workup but this is unlikely to be in sufficiently high concentrations to show a toxic effect. Rearrangement to the simple

arsines and diarsines is another possibility that could add to the toxicity but this would probably occur no matter how the Salvarsan was produced if it was allowed to stand in solution for too long.

Overall, the Salvarsan produced using dithionite showed more impurities than the Salvarsan produced using hypophosphorous acid.

3.4.4 Oxidation of Salvarsan

When solutions of Salvarsan are made up and left exposed to air, oxidation occurs reasonably quickly and after 24 hours peaks are visible at m/z 218.0 which corresponds to the expected oxidation product $[\text{RAs}(\text{OH})_2+\text{H}]^+$ which at higher source energies loses water to form $[\text{RAsOH}]^+$ (m/z 200.0) and then another molecule of water to give a peak at m/z 182.1 presumably generating an ion of the type shown in Figure 3-21.

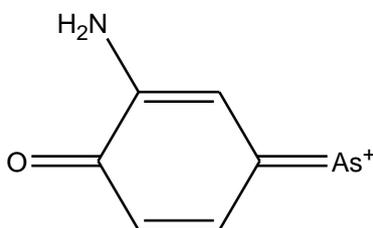


Figure 3-21 – Proposed structure for m/z 182.1

This corresponds to the proposal that Salvarsan is oxidized to give Mapharsen over a period of time, a reaction that is important for understanding the biological activity of Salvarsan.

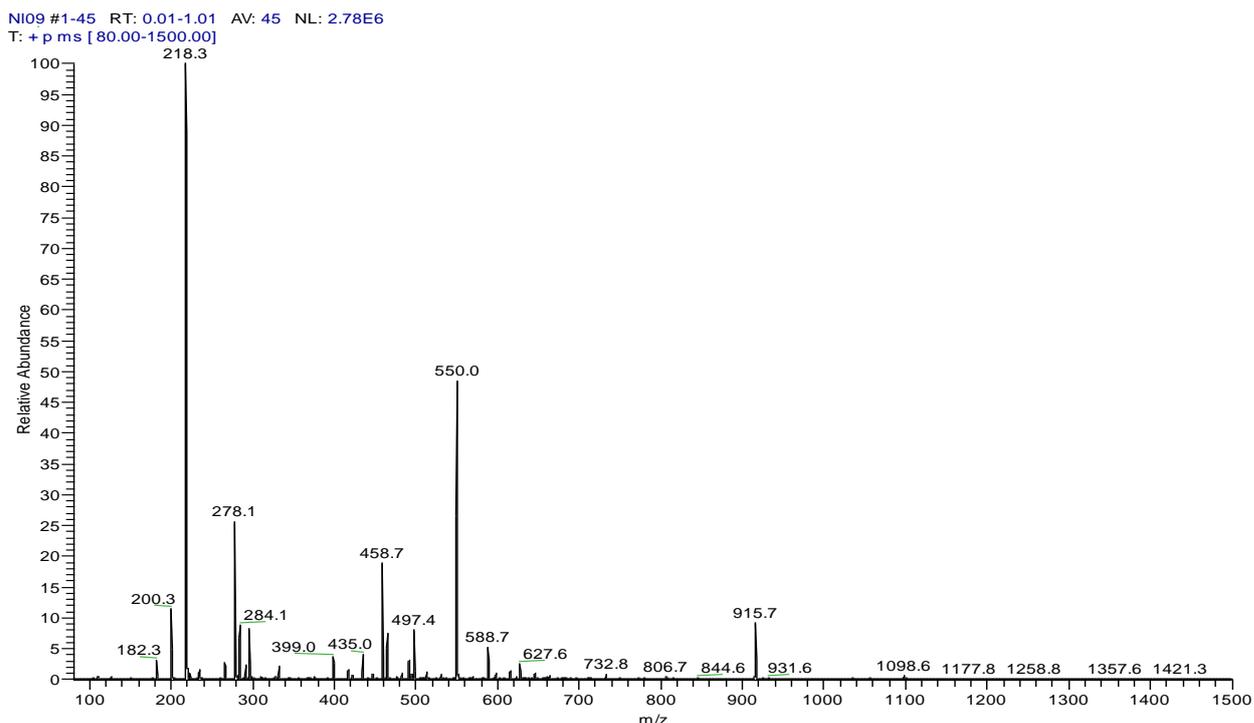


Figure 3-22 - Mass spectrum showing the 218 peak arising from oxidation

3.4.5 Mass spectrometry of other Salvarsan related compounds

The closely related compound, 3-amino-4-methoxyphenylarsenobenzene, was analysed and found to be the same as Salvarsan in solution except with the addition of the methyl groups, giving $[(R^{me}As)_n+H]^+$ as the dominant peaks. Figure 3-23 shows this spectrum with $[(R^{me}As)_3+H]^+$ at m/z 592, $[(R^{me}As)_4+H]^+$ at m/z 789, $[(R^{me}As)_5+H]^+$ at m/z 989, $[(R^{me}As)_6+H]^+$ at m/z 1182 and $[(R^{me}As)_7+H]^+$ at m/z 1379.

Mixed compounds were prepared (see 3.3.7) by reducing other arsonic acids in with 3-amino-4-hydroxyphenylarsonic acid and these showed similar spectra, with the expected mixtures of R groups on the arsenic rings.

NL01 #1-36 RT: 0.01-0.98 AV: 36 NL: 8.54E5
T: + p Full ms [150.00-2000.00]

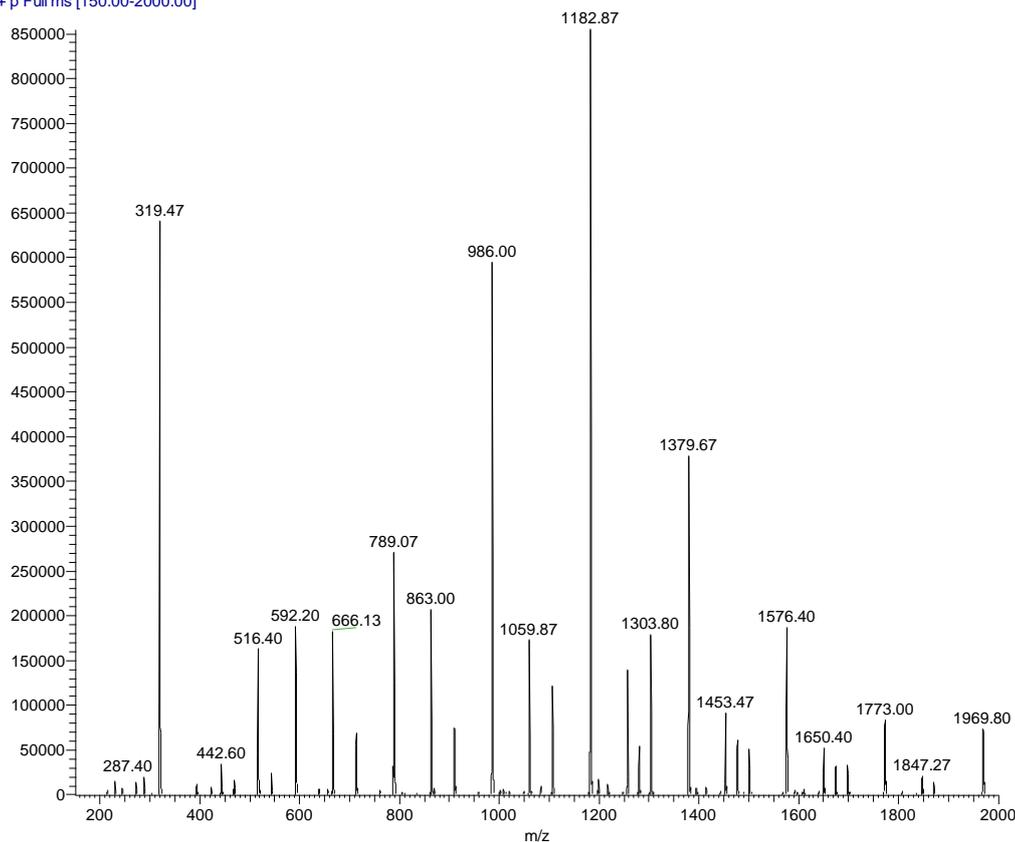


Figure 3-23 - Spectrum of 3-amino-4-methoxyphenylarsenobenzene

Most of the other arsenobenzenes prepared during the course of this work proved totally unsuitable for analysis by ESMS due to their insolubility in common solvents and the lack of functional groups which would allow for chemical ionization.

3.4.5.1 Mass spectrometry of “Arsphenamine polyarsenide”

In 1923 Christiansen reported the preparation of an “Arsphenamine polyarsenide”

This was essentially Salvarsan which had been reduced with a proportion of sodium arsenite in the reduction and this somehow was incorporated into the product, which was an orange colour, in comparison to the yellow of Salvarsan.

This was examined by ESMS and appears to consist of $[\text{R}_2\text{As}_4\text{O}_6\text{H}_2]^+$ m/z 613.8 and $[\text{R}_4\text{As}_4\text{O}_2\text{H}]^+$ m/z 764.7.

Fragmentation of the m/z 613.8 peak gives rise to peaks at m/z 504.7 $[\text{RAs}_4\text{O}_6\text{H}]^+$ and m/z 395.9 which cannot be unambiguously assigned.

This is an interesting material as it seems to contain more than one arsenic unit per each organic unit, which would merit a more detailed examination as it could contain novel structures.

3.4.6 Rearrangement of R groups

From the mass spectrometry results it appeared that the R groups were labile to some extent as peaks were seen which could only be explained by migration of the R groups, but it was not known if this was taking place as artefacts of the mass spectrometry technique or in solution.

Several experiments were designed to explore this further.

Firstly, a solution containing 0.5 mg/mL 3-amino-4-methoxyphenylarsenobenzene ($\text{R}^{\text{me}}\text{As}$)_n and a solution containing 0.5 mg/mL Salvarsan (3-amino-4-hydroxyphenylarsenobenzene) ($\text{R}^{\text{OH}}\text{As}$)_n were mixed and any scrambling of R groups was followed by mass spectrometry. This was initially carried out at room temperature and then at 100°C.

Each solution was analysed separately before mixing and they each gave the expected series of peaks, assigned as $(\text{RAs})_n$

On mixing the solutions together the methylated compound completely dominated the spectra with the peaks from Salvarsan becoming negligible – this must reflect relative

protonation of the different compounds possibly due to blocking by internal hydrogen bonding within the Salvarsan molecules making them less basic.

After 26 hours at room temperature the scrambling of R groups began to be seen – most clearly in the $[\text{R}_2\text{As}]^+$ peak which was roughly 50% m/z 319.5 $[\text{R}^{\text{me}}_2\text{As}]^+$, 40% m/z 291.5 $[\text{R}^{\text{OH}}_2\text{As}]^+$ and 20% m/z 305.6 $[\text{R}^{\text{OH}}\text{R}^{\text{me}}\text{As}]^+$ and after 48 hours scrambling was very obvious with 15% m/z 319.5 $[\text{R}^{\text{me}}_2\text{As}]^+$, 40% m/z 291.5 $[\text{R}^{\text{OH}}_2\text{As}]^+$ and 45% m/z 305.6 $[\text{R}^{\text{OH}}\text{R}^{\text{me}}\text{As}]^+$.

At 48 hours all other peaks in the spectra reflected this scrambling.

The experiment was repeated at 100°C under reflux conditions and scrambling was obvious after 1.5 hours and the main peaks were approaching a normal distribution after 18.5 hours.

A similar experiment was carried out in which Salvarsan was mixed with a mixed 4-aminophenyl and 3-amino-4-hydroxyphenylarsenobenzene compound. The mixture was examined by ESMS and then stirred or refluxed and checked by ESMS on a regular basis.

As expected the R groups scrambled causing the distribution of the major clusters of peaks to change. Under reflux conditions the final spectrum (about 45 minutes after the initial spectrum was obtained) showed all the peaks expected for Salvarsan and the reaction showed a red precipitate which appeared to be the same as the red precipitate obtained when 4-aminophenylarsonic acid was reduced on its own. In this case the equilibrium had been affected by the solubility of one of the products.

The conclusion of these experiments is that the R- groups on the $(\text{RAs})_n$ rings are relatively labile, even in aqueous solution at room temperature.

Lability increases as the temperature rises as would be expected.

There are two possibilities for this scrambling process. The first is exchange of R groups directly between rings as shown in Figure 3-24, presumably by bridging aryl groups.

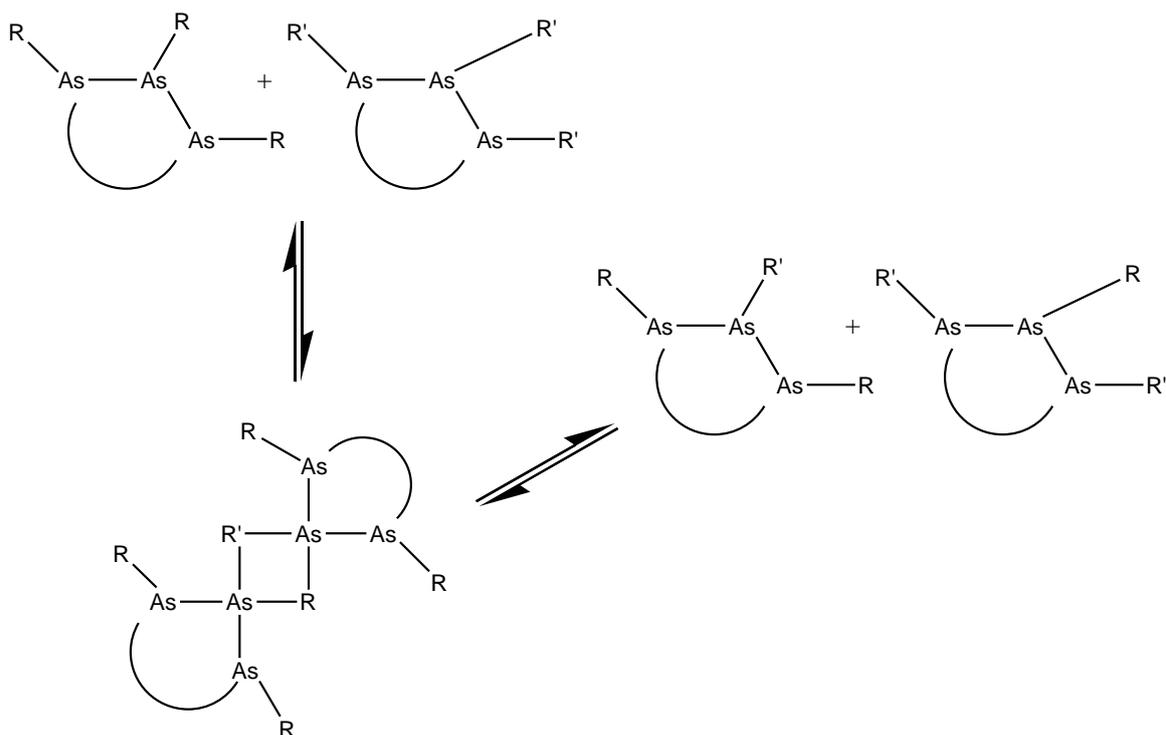


Figure 3-24 - Rearrangement via Bridging R groups

This type of exchange is common for other organometallic species such as R_4Sn species¹⁵¹ and Breunig *et al*¹⁵² demonstrated scrambling of R groups on distibenes.

The present case is unusual in that it is occurring in aqueous solutions.

A less likely option is that the $(RAs)_n$ are opening and exchanging RAs groups. This is counter indicated in an experiment where Salvarsan was dissolved and heated in sealed ampoules for a week.

Mass spectrometry after heating was carried out and showed essentially the same distribution of $[(RAs)_n+H]^+$ peaks as the original solution. If rings were opening and

reforming a change in the relative quantities might have been expected as they would have rearranged to the most thermodynamically stable size, which is unlikely to be the (RAs)₃ species due to the strain on the As-As bonds.

However this rearrangement is occurring it still seems to preclude the possibility of being able to isolate any specific ring size via physical methods.

More work is required to understand the systems and mechanisms involved in these rearrangement reactions.

3.5 Conclusions

Salvarsan was synthesized successfully as planned via several different methods.

It proved to be an interesting substance and was analysed in detail by electrospray mass spectrometry. Ehrlich's As=As structure can finally be put to rest as Salvarsan consists of small cyclic polymers of the type (RAs)_n, where n is 3 or greater, with most of the rings being *n*=3 or 5.

ESMS proved to be the most useful technique for studying these mixtures and Salvarsan prepared by several different methods was compared, along with a historical sample from the Hoechst factory. These samples were largely similar, but differed in several of the minor peaks – which appear to be impurities arising from the mode of synthesis. As was predicted, sulfur containing entities were among these impurities.

An especially interesting feature of these compounds is the rearrangement reactions they undergo in aqueous solution, which can be monitored in real time by ESMS. This alone qualifies these compounds for further study.

Several other related cyclopolyarsines were also synthesized but on the whole, these resisted analysis by the available techniques mainly due to limited solubility.

4 Chapter Four - Mapharsen and As(III) compounds

4.1 Mapharsen background

The last arsenical compound to be marketed as a remedy for syphilis was Mapharsen (3-amino-4-hydroxyphenylarsonous acid – 3-NH₂-4-OHC₆H₃As(OH)₂, see Figure 4-1), introduced by Parke Davis in 1937. This compound had previously been investigated by Ehrlich but had been found to be too toxic for use.¹⁰⁵ The history of Mapharsen is discussed in detail in 1.6.5.

Mapharsen is one of the most important compounds in the Salvarsan story – as it appears to be the active compound that Salvarsan is oxidized to in biological systems and the compound that exhibits antibacterial effects – in effect Salvarsan appears to act as a slow release form of Mapharsen.

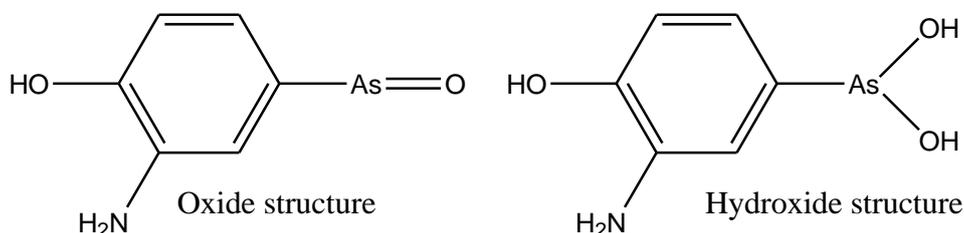


Figure 4-1 - Structures of Mapharsen.

4.2 Introduction to arsonous acids

As(III) compounds (Arsonous acids) are a poorly known class of compounds which have historically been described as “oxides” and assigned the structure RAs=O. It is more likely that they can exist in rings of (RAsO)_n through to RAs(OH)₂ depending on the pH of the solution and the substituents on the ring.

4.3 Synthesis of arsonous acids

Phenylarsonous acids are generally prepared by the hydrolysis of the corresponding phenyldichloroarsine although there are several exceptions to this, the most noteworthy being the preparation of the *p*-aminophenylarsonous acid by reducing the *p*-aminophenylarsonic acid with phenylhydrazine. This reaction doesn't appear to work with any of the other phenylarsonic acids.

In many of the recorded preparations for these compounds the arsonic acids can also be prepared by reduction with sulfur dioxide in basic solution.

The dichloroarsines can be easily prepared by reduction of the corresponding arsonic acid with sulfur dioxide gas in an solution of concentrated hydrochloric acid.

They precipitate as white solids (or in some cases, oils) and can be directly hydrolyzed to the arsonous acid or isolated as the dichloride.

Organic arsenic dichlorides are known to be vesicants, with phenyldichloroarsine being used in World War One as a blister agent.

4.3.1 Preparation of 3-amino-4-hydroxydichloroarsine¹⁵³

=3-Amino-4-hydroxyphenylarsonic acid (11.6 g, 0.05 mol) was added to a solution of concentrated hydrochloric acid (24 mL) in methanol (30 mL). Potassium iodide (100 mg) was added as a catalyst. Sulfur dioxide was bubbled through the solution for 30 minutes, during which time the dichloroarsine precipitated as fine white plates.

These were filtered, washed with concentrated HCl and dried under vacuum.

Yield – 10.1 g, 80%

Elemental analysis: found C 24.33, H 2.71, N 4.49%; calcd for C₆H₆AsNOCl₂·HCl: C 24.92, H 2.44, N 4.85%.

4.3.2 Preparation of 4-aminophenyldichloroarsine

4-Aminophenylarsonic acid (10.9 g, 0.05 mol) was added to a solution of concentrated hydrochloric acid (30 mL) in methanol (30 mL). Potassium iodide (100 mg) was added as a catalyst. Sulfur dioxide was bubbled through the solution for 30 minutes, during which time the dichloroarsine precipitated as a white solid.

This was filtered, washed with concentrated HCl and dried under vacuum.

Yield – 8.3 g, 70%

Elemental analysis: found C 24.70, H 3.25, N 4.70%; calcd for $C_6H_6AsNCl_2 \cdot HCl$: C 26.38, H 2.59, N 5.13%. Calcd for $C_6H_6AsNCl_2 \cdot HCl \cdot H_2O$: C 24.65, H 3.10, N 4.79%

4.3.3 Preparation of 3-nitro-4-hydroxydichloroarsine

3-Nitro-4-hydroxyphenylarsonic acid (13.14 g, 0.05 mol) was added to a solution of concentrated hydrochloric acid (24 mL) in methanol (30 mL). Potassium iodide (100 mg) was added as a catalyst. Sulfur dioxide was bubbled through the solution for 30 minutes, during which time the dichloroarsine precipitated as an orange oil.

The reaction mixture was cooled in an ice bath to try and get the dichloroarsine to solidify but this was not successful.

The excess aqueous liquid was therefore decanted and the dichloroarsine hydrolyzed with concentrated ammonia solution until neutral to litmus.

The solution was stored at 4°C overnight and a small quantity of orange plates precipitated out. These were filtered and dilute HCl was added to the filtrate causing a pale yellow solid to precipitate. This was collected and dried under vacuum.

Crystals were obtained through cooling a concentrated aqueous solution.

4.3.4 Preparation of 3-amino-4-hydroxyphenylarsonous acid (Mapharsen)

Water (10 mL) was degassed in a Schlenk flask and kept under nitrogen. 3-Amino-4-hydroxydichloroarsine (2 g) was added. The solution was then dehydrated under vacuum to give a white solid.

A sample of the original Parke Davis Mapharsen was generously provided by Pfizer from their archives for comparison with the material produced here.

4.3.5 Preparation of 4-aminophenylarsonous acid

Water (10 mL) was degassed in a Schlenk flask and kept under nitrogen. 4-amino dichloroarsine (2 g) was added. The solution was then dehydrated under vacuum to give a white solid.

4.3.6 Preparation of 4-aminophenylarsonous acid¹⁵⁴

p-Aminophenylarsonic acid (21.7 g, 0.1 mol) was added to methanol (115 mL) and heated to reflux to totally dissolve the acid. Phenylhydrazine (21.6 g, 0.2 mol) was added and the mixture refluxed for 1 hour.

This mixture was then distilled from a boiling water bath until nothing else distilled.

The residual oil was extracted with water (170 mL) and sodium hydroxide solution (120 mL, 2 mol L⁻¹) and shaken with ether to remove any organic impurities.

Ammonium chloride solution (100 mL, 5 mol L⁻¹) was then added and crystals formed after a week at 4°C.

These were filtered and purified by recrystallisation from hot water.

ESMS (methanol solution) – m/z 252.2 $[\text{NH}_2\text{C}_6\text{H}_4\text{As}(\text{OCH}_3)_2 + \text{Na}]^+$, m/z 481.1 $[(\text{NH}_2\text{C}_6\text{H}_4\text{As}(\text{OCH}_3)_2)_2 + \text{Na}]^+$
 Calculated - m/z 252.0, $[\text{NH}_2\text{C}_6\text{H}_4\text{As}(\text{OCH}_3)_2 + \text{Na}]^+$ and m/z 481.0, $[(\text{NH}_2\text{C}_6\text{H}_4\text{As}(\text{OCH}_3)_2)_2 + \text{Na}]^+$.

4.4 Analysis of Mapharsen

Table 4.1 - Microanalysis results

Sample Experimental	C		H		N	
Parke-Davis Mapharsen	29.85		3.44		5.75	
Waikato Mapharsen	25.02		2.72		4.68	
Calculated values		Difference		Difference		Difference
$\text{HONH}_2\text{C}_6\text{H}_3\text{As}(\text{OH})_2$	33.18	-3.33	3.72	-0.28	6.45	-0.70
$\text{HONH}_2\text{C}_6\text{H}_3\text{As}(\text{OH})_2 \cdot \text{HCl}$	28.46	1.39	3.59	-0.15	5.54	0.21
$\text{HONH}_2\text{C}_6\text{H}_3\text{As}(\text{OH})_2 \cdot \text{HCl} \cdot \text{H}_2\text{O}$	26.57	3.28	4.09	-0.65	5.17	0.58
$\text{HONH}_2\text{C}_6\text{H}_3\text{As}(\text{OH})_2 \cdot \text{H}_2\text{O}$	30.64	-0.79	4.29	-0.85	5.96	-0.21

The column above marked difference is the difference between the calculated values and the Parke Davis compound.

As can be seen in Table 4.1, the Mapharsen sourced from Parke-Davis corresponds most closely to the monohydrate, while the Waikato sample is much less pure – as one would expect as the method used to prepare it would leave far more HCl remaining in the product.

The method used to produce the Parke Davis sample is unknown but it was presumably isolated from a neutral or basic solution.

4.5 Mass spectrometry

The sample of Mapharsen from Parke Davis was analyzed on the Bruker MicroTOF machine, which allows for highly accurate results.

For the high precision analyses, the sample of Mapharsen was made up to approximately 2 mg/mL in distilled water and centrifuged carefully to remove any small particles of insoluble material. This sample was introduced into the machine via a syringe pump at 180 $\mu\text{L min}^{-1}$

The peaks were assigned as accurately as possible with some peaks of lower intensity being tentatively assigned with help from Bruker's peak calculation tool.

Data are tabulated in Table 4.2

Table 4.2 - Mass spectral peak assignments for Mapharsen

Major peaks	Assignment	Calculated
181.8578	$[\text{C}_6\text{H}_3\text{ONH}_2\text{As}]^+$	181.9582
199.9680	$[\text{C}_6\text{H}_3\text{OHNH}_2\text{As}+\text{H}]^+$	199.9687
217.9793	$[\text{C}_6\text{H}_3\text{OHNH}_2\text{As}(\text{OH})_2+\text{H}]^+$	217.9793
291.0100	$[(\text{NH}_2\text{OHC}_6\text{H}_3)_2\text{As}]^+$	291.0109
302.9740	$[(\text{NHOC}_6\text{H}_3)_2\text{AsO}]^+$	302.9745
309.0208	$[(\text{NH}_2\text{OHC}_6\text{H}_3)_2\text{AsOH}+\text{H}]^+$	309.0215
320.9844	$[(\text{NH}_2\text{OC}_6\text{H}_3)_2\text{AsO}_2]^+$	320.9851
345.8363	-	
362.9097	-	
380.9196	$[(\text{NH}_2\text{OC}_6\text{H}_3)_2\text{As}_2\text{OH}]^+$	380.9196
398.9295	$[(\text{NH}_2\text{OHC}_6\text{H}_3)_2\text{As}_2\text{O}_2\text{H}]^+$	398.9302
418.8699	-	
438.9220	-	
454.8935	-	
471.9612	$[(\text{NH}_2\text{OC}_6\text{H}_3)_3\text{As}_2]^+$	471.9618
489.9720	$[(\text{NH}_2\text{OHC}_6\text{H}_3)_3\text{As}_2\text{O}]^+$	489.9724
543.8600	$[\text{C}_{18}\text{H}_{13}\text{As}_3\text{N}_3\text{O}_3]^+$	543.8599
561.8693	$[\text{C}_{18}\text{H}_{15}\text{As}_3\text{N}_3\text{O}_4]^+$	561.8705
579.9749	-	
597.8898	$[(\text{NH}_2\text{OHC}_6\text{H}_3)_3\text{As}_3\text{O}_3+\text{H}]^+$	597.8916

The major peaks in this spectrum correspond to $[\text{RAs}(\text{OH})_2+\text{H}]^+$ (m/z 217.978) and its two daughter peaks $[\text{RAsO}+\text{H}]^+$ (m/z 199.969) representing loss of H_2O in the mass spectrometer and $[\text{C}_6\text{H}_3\text{ONH}_2\text{As}]^+$ (m/z 181.958) representing the loss of another molecule of H_2O and formation of an *o*-quinoimine ion. This last peak can be structurally assigned as the resonance forms shown in Figure 4-2, which will give stability to this species in the mass spectrometer

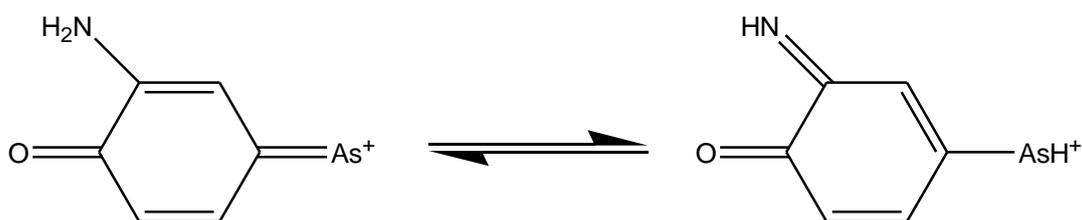


Figure 4-2 - Resonance forms of the proposed *o*-quinoimine ion

The peak at 291.010 is the same R_2As peak seen in the Salvarsan spectra and indicates either that the R groups have some lability in this sample too or that there was a small amount of $\text{R}_2\text{As}(\text{OH})$ or some other related species in the solution which could give the very stable R_2As^+ ion.

There is a peak which corresponds to the *o*-quinoimine structure shown in Figure 4-3 at m/z 302.974

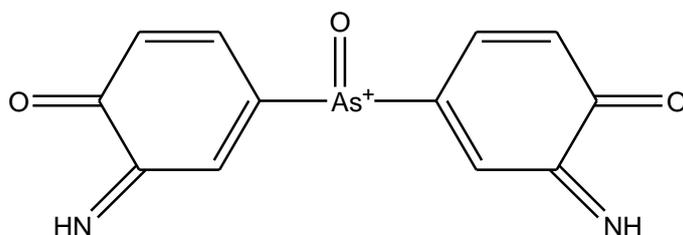


Figure 4-3 - Proposed structure for the peak at m/z 302.974

The exact nature of this peak is unknown, but the structure has the possibility of other resonance forms which could give it stability.

The peak at 309.021 (Figure 4-4) represents the species which would easily lose water to give the m/z 291 peak in the mass spectrometer.

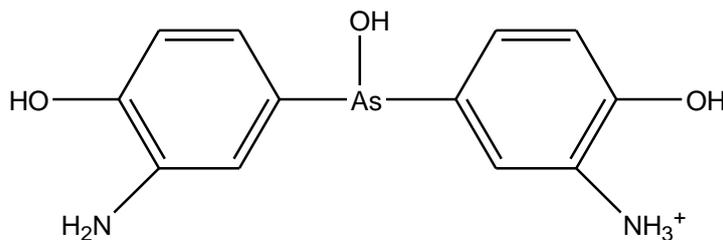


Figure 4-4 - Proposed structure for m/z 309.021

It is not known if these R_2As peaks are in the sample, or are formed in the mass spectrometer.

The peak at 320.984 (Figure 4-5) appears to be related with an extra oxygen and is an As(V) compound.

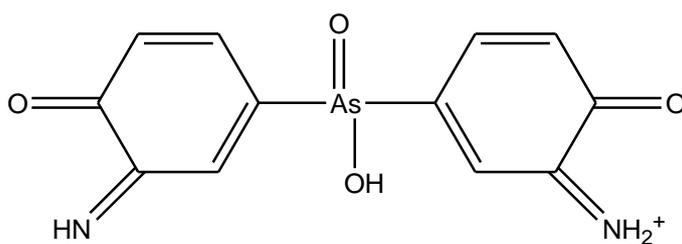


Figure 4-5- Proposed structure for m/z 320.98

Dimeric species are also seen. A possible structure for the peak at m/z 380.920 is shown in Figure 4-6

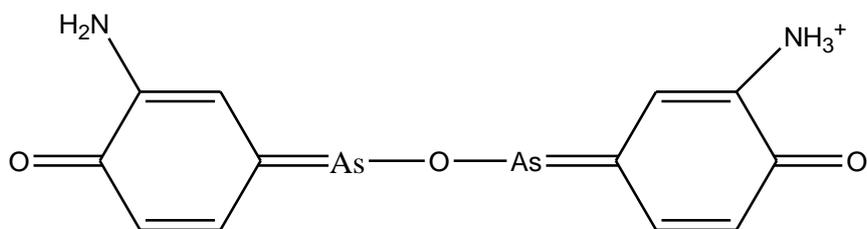


Figure 4-6 – Proposed structure for m/z 380.920

Most of the remaining peaks are hard to generate sensible formulae for and are only tentatively assigned – there is also the possibility of the arsenic end of the molecule reacting with the phenolic OH on the R group to generate more complex species.

The final peak at m/z 597.890 seems to correlate well to the $R_3As_3O_3H^+$ peak which is the arsoxane structure shown in Figure 4-7. There are precedents for these cyclic species¹⁵⁵ although their formation appears to be dependent on the pH of the solution.

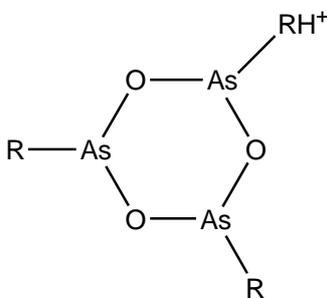


Figure 4-7- Arsoxane structure proposed for m/z 597.890

4.6 Mass spectrometry of $NO_2(HO)C_6H_3As(OH)_2$

This compound was analysed on the MicrOTOF and gave a complex spectrum consisting of cyclic species and what appear to be linear polymers.

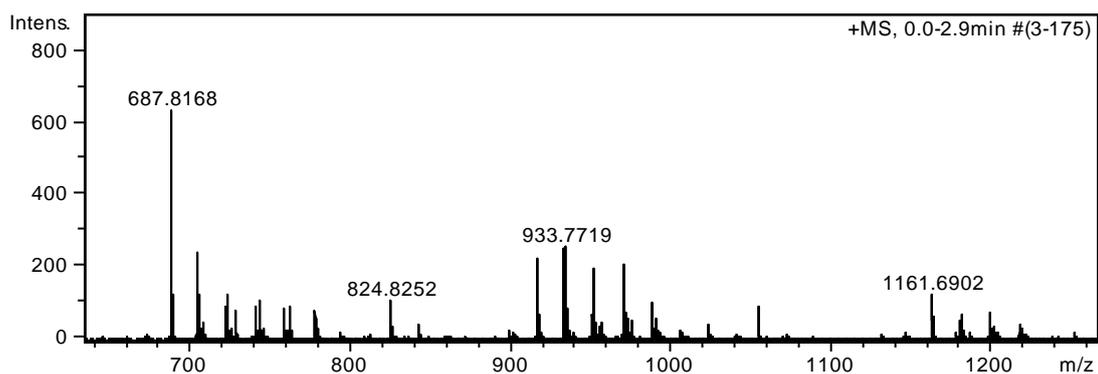


Figure 4-8 - Mass spectrum of $\text{NO}_2\text{HOC}_6\text{H}_3\text{As}(\text{OH})_2$

The species $[(\text{NO}_2\text{HOC}_6\text{H}_3\text{AsO})_3+\text{H}]^+$ is the major peak at m/z 687.817 (calculated – 687.814) and this is followed by a series of smaller peaks, each representing addition of O or OH.

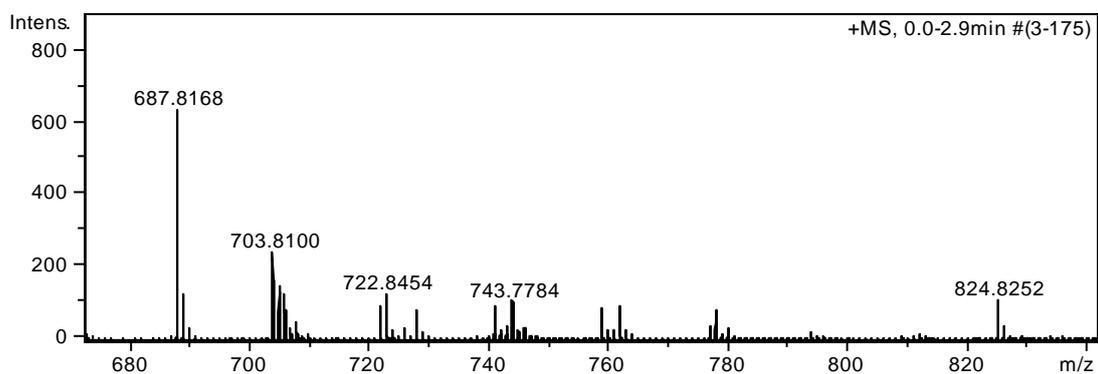


Figure 4-9 - Blowup of peaks

The peak at 824.825 is $[(\text{NO}_2\text{HOC}_6\text{H}_3)_4\text{As}_3\text{O}_3]^+$ (calculated – 824.826) and is a similar motif to what was seen for some of the rearrangement peaks in Salvarsan.

The species $[(\text{NO}_2\text{HOC}_6\text{H}_3\text{AsO})_4+\text{H}]^+$ is seen at 916.753 (calculated – 916.750). As is shown below in 4.7, this was the only species that crystallised, despite being a minor component in solution.

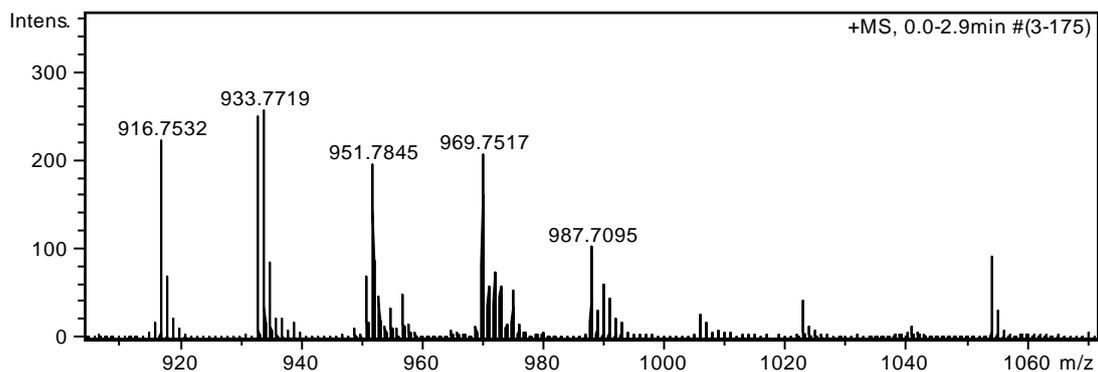


Figure 4-10 - Blowup of higher peaks

Further detail on the other species was hard to obtain as the peaks which appear to represent linear species get more complex as the addition of OH groups increases, making it difficult to get accurate masses due to overlap.

4.7 Crystal structure of *cyclo-Tetra- μ -oxido-tetrakis[3-nitro-4-hydroxyphenylarsenic(III)]* ($\text{NO}_2(\text{HO})\text{C}_6\text{H}_3\text{AsO}$)₄

The crystals analysed here were grown by chance by the method given in 4.3.3.

X-ray intensity data for this compound were obtained on a Bruker SMART CCD diffractometer, and were processed using standard software. Crystal data and refinement details are summarized in Table 4.3. Corrections for absorption were carried out using SADABS. The structures were solved and refined using the SHELX programs, operating under WinGX. All H atoms were positioned geometrically and refined using a riding model with C-H = 0.95 Å, $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C})$ for aromatic and O-H = 0.84 Å, $U_{\text{iso}}(\text{H}) = 1.5U_{\text{eq}}(\text{O})$ for the OH groups. As only very small needle crystals were available the data set was weak and so R_{int} and the final agreement factors are higher than usual. The highest residual electron density was 0.87 Å from atom As₁.

Table 4.3 - Crystal data and refinement details for cyclo-Tetra- μ -oxido-tetrakis[3-nitro-4-hydroxyphenylarsenic(III)]

Formula	C ₂₄ H ₁₆ As ₄ N ₄ O ₁₆
M _r	916.09
T(K)	93(2)
crystal system	Monoclinic
space group	P2 ₁ /c
a (Å)	7.1289(2)
b(Å)	31.6743(9)
c(Å)	13.0217(4)
α (deg)	90
β (deg)	98.286(1)
γ (deg)	90
V(Å ³)	2909.64(15)
Z	4
ρ (g cm ⁻³)	2.091
μ (mm ⁻¹)	4.642
Size (mm ³)	0.16 x 0.10 x 0.05
F(000)	1792
θ_{\max} (deg)	25.7
Reflns collected	16032
Tmax, min	0.801, 0.524
Unique reflns	5490 [R(int) = 0.1135]
Parameters	433
R ₁ [I > 2 σ (I)]	0.0683
wR ₂ (all data)	0.1344
GOF on F ²	1.072

Aryl arsenoxides of empirical formula RAsO exist as either hydrates RAs(OH)₂ in the case of *p*-NH₂C₆H₄As(OH)₂ or as cyclic (RAsO)_n compounds, where n = 4 or 5.

There are four published crystal structures of these compounds and selected data from these structures is presented in Table 4.4

Table 4.4 - Selected data from known (RAsO)_n compounds

R group.	CCDC Number/ Space group	As-O (Å)	As-C (Å)	As-O-As (°)	O-As-O (°)	Conformation.
2,4,6-(CH ₃) ₃ C ₆ H ₂ (RAsO) ₄	FAWNOG P2 ₁ /c	1.811 1.794 1.794 1.793 1.790 1.789 1.781 1.767	1.989 1.978 1.973 1.942	118.56 118.23 117.73 114.28	100.55 99.39 98.11 97.67	Twisted boat
C ₆ H ₅ (RAsO) ₄	HAVMEW C2/c	1.824 1.819 1.813 1.802 1.800 1.786 1.784 1.755	1.972 1.934 1.931 1.917	126.67 118.96 118.03 116.32	100.41 99.04 98.67 98.58	Chair-boat
CH ₃ (RAsO) ₄	VOLPUH P2 ₁ /n	1.820 1.803 1.793 1.793 1.785 1.782 1.784 1.779	1.933 1.930 1.929 1.905	124.04 119.90 118.80 118.75	100.19 98.20 98.16 97.52	Chair boat
3-F ₃ C (RAsO) ₄	WALZEP I4 ₁ /a	1.805 1.797	1.965	124.08	99.37	Twisted boat
3-NO ₂ -4- OHC ₆ H ₃ (RAsO) ₄	This work P2 ₁ /c XILKEJ	1.816 1.810 1.808 1.800 1.799 1.799 1.790 1.789	1.966 1.962 1.946 1.933	123.78 123.08 119.34 118.84	101.04 99.60 98.80 95.83	Chair boat.
Et (5) (RAsO) ₅	TURGES	1.821 1.817 1.814 1.813 1.808 1.805 1.796 1.791 1.784 1.783	1.956 1.947 1.944 1.934 1.933	122.77 119.67 119.62 119.18 118.74	98.69 96.32 96.17 95.83 95.20	Crown

The $(\text{NO}_2(\text{HO})\text{C}_6\text{H}_3\text{AsO})_4$ compound is very similar to the other compounds, The structure is illustrated in Figures 4-11 and 4-12 and consists of an eight membered ring of alternating As-O bonds giving a chair-boat conformation. Of the known $(\text{RAsO})_4$ species two other have the same conformation with the other two as twisted boat, indicating flexibility in the ring.

The OH group is internally H-bonded to the adjacent NO_2 groups so the intermolecular interactions are weak C-H---O and As---O ones. There are some short intermolecular O---O interactions involving the NO_2 groups.

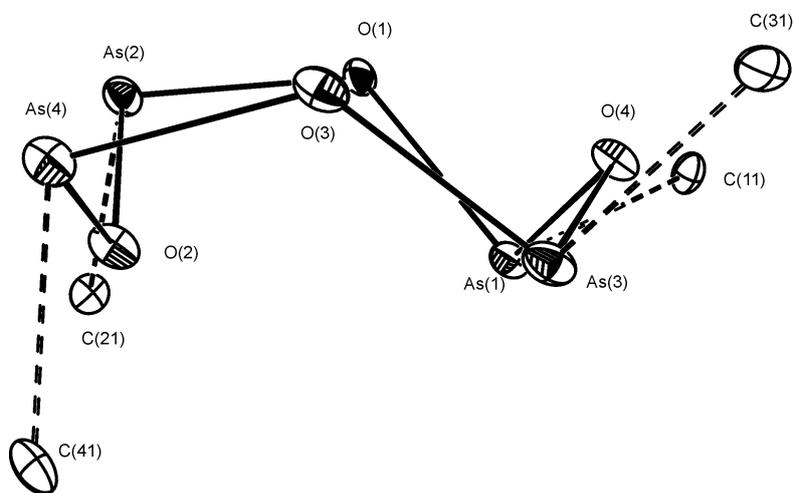


Figure 4-11 - View of the chair-boat conformation

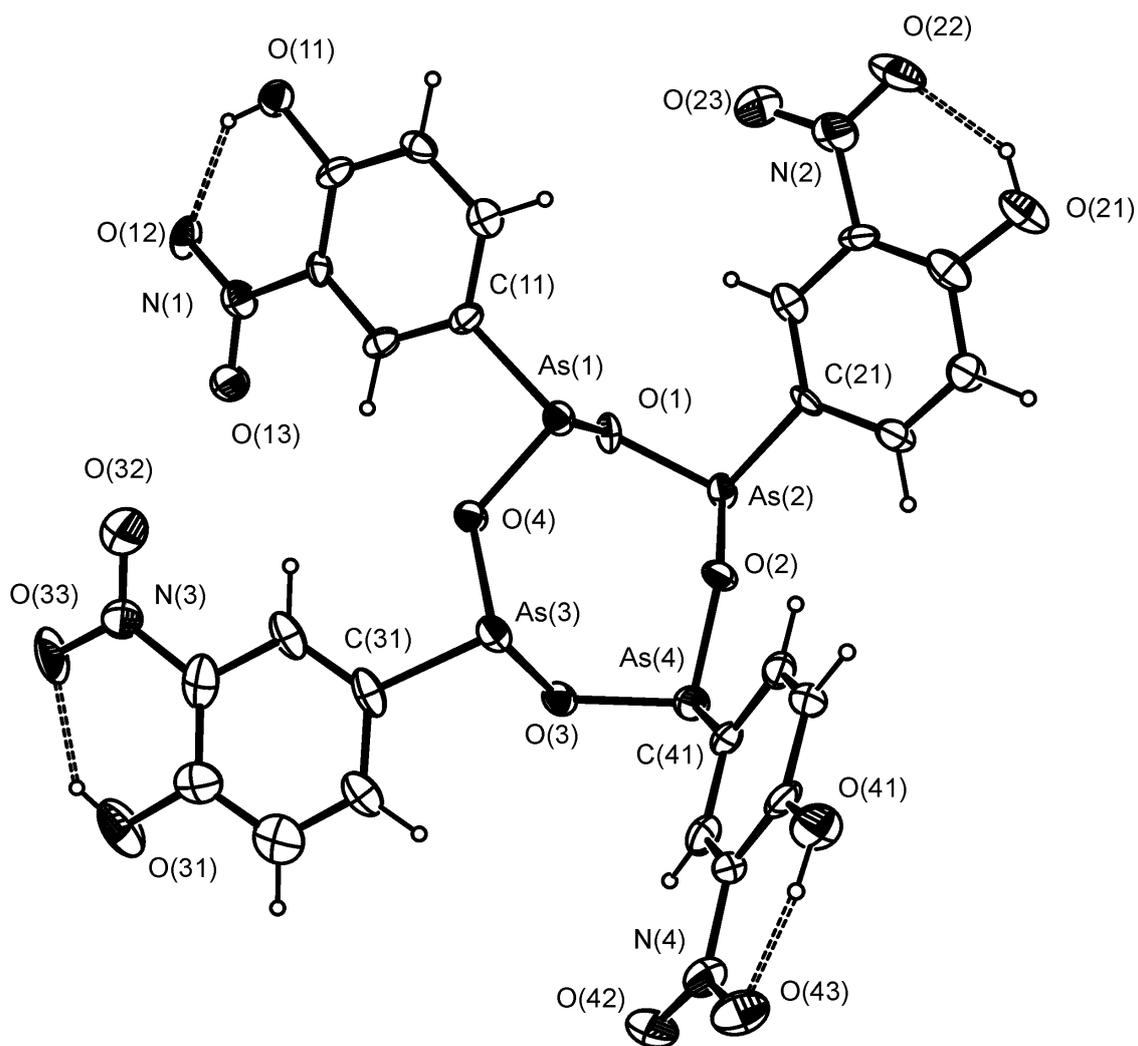


Figure 4-12 - Crystal structure of $(NO_2(HO)C_6H_3AsO)_4$

4.8 Preliminary protein binding studies

Although it is now widely accepted that the As(III) compound (Mapharsen) is the actual biologically active species derived from Salvarsan, there is still no understanding of how this was able to control the syphilis spirochete. During the course of this study it was proposed that thioredoxin (or thioredoxin reductase) could be the target. This was based on the known affinity that RAs(III) moieties have for attaching to S atoms (e.g. as in BAL, British Anti-Lewisite¹⁵⁶) and the knowledge that thioredoxin contains two S atoms from cysteine residues that are vital for the redox activity of the protein.¹⁵⁷

Thioredoxins are a class of small proteins which are found in all organisms and serve vital roles in many biological processes by facilitating the reduction of other proteins.

These compounds are known to be the hydrogen donor for ribonucleotide reductase, an essential enzyme for DNA replication. There are many different variations with mammalian thioredoxins showing differences from its bacterial counterparts.¹⁵⁷

The best known thioredoxin is from *E. coli*.

All thioredoxins contain an active site with a redox active disulfide and function in electron transfer via the reversible oxidation of two vicinal protein-SH groups to a disulfide bridge.¹⁵⁷

From model compounds, e.g. the phenylarsenic(III) adduct of dithiooctanoic acid (shown in Figure 4-13),¹⁵⁸ it can be seen that the ideal chelating agent for an RAs fragment would have two S atoms at approximately 3.4 Å apart, allowing As-S distances of 2.23 Å subtending an angle of around 99°. ¹⁵⁸

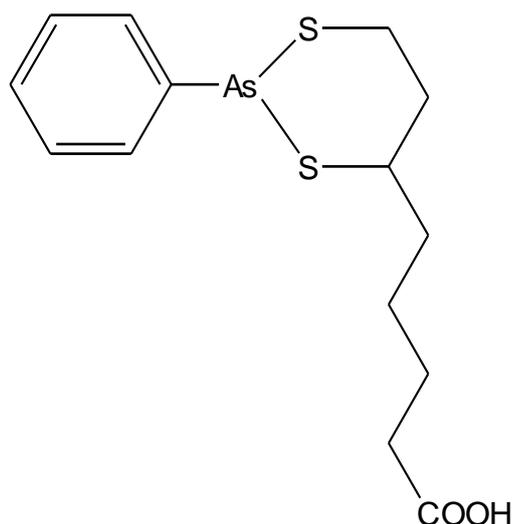


Figure 4-13 - The structure of the PhAs adduct of 1,3-dithiooctanoic acid¹⁵⁷

The structures of both the oxidised and reduced forms of thioredoxin are well established¹⁵⁹ and the reduced form has S...S distances of between 3.1 Å (human thioredoxin^{159b}) and 3.8 Å (E.Coli thioredoxin^{159b}) so appears to be an ideal target for attaching the Mapharsen through a reaction of the type shown in Figure 4-14

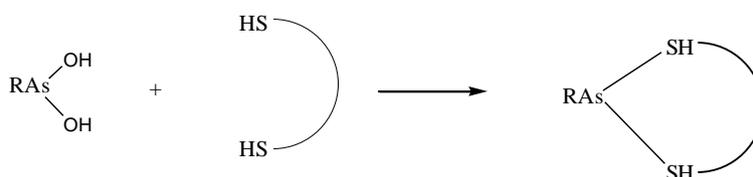


Figure 4-14 – Proposed sulfur bonding

4.8.1 Experimental

The MicroTOF was set for optimized for proteins and the sample was introduced via a syringe pump running at 180 $\mu\text{L min}^{-1}$.

All the spectra were deconvoluted using the charge state ruler with the standard Bruker software or automatically. When automatic deconvolution was used many minor peaks became apparent.

To assess the formation of such an adduct, ESI-MS studies on thioredoxin in the absence and presence of Mapharsen were undertaken.

The acquisition of a Bruker MicrOTOF allowed for this work to be done as it allowed for proteins to be examined with a high degree of accuracy.

Thioredoxin was used as obtained from Sigma (E. coli, recombinant, expressed in E. coli)

A solution of 0.1 mg mL^{-1} Parke-Davis Mapharsen was made up in cold distilled water.

This was frozen until it was needed and no discoloration was observed.

A solution of 0.1 mg mL^{-1} thioredoxin was made up in the same way.

Just prior to running the spectra a drop of 0.2% formic acid was added to provide the necessary H^+ ions to ionize the compound well in the spectrometer.

A spectrum of thioredoxin was recorded as a control.

1 mL of the Mapharsen solution was added to 1 mL of the thioredoxin solution and the mixture shaken well to mix. A spectrum was then recorded.

The mixture was left to sit at room temperature with a new spectrum being recorded every hour.

After 1 hour, the spectra had changed significantly and after two hours the reaction was complete and no further changes were observed.

Ideally this experiment should have been carried out at a biological pH in a buffer solution but buffer solutions are rarely MS friendly, and a slightly acidic solution was necessary to generate strong $[\text{M}+\text{nH}]^{\text{n}+}$ signals from the thioredoxin.

The mass spectrum of thioredoxin before deconvolution is shown in Figure 4-15.

Deconvolution is the process by which the computer “unpacks” the raw spectrum with multiple charges to get a single peak. Figure 4-17 shows the results after deconvolution, giving a peak which shows the actual molecular weight of thioredoxin.

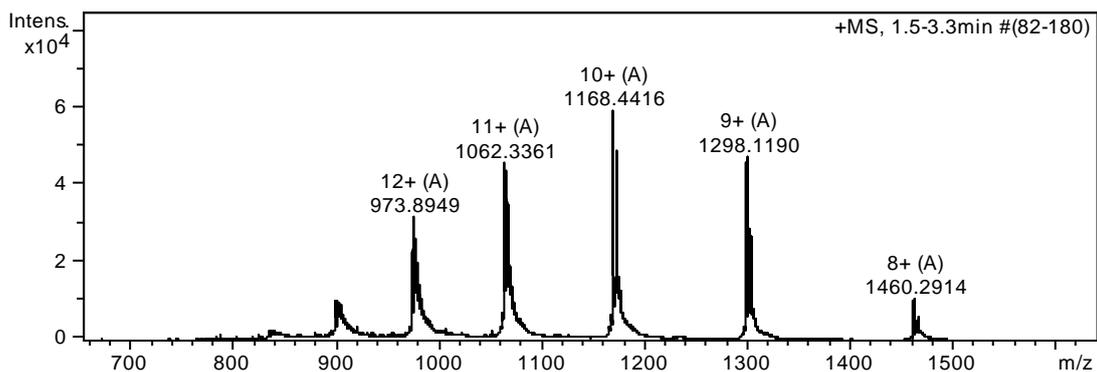


Figure 4-15 - The spectrum of thioredoxin before deconvolution.

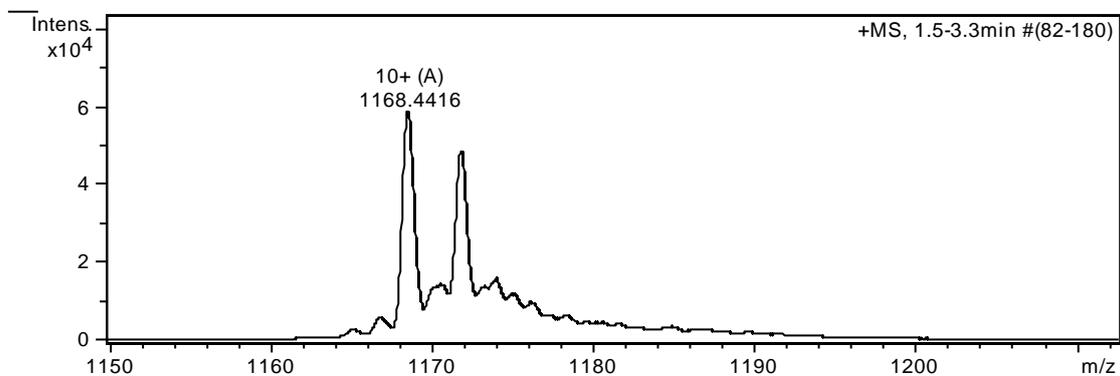


Figure 4-16 - Blow up of the 1168 (10+) peak.

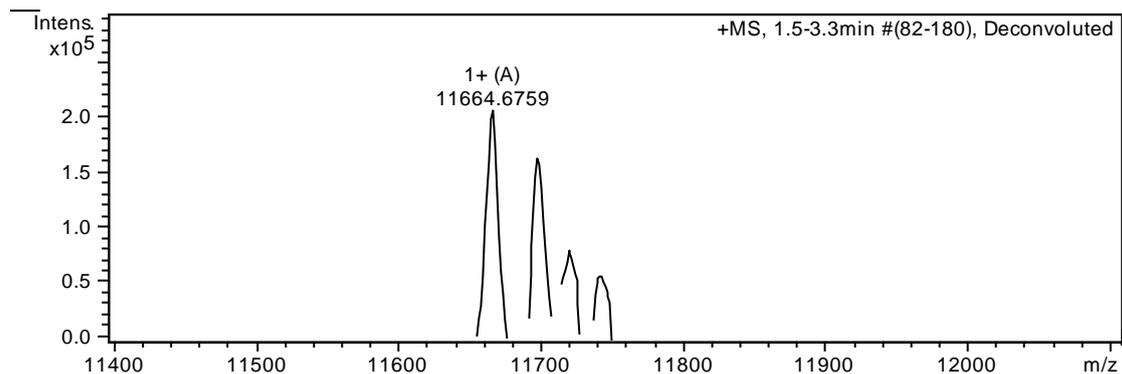


Figure 4-17 - Deconvoluted spectrum of thioredoxin showing major peaks.

E. coli thioredoxin has a molecular mass of 11675^{159a} and this is seen clearly in the deconvoluted mass spectrum. The other peaks are related compounds and were ignored for the purposes of this experiment.

After one hour, significant binding of the 3-amino-4-hydroxyphenylarsenic species had occurred and a new peak was observed at m/z 1186.7 in the spectrum before deconvolution.

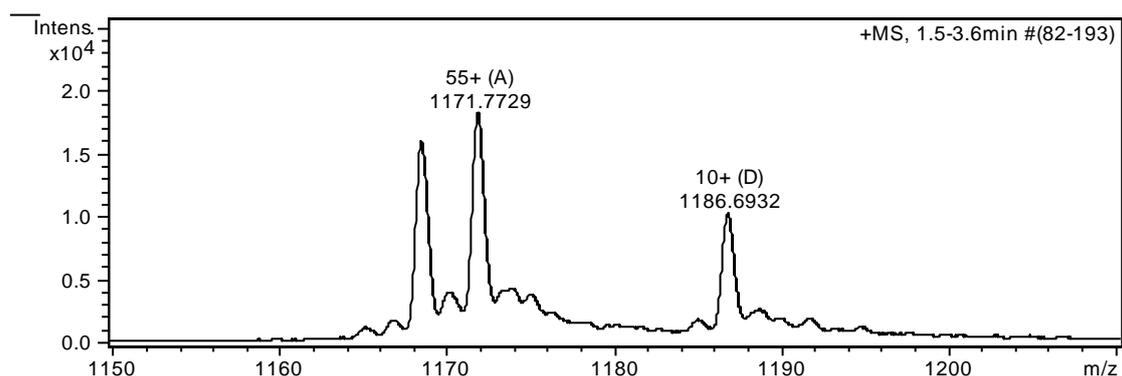


Figure 4-18 - Blow up of the 1168 (10+) peak after addition of Mapharsen (*T* = 1 hour)

Upon deconvolution of this spectrum the new peak (D) was found at m/z 11857.5. This corresponds to addition of the $\text{NH}_2(\text{OH})\text{C}_6\text{H}_3\text{As}$ ($\text{Mr} = 182.97$) unit onto the thioredoxin. This is added confirmation that the observed peak was not simply a H-bonded aggregate of the $\text{NH}_2(\text{OH})\text{C}_6\text{H}_3\text{As}(\text{OH})_2$ ($\text{Mr} = 216.97$) species onto the structure of the protein. Rather bonding with As-S covalent bonds has occurred. The peak representing thioredoxin can still be seen indicating that not all of the thioredoxin has reacted with the arsenic species, which was in excess.

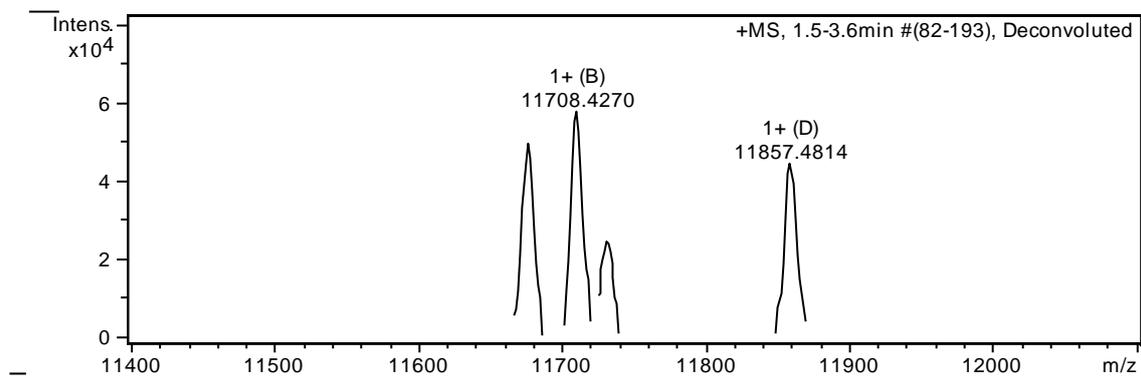


Figure 4-19 - Deconvoluted spectrum of Thioredoxin after addition of Mapharsen ($T = 1$ hour)

After two hours the thioredoxin + arsenic species had increased slightly.

The peak at m/z 1171.8 remains unchanged, suggesting that whatever this species was it wasn't interacting with the arsenous acid in any way.

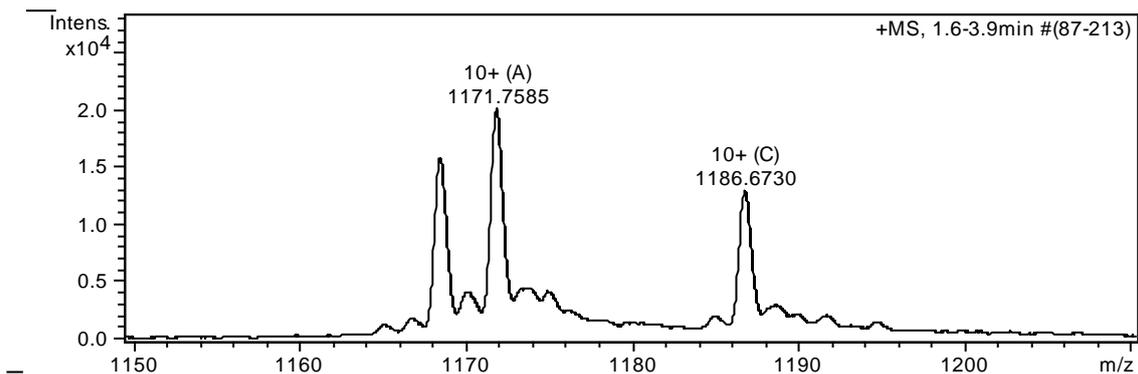


Figure 4-20 - Blow up of the 1168 (10+) peak after addition of Mapharsen ($T = 2$ hours)

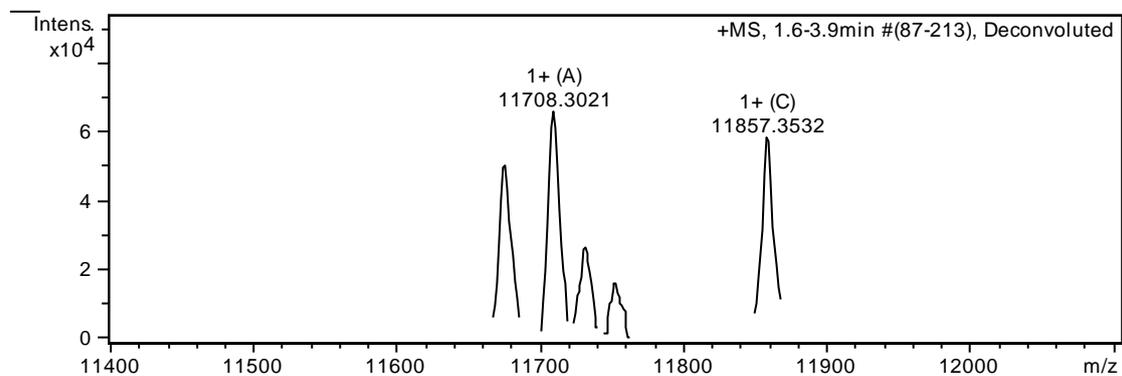


Figure 4-21 - Deconvoluted spectrum of Thioredoxin after addition of Mapharsen ($T = 2$ hours)

The *p*-aminophenylarsonous acid behaved in much the same way as the Mapharsen had with a new peak appearing at m/z 1185.08 in the spectrum before deconvolution.

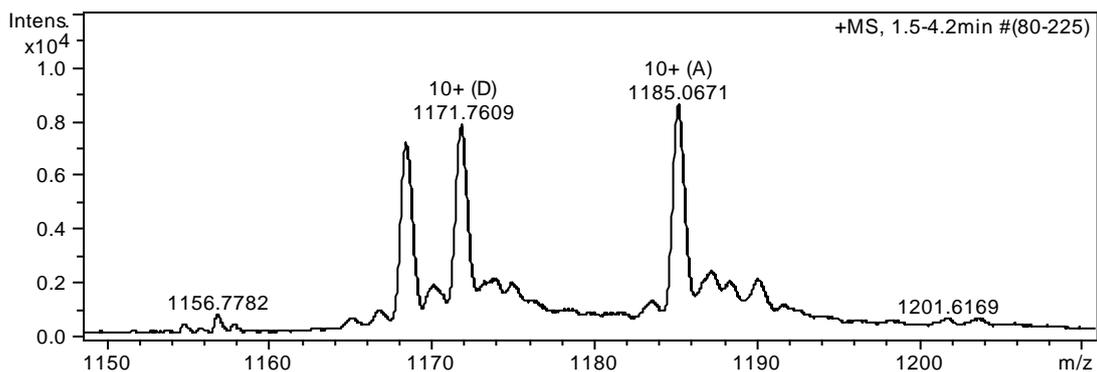


Figure 4-22 - Blow up of the 1168 (10+) peak after addition of *p*-aminophenylarsonous acid (*T* = 2 hours)

After deconvolution the new peak corresponds to a species with m/z 11841.3 which corresponds to the addition of $\text{NH}_2\text{C}_6\text{H}_4\text{As}$ ($\text{Mr} = 167.0$) to the thioredoxin peak.

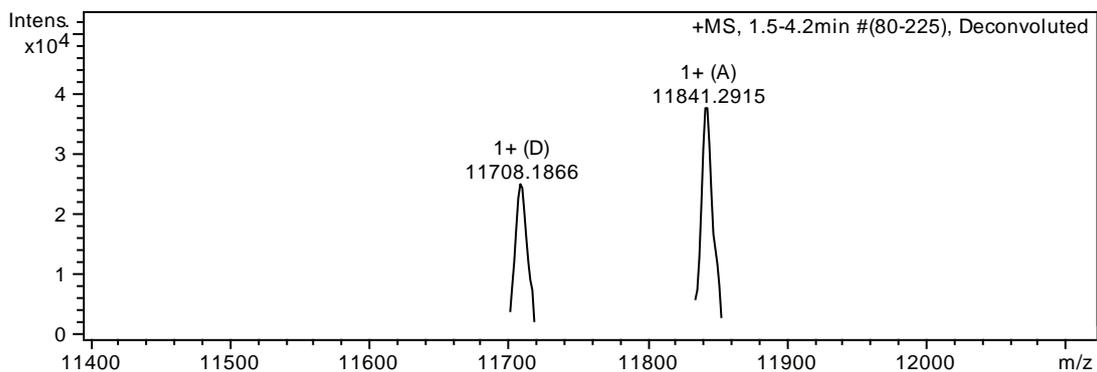


Figure 4-23 - Deconvoluted spectrum of Thioredoxin after addition of *p*-aminophenylarsonous acid (*T* = 2 hours)

It is interesting to note that in these spectra there remains a remnant of thioredoxin, even if the deconvolution software didn't pick it up (as in Figure 4-16 – the peak from unreacted thioredoxin at m/z 1168 can still clearly be seen in Figure 4-22 to the left of the m/z 1171 peak)

This unreacted thioredoxin is possibly the oxidized form and therefore unable to react with the arsenic species as the oxidized form would not have free S-H groups to react with the arsenic species.

In a more detailed study published during the course of this project others have used ESI-MS to study the interactions of $\text{PhAs}(\text{OH})_2$ with sulfur-containing biomolecules such as glutathione, isotocin as well as thioredoxin and have obtained semi-quantitative binding constants (K values of $4\text{-}12 \times 10^5$ for thioredoxin, though the analysis is not straightforward).¹⁶⁰

Similar mass spectrometric studies have also shown that Pt(II) binds strongly to the chelating S atoms from the Cys-Ala-Pro-Cys fragment of reduced thioredoxin, a reaction that is relevant to the resistance of cancer cells to Cisplatin since thioredoxin is over-expressed in some cancer cells.¹⁶¹

While all these studies clearly establish strong binding of $\text{RAs}(\text{OH})_2$ species to thioredoxins, further studies would be needed to establish that this is the target for the activity of Mapharsen against the spirochete.

5 *Appendices*

5.1 Appendix One – Note on the nomenclature of organic arsenic compounds in this thesis.

Over the course of history the arsenic compounds discussed in this thesis have received many different names – confusion has arisen between arsenic, arsonic, arsonous and arsinic acids and between the various commercial and trivial names the compounds have been given.

One of the earliest mentions of this confusion is a letter in *Industrial and Engineering Chemistry* by W.A. Silvester¹⁶² who draws attention to the different terminology being used in different countries for what are now known as arsonic acids.

The British system historically used the name arsinic acids – for example RAsO_3H_2 was known as an alkyl or aryl arsinic acid and the $\text{R}_2\text{AsO}_2\text{H}$ compounds were termed dialkyl (or aryl) arsinic acids.

In a reply to this letter, Raiziss points out that this system makes no distinction between primary and secondary compounds and suggests the American system whereby RAsO_3H_2 was known as an alkyl or aryl arsonic acid and the $\text{R}_2\text{AsO}_2\text{H}$ compounds were termed Dialkyl (or aryl) arsinic acids.

Doak further clarifies the nomenclature in his monograph - $\text{RAsO}(\text{OH})_2$ are defined as aryl or alkyl arsonic acids, compounds of the form $\text{R}_2\text{AsO}(\text{OH})$ are defined as aryl or alkyl arsinic acids

Compounds of the form $\text{RAs}(\text{OH})_2$ are defined as arsonous acids. This terminology is supported by IUPAC today.¹⁶³

Compounds of the form $RA_s=O$ have been historically called arsenic oxides but there exists little evidence that these compounds exist and they are more likely to exist as compounds of the type $(RA_sO)_n$ which have historically been called arsenoso compounds. IUPAC recommends these compounds be called arsoxane compounds and this nomenclature is now widely accepted.

IUPAC recommendations suggest that arsines should be correctly termed arsanes (cf. methane) which means that cyclopolyarsines are correctly termed cyclopolyarsanes.

This recommendation has not received much use in recent publications and the traditional use of “arsine” seems to remain in common usage and is retained in this thesis.

The nomenclature used in this thesis is as follows:

$RA_sO(OH)_2$ – arsonic acid – $C_6H_5AsO(OH)_2$ – phenylarsonic acid

$RA_s(OH)_2$ – arsonous acid – $C_6H_5As(OH)_2$ – phenylarsonous acid

$(RA_s)_n$ – cyclopolyarsane – $(C_6H_5As)_5$ – cyclopentaphenylarsine

$(RA_sO)_n$ – cyclopolyarsoxane – $(C_6H_5AsO)_2$ – cyclodiphenylarsoxane

5.2 Appendix Two – A brief introduction to Electrospray Mass Spectrometry

One of the major analytical techniques employed in this work has been Electrospray Mass Spectrometry (ESI-MS).

Earlier studies¹¹⁰ examined some cyclopolyarsines using electron impact mass spectroscopy and although they may have seen some cyclic species, these were thought to be fragments due to the harshness of this technique.

ESI MS has the advantage of being a very soft ionisation technique which samples directly from a solution of the compound under investigation, because of the “softness” of the technique entire molecules can be observed.

ESI MS works by having a solution of the compound fed into the mass spectrometer at a fixed rate, via a pump (or via a sampling loop). The type of pump varies according to the different machines used. Modern machines tend to use syringe pumps. The sample is fed into a fine capillary and squirted into the sample chamber into a jet of inert gas. This causes the stream of analyte to disperse into a very fine spray. The temperature at this stage can vary with the solvent employed but is usually somewhere around the boiling point of the solvent. The fine spray of analyte is therefore constantly losing solvent and as these micro-droplets contain ions they continue to disperse as the overall charge in the droplets becomes too great, eventually giving rise to single ions which are what is being detected by the machine. The spray is generally drawn by a vacuum into a heated capillary tube which feeds into the detector of the machine. For more information see Henderson and McIndoe.¹⁶⁴

5.3 References

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5.4 Appendix Four – Papers published from this thesis.

Four papers have been published from the work carried out in this thesis. They are:

The composition of Ehrlich's Salvarsan; resolution of a century-old debate, N. C. Lloyd, H. W. Morgan, B. K. Nicholson, and R. S. Ronimus, *Angew. Chem., Int. Ed.*, **44** (2005) 941-944. (*Highlighted in Chem. Eng. News*, 83 (25) (2005) p116).

Salvarsan – the first chemotherapeutic compound, N. C. Lloyd, H. W. Morgan, B. K. Nicholson, R. S. Ronimus and S. Riethmiller, *Chemistry in New Zealand*, **69** (2005) 24-27. (Reprinted in *ChemNZ*, No. 99, May 2005).

cyclo-Tetra- μ -oxido-tetrakis[3-nitro-4-hydroxyphenylarsenic(III)], N. C. Lloyd and B. K. Nicholson, *Acta Cryst.*, **E63** (2007) m2573.

Substituted phenyl arsonic acids; structures and spectroscopy, N. C. Lloyd, H. W. Morgan, B. K. Nicholson, and R. S. Ronimus, *J. Organometal. Chem.*, **693** (2008) 2443-2450.

