SALVARSAN – THE FIRST CHEMOTHERAPEUTIC COMPOUND

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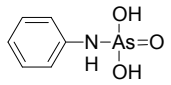
Early History

If defined as the use of a specific compound discovered as the result of a systematic search for a cure for a specific disease, then chemotherapy is less than a century old. The first example was the introduction in 1910 by Paul Ehrlich[1,2] of an organoarsenic compound he named *Salvarsan*, which provided the first real cure for the extremely unpleasant disease syphilis (caused by the parasitic spirochete *Treponema pallidum*). As is well-known, the compound was also called Ehrlich 606 because it was the 606th compound tested by Ehrlich in conjunction with his colleagues Bertheim (synthesis) and Hata (biological testing) (Figs. 1 & 2).[3,4]

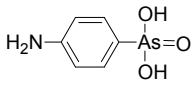
Salvarsan's origins can be traced to 1863, when Pierre Bechamp (famous for his discovery of the Bechamp process for the cheap production of aniline) isolated a compound from a reaction between arsenic acid and aniline. He characterised this compound as **1**, the anilide of arsenic acid. It was shown later that Bechamp's compound was less toxic that inorganic arsenic compounds and so it was given the informal name of *Atoxyl*.[3,4] In 1905, Thomas published a paper[5] which showed that Bechamp's compound was effective in the treatment of sleeping sickness, the greatest cause of death in Africa at the time. This paper caught the attention of Paul Ehrlich.

Ehrlich was born in 1854 in what is now Strzelin, Poland. He started his scientific career as a medical student, and became interested in the new dyes which were becoming available at the time. He started working in the field of immunology, convinced that *the body's immune system could be fortified by chemical means*. His interest in dyes – specifically their ability to selectively stain microbes – is what sparked his interest in chemotherapy. In simple terms, the idea was that if a dye could selectively target a micro-organism, and the functional groups

responsible could be included in a molecule that was also toxic to the micro-organism then you would have a specific chemotherapeutic agent.[6]

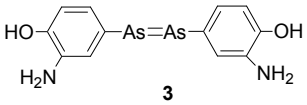


1 - Bechamp's Atoxyl



2 - Ehrlich's Atoxyl

When Alfred Bertheim arrived in Ehrlich's laboratory he worked on Bechamp's Atoxyl **1**, which was still thought to be an anilide of arsenic acid. It was quickly realised that the structure was incorrect and structure **2** was adopted, but not without controversy.[3] Ehrlich's lab then embarked on a systematic study of different compounds, starting from Atoxyl in order to find compounds with improved activity specifically against syphilis. The best candidate was *Salvarsan* for which Ehrlich assigned structure **3** (Fig. 3).[1]

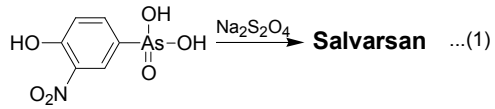


3

The introduction of Salvarsan was a major event, and the compound rapidly became the most widely prescribed drug in the world (necessitating emergency synthesis programmes in England, the US, and Japan after 1914 when the supply of German Salvarsan from the Hoechst plant was interrupted by the war). By 1920 Salvarsan had been shown to be effective against other parasitic disease[6] and 2 million doses a year were produced in the US alone.[7] Demand for Salvarsan remained very strong until 1943 when penicillin became available – an equally effective and more pleasant remedy.

Preparation of Salavarsan

Despite its wide use, Salavarsan has remained an enigmatic material. Ehrlich's synthesis involved the reduction of the substituted phenylarsonic acid with dithionite (Eq. 1)[1] in a reaction that was not straightforward; the products had variable toxicity such that up to 50% of batches from Ehrlich's operation were reported to be rejected.[8] This has been attributed to the presence of unidentified sulfur-containing impurities that were derived from the dithionite.[9] An alternative, two-step synthesis was more reliable (Eq. 2).[10]



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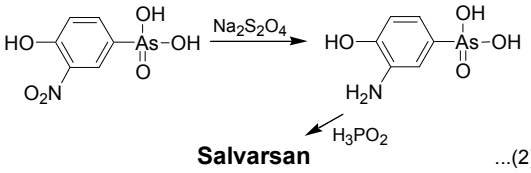


...(2)

Prepared in this way, Salvarsan is a pale-yellow powder. The neutral form is very easily oxidized so it was isolated as the HCl salt, which can be handled for short periods in air without deterioration. Administration of the drug by the physician was not straightforward – the typical average dose of 0.4 g of the salt was dissolved in water, neutralized by adding the exact calculated amount of aqueous NaOH, filtered to remove any undissolved material, and then made up to *ca.* 300 mL with saline solution. This solution was warmed to physiological temperature and injected. Ans all this with minimum exposure to air to avoid oxidation! Needless to say, there were many cases of medical misadventure, when the process was not followed exactly.

What is Salvarsan?

It is intriguing that despite its long history, and commercial and medical importance, the chemical constitution of Salvarsan is still undecided (try introducing a new pharmaceutical today without knowing exactly what it consists of!). The empirical formula of the material was well-established by Ehrlich's team as the arsenic(I) species, $RAs.HCl.H_2O$ (R = 3-amino-4-hydroxyphenyl). While this is not in question, Ehrlich had assigned the structure **3**, with an As=As double bond, to the base (Fig. 3). This seemed reasonable at the time, since corresponding congeneric azo compounds RN=NR were well established. However, as inorganic chemistry developed it became apparent that this formulation was untenable[11] since As=As double bonds (unlike those of nitrogen) are only stable in exotic molecules with extensive steric protection.[12] Various attempts at measuring physical properties have been made over the years, but full chemical characterization has been elusive. Textbooks and reviews still quote **3**, the original incorrect Ehrlich structure;[13] various other suggestions for polymeric or oligomeric structures have been proposed but with little supporting evidence.[14] The reasons why Salvarsan has proved so

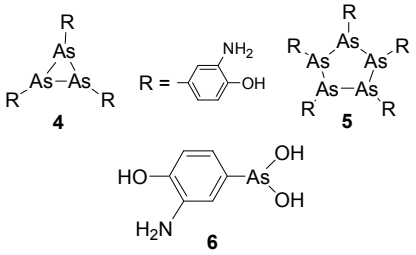
intransigent include: i) it is actually a mixture of species, ii) it can only be isolated as an amorphous powder that precludes X-ray crystallography, iii) as a free base it is oxidized very readily to As(III), iv) in aqueous solution it is reactive and appears to undergo strong intermolecular hydrogen-bonding interactions that give gels at particular concentrations/pH values, and v) it is non-volatile so traditional mass spectrometry does not provide useful information. It is, therefore, a particularly difficult compound to deal with.

Why the current interest?

Given its widespread use as a cure for syphilis, surprisingly little is known about the mode of action. Discovering this and site of action for a compound of uncertain composition is not easy. Since it is effective against a range of obligate pathogens, it must presumably affect a target present in the pathogen but absent (or reduced) in the human host. Advances in molecular biology and in our understanding of the structure of the active components of Salvarsan create new opportunities to detect the target and understand the mode of action. In 1997 the complete genome of the syphilis organism was sequenced[15] so that, in theory, every protein the pathogen synthesises can be identified and by molecular techniques it can be cloned and expressed in large quantities in a suitable benign host, *e.g. E. coli*. Doing this for every protein would be an enormous task but two approaches can make it simpler. Firstly, one can mine the sequence database to look for key enzymes in the genome of the pathogen that have a different structure from equivalent enzymes in the human genome – these become prime targets for selective toxicity. Secondly, because we can now produce large quantities of the active component of Salavarsan in pure form, one can manufacture affinity columns with the active component attached. By passing protein extracts from the pathogen through this column those that interact will be separated from proteins which have no affinity. The former proteins can then be eluted, their amino acid sequence determined, and the corresponding gene (and possibly enzyme activity) identified from the genome sequence. Finally, tests of inhibition of these enzymes by Salavarsan can be conducted following cloning and expression. Therefore, we decided to reinvestigate the chemistry of Salvarsan and related species to establish their true chemical identity and to see if the target enzymes and mode of action could be established.

Waikato studies

The initial goal was to establish a reliable synthesis of Salvarsan. This was no trivial exercise but conditions have been established whereby both the direct Ehrlich method and the modified one (Eq. 1 & 2) give a consistent material that analyzes correctly for $RAs.HCl.H_2O$. In studies detailed elsewhere,[16] we used electrospray mass spectrometry to examine our material. This relatively new technique has the ability to analyse species directly from solution without the need for intrinsic volatility, and the chemical ionization involved occurs under very mild conditions so that ions are transferred generally without fragmentation. This makes interpretation relatively straightforward, even for mixtures, since each peak can be related to a single parent molecule or ion.[17] For Salvarsan this showed the compound to be a complex mixture with the main constituents as small ring cyclopolyarsines $[RAs]_n$ (n = 3-6), *e.g.* **4** and **5**, together with small amounts of larger rings. Variable amounts of partially oxidized materials $[RAs]_nO_{1-2}$, some sulfur-containing impurities $[RAs]_nS$ as well as the fully oxidized arsenic(III) compound $RAs(OH)_2$, **6**, wee also present. Compound **6** is particularly important since it is generally assumed to be the active form, generated by *in situ* oxidation of Salvarsan, though this has still to be fully proved.



To provide a link to Ehrlich we were fortunate to be supplied with some original Salvarsan from the archives of the Georg Speyer Haus (the Frankfurt research institute established for Ehrlich by Frau Speyer) (Fig. 3). This was examined by electrospray mass spectrometry under the same conditions used for our own material; the resulting patterns were closely similar, showing that our syntheses had reproduced the original ones, and that Ehrlich's Salvarsan had survived essentially unchanged for 80 years or so.

With a reliable source of Salvarsan we can turn our attention to further characterization of it and its close variants, and carry out tests to see if its mode of biological activity can be determined. Despite interest in this area having a strong historical component, the information obtained may

lead to new disease treatments as organoarsenic drugs are still used for treating some Third World parasitic diseases,[18] and are reported to even cure Chronic Fatigue Syndrome in falcons and parrots![19] With developing resistance to antibiotics, Salvarsan itself may even one day return as a treatment for syphilis.

Acknowledgements

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References and Notes

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Figure 1. Ehrlich and Hata.

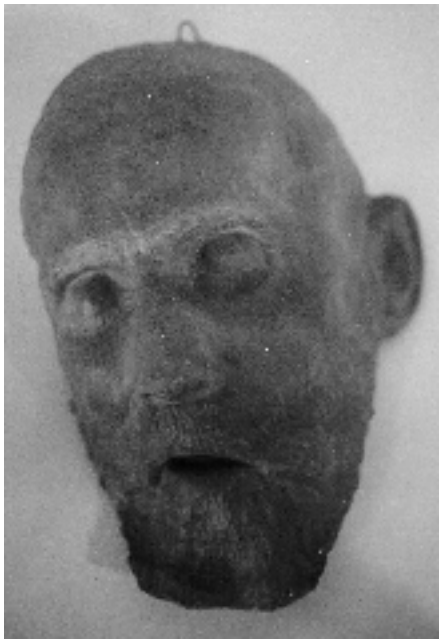

Figure 2. Ehrlich's death mask exhibited on the 150th anniversary of his birth at the World Conference on Dosing with Anti-infectives, Nurnberg, Sept. 2004.

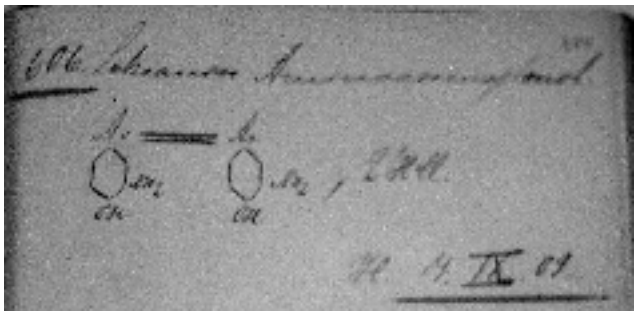

Figure 3. Entry in Ehrlich's book assigning **2** to "606";7 Sept. 1909.

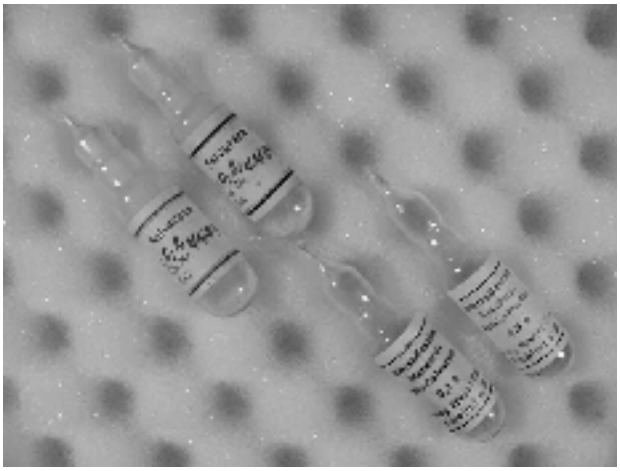

Figure 4. Ampoules containing Salvarsan and Neosalvarsan from Ehrlich's laboratory.