Dyes and Pigments 88 (2011) 231–234

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Review

# On the 75th anniversary of Prontosil

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ARTICLE INFO

*Article history:*
Received 21 June 2010
Accepted 23 August 2010
Available online 20 September 2010

*Keywords:*
Azo dyes
Antimicrobials
Domagk
Prontosil
Sulphonamides

ABSTRACT

While much of the credit for the beginning of the "antibiotic era" is given to Fleming, the first clinically available antibacterial agents were the sulphonamides, discovered as by-products of the azo dye, Prontosil. This was given its general release, i.e., outside Germany, in 1935 and rapidly became associated with "miracle" cures, particularly in skin diseases, pneumonia and childbed fever. While the discovery of sulphanilamide as the active agent in Prontosil led to the explosion in "sulpha" drugs other, related, agents such as Marfanil and the thiosemicarbazides were also developed by the Bayer chemists, and knowledge of the breakdown of the azoic bond specifically in the colon has also led to the introduction of drug delivery approaches to that organ.



## 1. Introduction

The "Antibiotic Era" is often held to have begun with the discovery of penicillin by Alexander Fleming. However, Fleming's original discovery of the anti-staphylococcal action of *Penicillium notatum* "mould juice" occurred in 1928 and the significant clinical introduction of penicillin was not a reality until 1944. In terms of useful antimicrobial drugs, the major contribution during this hiatus was provided by the sulphonamides. This class was derived from a family of azo dyes, the activity of which was first publicised in 1935.

In the early part of the 20th Century the nascent pharmaceutical industry – which itself did not begin to thrive until after the establishment of the sulphonamide class – was closely allied to producers of synthetic dyes, drug research laboratories often screening dyes as lead compounds. Such was the case at the German conglomerate, IG Farben, which had effectively continued the work of Paul Ehrlich in investigating dyes as antimicrobial agents – indeed, one of Ehrlich's students, Roehl, was employed by Bayer (part of IG Farben, along with AGFA, BASF and Hoechst) and developed some of the early antimalarials, work which eventually realised the globally important chloroquine [1].

Gerhard Domagk was not one of Ehrlich's students, but his experiences as a medic during the First World War provided him with enormous drive to fight infectious disease. Domagk began his search for anti-infectives, again working for Bayer, in the late 1920s.

Plainly, screening dyes for antimicrobial use requires a constant supply of test compounds. Fortunately for Domagk, as for Roehl, the research chemists tasked with the supply were highly talented. Werner Schulemann, Friedrich Mietzsch, Hans Mauss and Joseph Klarer were involved in much of Bayer's better drug discovery chemistry, Mietzsch and Klarer being principally involved in providing those azo dyes which culminated in Domagk's success.

In the 1930s, azobenzene derivatives (Aryl–N=N–Aryl) were well established as dyestuffs, mainly for the textile industry, which was still a thriving concern at that time. However, despite the belief that drug action must be related to dyeing facility, the target cells becoming the substrate, the colour of the compounds synthesised for Domagk's experimentation was not of major concern. However, because of the improved wash- and lightfastness of textile azo dyes containing it, the presence of the sulphonamido group ($-SO_2N<$) was identified as an important indicator of potential drug activity from an early stage. Indeed, Mietzsch had a commendably modern approach to his particular task of antibacterial drug discovery, which was effectively that of molecular design: "..the right substituents in the right positions on the azo group" [2].

For the various chemists at Bayer/IG Farben involved in the search for improved therapeutics based on dyes, the lead compound in antimalarial research was the phenothiazine dye, methylene blue. This was converted into a more effective agent by altering one of the dimethylamino auxochromes to one having basic character. This was achieved by connecting a second amino residue to the auxochromic group by way of an inert alkylene linker. The resulting aminoalkylamino side chain, and its later derivatives, was attached to several different dye classes, including

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doi:10.1016/j.dyepig.2010.08.012

phenoxazines, triarylmethanes and acridines, from which the widely used acridine, Atabrine (Mepacrine, Quinacrine), was produced [3].

Azo dyes (Fig. 1) had, in fact, already been suggested and trialled *in vitro* as antiseptic agents as early as 1913. Eisenberg reported excellent results for the simple dye chrysoidine [4], as did Ostromislensky for pyridium and serenium [5] The latter two were still in use as urinary antiseptics in the 1930s [6], and pyridium remains a commonly prescribed preparation [7]. However, such results were not observed in Bayer's animal testing [3].

Perhaps not surprisingly, then, the initial azo-compound given to Domagk for testing, KL695 (KL = Klarer), contained both the sulphonamide and aminoalkylamino residues (Fig. 2). The dye KL730, which became known as Prontosil, was a simple analogue of the azo dye chrysoidine [8], having the straightforward addition of a sulphonamide group (Fig. 2).

While it is difficult, in hindsight, to be impressed by the antibacterial effects of these two simple compounds, this remains one of the major scientific breakthroughs of the 20th Century. It should not be forgotten that Domagk and his associates were working in an era where there was, as yet, little knowledge of drug metabolism and it is probable that they had little regard for the reactivity of the azoic linkage in mammalian systems. However, the activity of these compounds, and others, led to the patenting of several series, starting at the end of 1931, with Mietzsch and Klarer given as inventors. It should also be noted that Domagk's award of the Nobel Prize for Medicine in 1939 (received in 1947) was made to him alone, and not shared with the drugs' inventors.

As mentioned, the discovery of the antibacterial activity of these azo dye sulphonamides was made around 1930–31, as the result of an industrial medicinal chemistry project. Given the commercial nature of the work, the data were held in-house pending further investigations. However, the results were unusual in that there was a lack of antibacterial activity *in vitro*, the dyes being successful in Domagk's model for human septicaemia, mouse peritonitis induced by a clinical isolate of *Streptococcus pyogenes*. The extravagance of candidate screening by duplicating *in vitro* tests in animals would be difficult to justify in the 21st Century, but it is fortunate that it occurred in this case. Domagk's first experiment, using twenty-six infected mice, resulted in the twelve which received Prontosil surviving, whereas the remaining controls died.

KL730, initially known at Bayer as *Streptozon*, underwent many months of animal testing. It was presented and discussed at scientific meetings within Germany, and several preliminary literature reports of its activity appeared. One example concerned the cure of a 10-month old baby boy, dying from staphylococcal septicaemia [9], and cures were also claimed for other typically staphylococcal or streptococcal diseases, such as erysipelas, scarlet fever and puerperal fever. Again, it is difficult, even in these days of hospital superbugs, to appreciate the impact of these findings. However, the fact that such diseases are relatively unknown today is due, in part, to the initial inroads made by the sulphonamides.

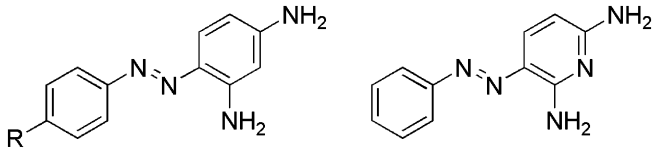


**Fig. 1.** Early, *in vitro*-active antiseptic azo dyes.

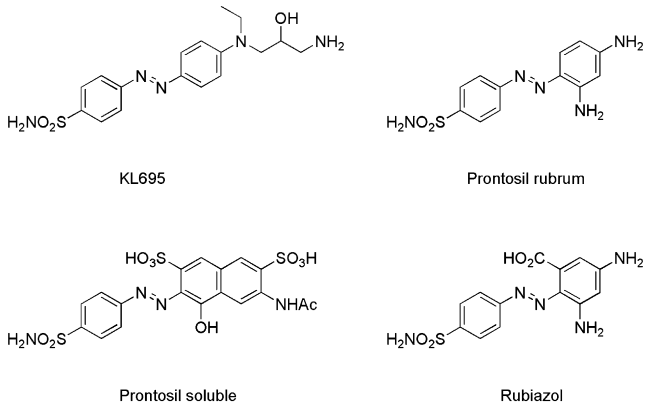


**Fig. 2.** Initial azo dyes having significant antibacterial action *in vivo*.

In the 1930s, despite considerable advances in diet and social conditions, puerperal, or childbed, fever was a significant threat, and one of the principal, and successful, initial trials of Prontosil (the name Streptozon had been dropped by 1935) against this disease was supervised by the English clinician Leonard Colebrook at Queen Charlotte's Maternity Hospital in London [10]. There were, of course, more famous incidences of cures – for example, Domagk's daughter Hildegard [11] and Franklin Delano Roosevelt Jr., one of the sons of the then US President, both from streptococcal septicaemia [12]. The proper, clinical introduction of Prontosil thus occurred in 1935, the same year as Domagk's landmark publication, understatedly entitled: A contribution to the chemotherapy of bacterial infections [13], and the presentation of the Prontosil work to the Royal Society of Medicine by Heinrich Horlein, Medical Director of IG Farben [3].

Prontosil (also known as *Prontosil rubrum* due to its dark red colour) was initially administered orally as a hydrochloride salt, and later as the free base as this was less staining. However, both preparations were relatively insoluble in water, and recourse was made to the library of prepared, patented compounds for a more soluble active derivative for use in injectable formulations. Prontosil soluble (Fig. 2, originally *Streptozon S*) was produced by the reaction of diazotised sulphanilamide with 2-acetamino-8-hydroxynaphthalene-3,6-disulphonic acid. This proved to be eminently suitable for use in aqueous media and allowed far more rapid administration of the drug in cases of serious disease, often in conjunction with oral Prontosil.

As noted, many azo derivatives were produced during the drug discovery phase by Mietzsch and Klarer – indeed Horlein claimed over one thousand derivatives by 1936 [3]. The molecular requirement for the relative 1,4-disubstitution pattern of the sulphonamide and azo moieties was established by this time, but it was the French team under Fourneau which made the quantum leap in suggesting that it was a dye metabolite common to all the successful derivatives which was, in fact, the active agent [14]. Sulphanilamide is formed via the reduction of the azoic bond in each case (Fig. 3).

In fact, the canine metabolism of an azo dye, Orange I, notably covering both the degradation of the –N=N– moiety and the identification of sulphanilic acid in the urine, had been reported as early as 1911 [15], while the presence of sulphanilamide in human urine following Prontosil administration was first described by Fuller in 1937 [16].

This metabolic activation of Prontosil and its congeners explained the lack of activity of the compounds during *in vitro* screening, and it was a logical step for the French team to test the precursor sulphanilamide itself for antibacterial efficacy. The resulting high activity of