Analysis of certain samples of phenazine dyes from the Historical Dyestuff Collection of the Technical University Dresden by liquid chromatography–mass spectrometry

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Abstract
By means of liquid chromatography–mass spectrometry and other analytical methods, a variety of different samples of the phenazine dye Chrysaniline hosted in the Historical Dyestuff Collection of the Technical University Dresden and registered mostly under the trade name Phosphine have been analysed. It was found that all the dyes analysed are mixtures of compounds with different numbers of methyl groups at their phenazine core. They result, hence, from the oxidation of technical aniline that contained several amounts of isomeric toluidines and traces of dimethylanilines.

Keywords
anilines, Chrysaniline, liquid chromatography, mass spectrometry, oxidation, phenazine dye, Phosphine

Date received: 23 April 2022; accepted: 8 June 2022

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**Introduction**

Aniline, as the parent compound of aromatic amines, was one of the first raw materials that was isolated from tar in rather low yield, but initially in sufficient amounts in the first third of the 19th century. Owing to its high reactivity, it found rapid interest as a starting material for the preparation of a large variety of products, especially for the preparation of organic dyes. For their synthesis, oxidation reactions using different oxidation reagents have been mainly used.

Depending on the reaction conditions applied and the quality of the starting materials used, different products were formed. For example, by oxidation of aniline ($\text{ANH}_2$) with potassium chlorate, Aniline Black was generated, as long as the starting aniline used is of rather pure quality (Scheme 1). During this reaction, an oxidative coupling between the amino group and its para-linked carbon atom occurs to yield primarily a $N$-phenyl-quinonediimine intermediate, which is able to react with further aniline. Thereby, an oligomer product is formed containing more than 24 aniline moieties at least.

As far as the aniline oxidation is performed with starting materials that contain different amounts of ortho- and para-toluidine and other oxidising reagents, such as potassium dichromate, some other products such as Mauveine, Parafuchsine and Chrysaniline were obtained. Their colouristic properties are demonstrated in Figure 1, in which pieces of wool were dyed with samples of these dyes (Figure 1).

However, the dyes used practically contain mostly different amounts of methyl-substituted derivatives, such as Fuchsine or Chrystoluidine. Therefore, subsequent to the synthesis of these dyes, more or less extended purification procedures were necessary for obtaining pure products. However, this time and cost-intensive procedure has lost its importance since improved synthetic methods for these dyes have been elaborated (Scheme 2). Thus, Fuchsine ($F$) and its methyl-free derivative Parafuchsine ($P$) can be prepared in rather pure quality by heating of 4,4'-diamino-diphenylmethane (1) with ortho-toluidine ($2a$, $R=\text{CH}_3$) or aniline ($2b$, $R=\text{H}$), respectively, in the presence of suitable oxidation agents, such as nitrobenzene or $\text{SnCl}_4$, and Chrysaniline ($C$) is prepared by the condensation of 3-amino-diphenylamine (3) with 4-aminobenzaldehyde (4) and subsequent oxidation of the dihydroacridine intermediate.

The classical synthetic procedure for preparation of these Fuchsine dyes was designed as ‘Fuchsine smelt’ (Fuchsinschmelze) and has been performed using arsenic acid as oxidation agent. Thereby, Chrysaniline ($C$) was obtained as by-product that could be separated generally as less-soluble nitrate. In this form, the Chrysaniline obtained was sold and in the meantime registered as C.I. Basic Orange (C.I. 46045).

For Mauveine ($M$), the formation of products that are formed in the course of the aniline oxidation was intensively studied. These studies indicate the presence of several methyl groups in the heterocyclic core of the Mauveine (see Scheme 3). Thus, besides the parent compound $M_0$, commonly called Pseudomauveine, compounds $M_2$–$M_4$, in particular, were found in different amounts in the reaction mixtures obtained.

Also for Fuchsine, several studies concerning the presence of different compounds in the solid products are reported (refer to Scheme 4). For example, in certain products of different producers, mainly Rosaniline ($F_1$) was present, whereas in some other samples, either mixtures of

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**Scheme 1.** Different organic dyes synthesised by oxidation of aniline/toluidine mixtures.
Pararosaniline (P), Rosaniline (F1) and Magenta II (F2) as well as New Fuchsine (F3) were found.\textsuperscript{21,22} In another study, in which the origin of the compounds was not discussed, besides 2.8\% of Rosaniline (F1) and 25\% of Magenta II (F2), about 71\% of New Fuchsine (F3) was observed.\textsuperscript{23} In an actual study, different samples of Fuchsine and some of its N-phenyl-substituted derivatives, which are hosted in the Historical Dyestuff Collection of the Technical University of Dresden, have been analysed and their composition from different compounds has been confirmed.\textsuperscript{20}

In contrast to Mauveine and Fuchsine, for Chrysaniline, nearly no analytical studies on the composition of the solid products are known at present. Therefore, no detailed information on the preparative procedures for this dye is available. However, it can be assumed that during the synthesis of Chrysaniline by starting with certain aniline/toluidine mixtures, similar reactions proceed to those during the synthesis of Fuchsine with the only difference, that instead of \textit{para}-toluidine the isomeric \textit{ortho}-toluidine plays an important role.\textsuperscript{24} Their behaviour during the Chrysaniline formation will be discussed subsequently more in detail.

In the Historical Dyestuff Collection of TU Dresden, besides several samples of Mauveine and Fuchsine,\textsuperscript{25} nearly one dozen different samples of Chrysaniline are also hosted; hence, their compositions have been studied in more detail now. These dyes, most of which are designed as Phosphine and were sold under different trade names, originated from different producers and came on the market in 1882. Owing to their ‘Schönheit und Echtheit sowie ihrer eminenten Verwendbarkeit zu Combinationsfarben’, these dyes were intensively praised and commercially used at this time,\textsuperscript{26–28} but they lost their importance later on. In the course of our analytical studies documented herein, certain information on the applied synthetic routes can be derived.

**Results and discussion**

All samples used for the liquid chromatography–mass spectrometry (LC-MS) measurements were dissolved in formic acid containing a mixture of water and methanol (60/40 v/v containing formic acid (0.1\% v/v)). Clear solutions with red, orange or yellow colour were obtained. In

\begin{align*}
\text{Scheme 2. Alternative synthetic routes to Fuchsine dyes (F) and Chrysaniline dyes (C).}
\end{align*}

\begin{align*}
\text{Scheme 3. Structures and names of the Mauveine dyes.}
\end{align*}
Figure 2, the colours of all the Chrysaniline dyes (1–11) dissolved in methanol with an arbitrary concentration are shown. Sample 12 shows a solution of Fuchsine.

In Figure 3, the thin layer chromatograms (TLCs) of the dyes studied are depicted. As can be seen, the purities of the samples are rather different. This indicates the presence of certain isomeric and homologous compounds in the samples studied, which has to be further confirmed by LC-MS measurements.

In Figure 4, the LC chromatograms of Samples 1, 2, 4 and 5 are shown as examples. The chromatograms of the other dyes are given in the Supporting Information. The figures show the total ion currents (TIC), representing the sum of the intensities of protonated compounds over the whole measured mass range, as black lines.

All the TIC chromatograms show numerous signals indicating the presence of different compounds in each sample. For each signal, the corresponding high-resolution mass spectrum exhibits the molecular weight and the elemental composition of the compound measured. As an example, the mass spectrum of the peak at about 4 min in the chromatogram of Sample 1 with a mass of 286.1344 is shown in Figure 5.

The exact measured mass of the protonated molecular ion of m/z 286.1316 confirms the elemental composition C_{19}H_{16}N_{3}^+ for this compound (the deviation from the theoretical exact mass 286.1344 fits the experimental error) and documents, therefore, the presence of Chrysaniline. This statement can be evaluated by fragmentation of the protonated molecular ion using an MS/MS experiment. In this experiment, the protonated molecular ion of the compound is separated by the first mass analyser, a quadrupole, then fragmented in a collision chamber in which the exact mass of the resulting ions is measured using a time-of-flight (TOF) analyser. As an example of this procedure, the MS/MS spectrum of the corresponding Chrysaniline is shown in Figure 6.

Most of the signals in the MS/MS spectra are assigned using their exact masses based on known fragmentation rules. Thereby, a fragmentation mechanism for the compound in question may be assumed. The mechanism is given in Figure 7 and confirms the structure of Chrysaniline.

In this way, the most intense signals in all the chromatograms are assigned to the appropriate compounds. The

| Nr | R¹ | R² | R³ | Usual name       | Cl. designation |
|----|----|----|----|-----------------|-----------------|
| P  | H  | H  | H  | Parafuchsine    | Cl 42500        |
|    |    |    |    | Pararosaniline  | Basic Red 9     |
| F1 | Me | H  | H  | Fuchsine        | Cl 42510        |
|    |    |    |    | Rosaniline      | Basic Violet 14 |
| F2 | Me | Me | H  | Magenta II      | Basic Violet 14 |
| F3 | Me | Me | Me | New Fuchsine    | Cl 42520        |
|    |    |    |    |                 | Basic Violet 2  |

Scheme 4. Structures and names of the Fuchsine dyes.
corresponding data are collected in the Supporting Information. An overview of the identified compounds is given in Table 1. Note, that the proposed structures for Chrysaniline (C), Fuchsine (F) and for the Fragments D exhibit only one possibility for the positions of the methyl groups; further isomers are possible. The actual positions of the methyl groups are not estimable by mass spectrometry. The same situation is valid also for the position of the amino group in fragment E, and its origin is unknown. Other than given, the external amino group can also be linked alternatively at the 9-phenyl group.

Therefore, efforts to separate the dye components in the samples by means of preparative column chromatography have been performed. However, owing to the very small differences in the polarity of the isomeric or homologous Chrysaniline compounds, no complete separation of the components in the samples was possible, as was evident from nuclear magnetic resonance (NMR) measurements (see the $^1$H NMR spectrum of the analysed Sample 1 in the Supporting Information SI 10).

Figure 4 contains additionally the ion chromatograms of the protonated molecular ions of the most abundant compounds in the samples (extracted ion current, EIC).
be determined more in detail using the peak areas of these signals. Therefore, in all cases, the peak area in the ion chromatograms was calculated for every compound and the values obtained were normalised to the signal with the highest peak area. The results are given in Table 2. Note, that only the signal intensities of the same compound can be compared between the different samples. Due to the varying ionisation efficiencies of different compounds, the intensities of the ions depend on the particular substance. In addition, in Table 2, the maximal numbers of isomers of each compound detected in the samples are given in brackets.

These numbers refer to the numbers of signals in the LC chromatograms with the same mass. They confirm the presence of isomers in the detected compounds. For example, the four signals detected in the ion chromatograms for the Samples 2 and 4, and depicted in Figure 3, indicate the presence of four different isomers in these probes. However, it is not clear if all the isomers in these samples could be detected precisely because the resolution of the chromatographic device and its sensitivity is restricted.

From these data, valuable information on the practised routes for the preparation of the Chrysaniline dyes can be
Table 1. Compounds detected as their protonated molecular ions in the chromatograms of the analysed dye samples, assigned by their exact masses.

| Structure | Formula | M+  | Structure | Formula | M+  |
|-----------|---------|-----|-----------|---------|-----|
| ![C](image) | C_{19}H_{16}N_{3} | 286.1344 | ![D](image) | C_{13}H_{1,N_{3}} | 195.0922 |
| ![Me-C](image) | C_{20}H_{18}N_{3} | 300.1501 | ![Me-D](image) | C_{14}H_{13}N_{3} | 209.1079 |
| ![C](image) | C_{21}H_{20}N_{3} | 314.1657 | ![Me2-D](image) | C_{13}H_{13}N_{3} | 223.1235 |
| ![C](image) | C_{22}H_{22}N_{3} | 328.1814 | ![Me3-C](image) | C_{13}H_{15}N_{2} | 271.1205 |
| ![C](image) | C_{23}H_{22}N_{3} | 342.1970 | ![Me4-C](image) | C_{14}H_{18}N_{3} | 288.1501 |

The letters in the rings indicate the used starting compounds (o for ortho-toluidine, p for para-toluidine and d for dimethylaniline).

Table 2. Relative intensities of the protonated molecular ions of the compounds (refer to Table 1) detected in the dye Samples 1–11.

| Compound | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Maximum intensity |
|----------|---|---|---|---|---|---|---|---|---|----|----|------------------|
| C        | 1.00 | 0.20 | 0.11 | 0.26 | 0.49 | 0.07 | 0.29 | 0.07 | 0.25 | 0.14 | 0.28 | 1.98E+07 |
| Me-C     | 0.66 (1) | 0.62 (4) | 0.29 (4) | 0.47 (4) | 0.61 (4) | 0.20 (4) | 0.34 (4) | 0.21 (4) | 0.74 (4) | 0.44 (4) | 1.00 (4) | 1.44E+07 |
| Me2-C    | – | 0.66 (4) | 1.00 (5) | 0.60 (4) | 0.45 (4) | 0.17 (4) | 0.20 (4) | 0.13 (4) | 0.71 (4) | 0.47 (4) | 0.78 (3) | 1.27E+07 |
| Me3-C    | – | 0.64 (2) | 0.03 (2) | 1.00 (2) | 0.42 (2) | 0.06 (2) | 0.08 (2) | 0.01 (1) | 0.23 (2) | 0.41 (2) | 0.07 (1) | 8.63E+06 |
| Me4-C    | – | 0.47 (1) | – | 1.00 (1) | 0.30 (1) | – | – | – | 0.03 (1) | 0.34 (1) | – | 7.13E+05 |
| D        | 0.61 (1) | 0.59 (1) | – | 0.38 (1) | 0.72 (1) | – | 1.00 (1) | – | – | – | 0.64 (1) | 8.81E+05 |
| Me-D     | 0.35 (1) | 0.94 (2) | 0.39 (2) | 0.58 (2) | 0.80 (2) | 0.35 (2) | 1.00 (2) | – | – | – | 0.55 (2) | 5.91E+05 |
| Me2-D    | – | 0.40 (1) | 0.34 (1) | 1.00 (2) | 0.55 (2) | – | – | – | – | – | 4.49E+05 |
| F        | – | 0.60 (2) | – | – | 0.44 (1) | – | 1.00 (2) | – | – | – | 0.55 (2) | 2.49E+05 |
| Me-F     | – | 1.00 (1) | – | – | – | – | – | – | – | – | – | 1.84E+05 |

For normalisation, the signal with highest intensity for each ion was used. Additionally, the number of isomers detected for each compound is given in brackets.
Thus, in the chromatograms of all samples, besides the molecular ion peak at 286.1344 for the protonated Chrysaniline (C), further peaks with mass numbers higher than 14, 28, 42 and 56 mass units were detected. This indicates the presence of higher methyl homologues resulting from the use of aniline/toluidine mixtures for the Chrysaniline synthesis. Obviously, besides the parent aniline, isomeric ortho- and para-toluidines and some dimethylanilines, probably 2,4-dimethylaniline, have been used as starting materials for the syntheses of these dyes. Unfortunately, the positions of the methyl groups on the different rings cannot be exactly designated.

**Conclusion**

From the analytical measurements previously documented, two different mechanistic routes for the formation of Chrysaniline by means of oxidation of an aniline/toluidine mixture can be derived as alternatives for the formation of Mauveine (route a, Scheme 5). Thus, in the first route (route c in Scheme 5), ortho-toluidine was activated by the applied oxidation reagent at its ortho-linked methyl group with initial formation of a reactive radical cation $T_{o}NH_{2}^{+}$ that initiates via $T_{o}NH_{2}$ a coupling reaction with a second aniline molecule. Thereby, an intermediate of the structure $T_{o}ANH_{3}$ is formed from which the final Chrysaniline is obtained during the course of a repeated reaction with a second aniline molecule.

Alternatively, in a second route b, para-toluidine was activated on its para-linked methyl group with initial formation of the radical cation $T_{p}NH_{2}^{+}$ that initiates via $T_{p}NH_{2}$ a coupling with a further aniline or p-toluidine molecule. Thereby, the isomeric coupling products $T_{p}ANH_{3}$ or $T_{p}TNH_{3}$ are formed, from which $T_{p}ANH_{3}$ can serve as a Fuchsine educt and $T_{p}TNH_{3}$ as a Chrysaniline educt on their reaction with a further aniline or toluidine equivalent.

Although in the course of both alternative routes, methyl groups were introduced into the Chrysaniline core; in the route b, a methyl group of para-toluidine was introduced at the 7-position of the 3-amino-acridine moiety, whereas via route c, methyl groups were introduced only on the aryamine moiety linked at Position 9 of the acridine core.
Table 3. Information and the internal notation on the dyes used for the experiments.

| No. | Internal notation | Trade name | Firm | Year of production |
|-----|------------------|------------|------|--------------------|
| 1   | 3417             | Phosphine  | Grießheim\(^a\) | Before 1924        |
| 2   | 3414             | Phosphine A| CIBAb | 1928               |
| 3   | 3424             | Phosphine LB| Grießheim\(^a\) | Ante 1924          |
| 4   | 3425             | Phosphine LR| Grießheim\(^a\) | Ante 1924          |
| 5   | 3426             | Phosphine R | Grießheim\(^a\) | Before 1924        |
| 6   | 3427             | Phosphine RX| Grießheim\(^a\) | Before 1924        |
| 7   | 3430             | Xanthine   | Grießheim\(^a\) | Before 1924        |
| 8   | 3432             | Ledergelbbase O| Fabrwerke MLB\(^c\) | 1906             |
| 9   | 3436             | Ledergelb G | Grießheim\(^a\) | Before 1924        |
| 10  | 3435             | Ledergelb R | Grießheim\(^a\) | Before 1924        |
| 11  | 3422             | Phosphine O | Farbwerke MLB\(^c\) | 1922             |

They are designed according to the Colour Index as C.I. Basic Orange 15 (C.I. 46045).
\(^a\)Chemische Fabrik Grießheim-Elektron, Anilinfarbenfabrik K. Oehler, Offenbach, since 1905.
\(^b\)Gesellschaft für Chemische Industrie in Basel, since 1884.
\(^c\)Farbwerke vorm. Meister, Lucius & Brüning, Höchst, since 1867.

Unfortunately, from the LC-MS measurements, no decision on both these alternatives can be derived. Nevertheless, these measurements indicate unambiguously the use of an aniline/toluidine mixture and the application of the Fuchsine smelt as a synthetic route for the preparation of Chrysaniline dyes analysed and ruled out the use of 2-aminodiphenylamine (3) and 4-aminobenzaldehyde (4) as alternative starting materials (see Scheme 2) for the preparation of the analysed Chrysaniline dyes.

**Experimental**

**Samples**

For the experiments, the samples hosted in the Historical Dyestuff Collection of the Technical University Dresden were used. Information about these samples is collected in Table 3.

**Dyed textile strip**

The dyed textile strips depicted in Figure 1 were prepared by dipping the uncoloured textile strips into an aqueous solution of the corresponding dye with a concentration of ca. 10%.

**TLC**

A methanolic solution of the dye samples was deposited on a strip of alumina covered with silica and eluted with a mixture of methanol/ethyl acetate (2:1).

**Column chromatography**

To obtain a pure sample of Chrysaniline dye for \(^1\)H NMR measurement, a crude probe of Sample 1 (2 g) was dissolved in water (50 mL) and the precipitate obtained after addition of aqueous NaOH (5 mL, 10%) was isolated by filtration and dried in air. The dried sample was deposited on a column filled with silica and eluted with acetone. After evaporation of the solvent, the residue was examined by \(^1\)H NMR spectra (Figure SI 10).

**Sample preparation for LC-MS measurements**

The samples of the dyes were provided as powders. A 3.5 mg of each sample was dissolved in 10 mL of a mixture of water/methanol (60/40 v/v) containing formic acid (0.1% v/v). Water was purchased from Fisher Chemical, and methanol and formic acid were acquired from VWR Chemicals (both HiPerSolv CHROMANORM LC-MS). The resulting solution was diluted by a factor of 10 using the same water/methanol mixture and subsequently filtered with a 0.2 µm membrane syringe filter (Nalgene\(^\text{TM}\); Sartorius Stedim Biotech). The filtered solution was directly injected into the high-performance liquid chromatography (HPLC) system without any further preparation.

**LC-MS measurements**

Chromatographic separations were done using an Agilent 1260 Infinity HPLC system. As the column, an Agilent Poroshell 120 EC-C18 column with a length of 50 mm, a diameter of 4.6 mm and a particle diameter of 2.7 µm equipped with a corresponding precolumn cartridge was used. In all experiments, a gradient of water/methanol containing formic acid (0.1% vol%) from 40% to 100% methanol in 7 min was applied. The flow rate was 0.5 mL/min, and the column temperature was 40 °C. A sample volume of 2.0 µL was injected.

For detection, an Agilent 6538 UHD Accurate-Mass Q-TOF mass spectrometer with an electrospray ionisation (ESI) source was used. All samples were measured in positive ion mode with a capillary voltage of 4.5 kV, a fragmentor voltage of 80 V and a skimmer voltage of 45 V. As gas temperature, 325 °C was selected. The nitrogen gas flow was 8 L min\(^{-1}\) and the nebuliser worked with a pressure of 50 psig. Spectra were measured in the mass range of m/z 60–m/z 1000. In MS/MS experiments, the protonated...
molecular ion of the components was fragmented with an energy of 35 eV. For analysis of the substance with a prototated molecular ion at m/z 288, a fragmentation energy of 25 eV was enough to observe sufficient fragmentation. Prior to all measurements, the MS system was mass calibrated with a standard provided by Agilent.

Acknowledgements
The authors thank Reinhard Buchholz for his experimental support and Dr Thomas Prestel, Fakultät für Physik der TU Dresden, for the measurement of reflection spectra of the dyed strips and Frank Drescher for the support of LC-MS measurements.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Moreover, a financial support of the ‘Gesellschaft der Freunde und Förderer der Technischen Universität Dresden’ is gratefully acknowledged.

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Supplemental material
Supplemental material for this article is available online.

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