

THE PROCESS FOR 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE SYNTHESIS, AN INTELLECTUAL PROPERTY SEIZED IMMEDIATELY AFTER WORLD WAR II

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Introduction

The efforts to win World War II included use of intelligence units whose role was to find and detain German scientists then interrogate them for information regarding military information that might help the Allies in their war aims (1). This involved identification of targets by the Combined Intelligence Objectives Subcommittee (CIOS) which were pursued by Target Forces (T-Forces) whose role was to identify and intercept German scientists (2). These activities were expanded to include purely scientific and economic targets in response to several suggestions, including, for example, from Vannevar Bush, Director of the Office of Scientific Research and Development. Many reports of civilian interest were prepared and 2,720 reports published by 1948 (3). One of the many outcomes of this was the journey of civilian doctor Stanley Hall to Elberfeld on May 19, 1945—only 11 days after the end of the war in Europe—seeking medical research information on pesticides (4). An unexpected outcome of Dr. Hall's journey was information concerning a scientific tool familiar to many botanists, use of tetrazolium salts in seed viability testing.

Georg Lakon was the academic botanist who, under the guidance of Richard Kuhn, invented the method of tetrazolium testing for determining the viability of seed stocks so that a constant number of viable seeds may

be sown using a mixture of viable and dead, thereby optimizing use of land. Lakon used small amounts of various salts to optimize the method, in which viable seeds are recognized because of red color development, especially in embryo tissues. He published in German (5), and his work was only widely circulated in the west after the war (4, 6). While tetrazolium testing is a very useful procedure in the botany laboratory (7), the advance which made it a widely successful commercial test was the availability of a large-scale method for synthesizing the optimal tetrazolium salt (2,3,5-triphenyltetrazolium chloride). This method was one of the economic secrets revealed by CIOS interrogation of detained German scientists from *I.G. Farbenindustrie* at Elberfeld. Precisely how scientists were found, detained, and interrogated is unknown but it may have been a fraught process considering the times. Allied personnel may have been heavy handed, or not. German scientists may have been defiant, demoralized, or despondent, or not. The process certainly involved a great clash of cultures that is rarely seen in the conduct of science and the exchange of research results. The following translation illustrates an example of information produced by this kind of interrogation.

Methods

The source material (4) is not an interrogation transcript, but it is probably based upon interrogation of a

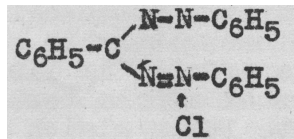
scientist or inventor named Leuchs and edited for clarity by CIOS agents. When the general scientific interest and value of this material was fully appreciated it was published to enhance post-war recovery of the Western Allies, along with a very great number of other chemical processes developed in Germany during the Nazi period (3). My aim was not to produce a literal translation of this record of Leuchs's work, but a description of his scientific methods. I attempted to understand what he did rather than produce a literary work (8), making up for any lack of linguistic skill by long familiarity with experimental science.

To try to identify Leuchs, I searched the USPTO database (9) for all patents where the inventor name included Leuchs and patents from 1925 to 1993 were used to draw a time line of relevant patent publications. These years were used to include men in their twenties in the 1940s who may have worked into their seventies. Patents from 1899 to 1945 were also used to include older scientists who may have been conscripted through lack of manpower. Scholarly citations for these scientists were also identified (10).

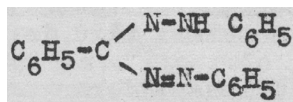
Results

The following is a translation of Leuchs's work, as described by the CIOS (4, Appendix 4). Editorial comments, for clarification and the like, appear in square brackets. Structures in the source document were simply typed in line, as shown in Figure 1, the first page of Appendix 4; however, in this translation bold italic numerals are used to designate structures.

Preparation of Triphenyltetrazolium Chloride (Tetrazolium Salt) [1]



1



2

To 400 g triphenylformazan [2], 1.7 liters dry spirit and 330 [sic, unit omitted] amyl nitrite, mixed in a 3-liter flask with stirring, 185 g of 37% alcoholic hydrochloric acid is added dropwise. Heat of reaction causes the temperature in the flask to rise to 50-55°. When the alcoholic hydrochloric acid has been added dropwise, stirring is continued for a further 1½-2 hours; then the flask is allowed to stand without stirring for 4 hours, until no further bubble development (nitrogen) is seen. The red color of the

APPENDIX 4

Preparation of Triphenyltetrazoliumchlorid (Tetrazoliumsalz)

$$\begin{array}{c} \text{N-N-C}_6\text{H}_5 \\ | \\ \text{C}_6\text{H}_5\text{-C} \\ | \\ \text{N=N-C}_6\text{H}_5 \\ | \\ \text{Cl} \end{array}$$

Zu 400 g Triphenylformazan $\text{C}_6\text{H}_5\text{-C} \begin{array}{l} \text{N-NH C}_6\text{H}_5 \\ \text{N=N-C}_6\text{H}_5 \end{array}$, 1,7 Ltr. Trockenspirit

und 330 Amylnitrit lsst man in einem 3 Ltr.-Kolben unter Rhren 185 g 37% alkoholische Salzsure zutropfen. Durch die Reaktionswrme steigt die Temperatur im Innern des Kolbens auf 50-55°. Wenn die alkoholische Salzsure zuge- tropft ist, wird noch 1 1/2 - 2 Stunden nachgerhrt, dann, ohne zu rhren, noch 4 Stunden stehen gelassen, bis keine Blasenentwicklung (Stickstoff) mehr zu bemerken ist. Die rote Farbe des Triphenylformazans ist verschwunden und eine tiefbraune Lsung entstanden. Diese Lsung wird darauf gelange mit Leitungswasser versetzt, bis sich deutlich eine lschicht (in der Hauptsache Amylalkohol) abscheidet, wozu ungefhr 6-8 Ltr. Wasser erforderlich sind. Die wssrig- alkohol. Lsung wird durch Ablassen von der lschicht getrennt und im Vakuum oder auf dem Wasserbad bis auf 1 - 1 1/2 Ltr. eingeengt. Zur Klrung behandelt man noch heiss mit Tierkohle und saugt ab. Das gelbliche Filtrat wird jetzt auf dem Wasserbad bis zur beginnenden Krystallisation weiter eingedampft, auf Eis gestellt, wodurch das wasserhaltige Rohprodukt des Triphenyltetrazoliumchlorids in fester Form erhalten wird. Zwecks Reinigung wird das Rohprodukt in heissem Wasser gelst (auf 1 Teil Rohprodukt hchstens 1 Teil Wasser). Die Lsung reagiert meist kongossauer, deshalb muss mit 20% Ammoniaklsung bis zum Verschwinden der kongos- sauren Reaktion (aber noch deutlich lackmussauer!) abgestumpft werden, wozu ca. 30-40 ccm Ammoniaklsung erforderlich sind. Ausserdem wird nochmals etwas Tierkohle zugegeben und noch warm abgesaugt. Das Filtrat wird in Eiswasser gestellt und unter Umrhren zur Krystallisation gebracht. Man erhlt dann ein fest farbloses Triphenyltetrazoliumchlorid, das abgesaugt zunchst 2 Tage lang bei gewhnlicher Temperatur unter Ausschluss von Licht und dann erst im Dampfschrank getrocknet wird. Triphenyltetrazoliumchlorid stellt ein schwach gelb- brunliches Krystallpulver dar, das bei 241° sich zersetzt und die erforderlichen Reinheitsprfungen erfllen muss. Ausbeute gegen 300 g = 67-68% d.Th.

Darstellung von Benzyliden-phenylhydrazon.

Zu 108 g Phenylhydrazin und 200 ccm Trockenspirit lsst

- 45 -

Figure 1. First page of Leuchs's work, as described by the CIOS (Ref. 4, Appendix 4).

triphenylformazan disappears and a deep brown solu- tion forms. This solution is mixed with tap water until a clear oily layer (mainly amyl alcohol) separates, and about 6-8 liters of water are required. The aqueous alcoholic solution is separated by draining the oily layer and concentrated under vacuum or on a water bath to 1-1½ liters. Clarify while hot with activated carbon, and vacuum filter. Evaporate the yellowish filtrate on a water bath until the beginning of crystal- lization, then place on ice, so that the aqueous crude product triphenyltetrazolium chloride is obtained in solid form. For cleaning, the crude product is dis- solved in hot water (to 1 part crude product add not more than 1 part water). The solution usually reacts with Congo Red, therefore it is blunted [sic, this sentence is not precisely clear in German or English] by 20% ammonia solution until the Congo Red acid reaction disappears (but it is still significantly acid, judged by litmus), and about 30-40 mL of ammo- nia solution is required. A little activated carbon is added again and the solution vacuum filtered while still warm. The filtrate is placed on ice water and

allowed to crystallize while stirring. The result is a nearly colorless triphenyltetrazolium chloride, which is vacuum filtered and dried for 2 days at room temperature, with exclusion of light, then in a steam cabinet. Triphenyltetrazolium chloride is a slightly yellow-brown crystalline powder, which decomposes at 241°, and met the required purity tests. Yield, about 300 g (67-68% theoretical yield).

Description of benzylidene-phenylhydrazone

To 108 g of phenylhydrazine and 200 mL dry spirit in a 1-liter flask add 106 g benzaldehyde dropwise, with stirring. When the benzaldehyde is added dropwise, boil 30 minutes, then cool on ice water, the benzylidene-phenylhydrazone is harvested by vacuum filtration and washed with cold dry spirit and a little ether. Drying benzylidene-phenylhydrazone is done in the light-protected desiccator [*sic*, this equipment had not been described] over calcium chloride, preferably using a water-jet vacuum. Yield 162 g, equivalent to 83% of theoretical yield. The desiccated material is transferred to brown bottles.

Description of Triphenylformazan

77 g aniline and 207 mL concentrated hydrochloric acid were diazotized with an aqueous solution of 59 g sodium nitrite, using a temperature not exceeding +10°. The diazonium solution [phenyldiazonium chloride] is filtered rapidly. During filtration or earlier, prepare a solution of 162 g benzylidene-phenylhydrazone in 350 mL pyridine fraction II [*sic*, this is a reference to an unknown fractionation method, information omitted from this document] and 350 mL dry spirit in a large beaker with stirring and cool the solution to 15-20°. Then at this temperature, add the filtered diazonium solution, dropwise with stirring. The temperature rises to about 25° when the diazonium solution is added dropwise, which does no harm. Higher temperatures should be avoided, however, by external cooling with ice. In order to prevent decomposition of the diazonium solution, set it up for use on ice water. After dropwise addition of the diazonium solution mix thoroughly for at least 2-3 hours more; the resulting red precipitate is raw triphenylformazan. Formula: [2]. This was vacuum filtered and washed several times with water; yield 205 g. For the purpose of further processing to triphenyltetrazolium chloride it is sufficient to boil this air-dried material with 15 parts by volume dry spirit. To this end, for example, 205 g of triphenylformazan was boiled with 3,075 mL dry spirit for 30-40 min, then left to cool overnight and finally vacuum filtered. This gives a shimmering violet crystalline product which has mp. 170-173° and is suitable for further processing. Yield: 80% of the crude product (164 g).

Preparation of Triphenyltetrazolium Chloride (Tetrazolium Salt) [1]

. . . [iteration of all the above] . . . of the crude product (164 g).

Conclusion

It is initially surprising for a scientist used to word processors that automatically highlight spelling mistakes to see glaring typing errors (e.g. *benzaldehyd* for *benzaldehyd* and *triphenyltetrazoliumschlorids* for *triphenyltetrazoliumchlorids*) which survive all the way through the publication process. However, I became used to spelling errors, occasional omission of units and inconsistent abbreviations (e.g., “*d.Th.*” and “*der Th.*” for theoretical yield). There were also mistakes in chemical diagrams. Compare structures **1** and **2** above, from the detailed appendix to the summary of the synthetic scheme from the main body of the CIOS report (Figure 2). In both of the former, C–N double bonds are shown as single bonds, and in structure **1** a line indicating a C–N bond is missing. (However, note that a hydrogen atom is missing from the triphenylformazan structure in Figure 2.) I corrected some of these errors, though I have in some cases translated imperfect grammar faithfully rather than correcting it (e.g., “*mindestens 2-3 Stunden weiter*” as “at least 2-3 hours more”). The greatest error is

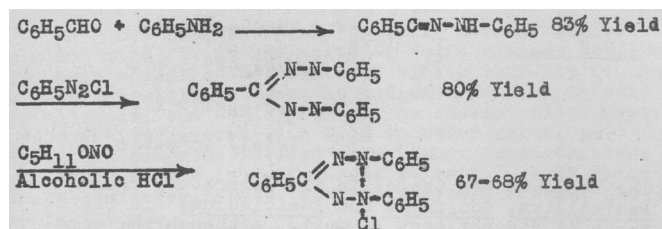


Figure 2. Summary of Leuchs's synthetic scheme, as described by the CIOS (Ref. 4, p. 16).

a large repetition of German text and structural formulae including imperfect copying of hyphenation, presumably of German text by an Anglophone typist (e.g., *wässrig-alkohol* contained a carriage return and hyphen in the first iteration and the hyphen was copied over so the word *wässrigalkohol* was written *wässrig-alkohol* mid page in the second iteration). I have abbreviated this large iteration and italicized the first and last few words. I have also included a summary of the synthetic scheme using more modern structural notation (Figure 3).

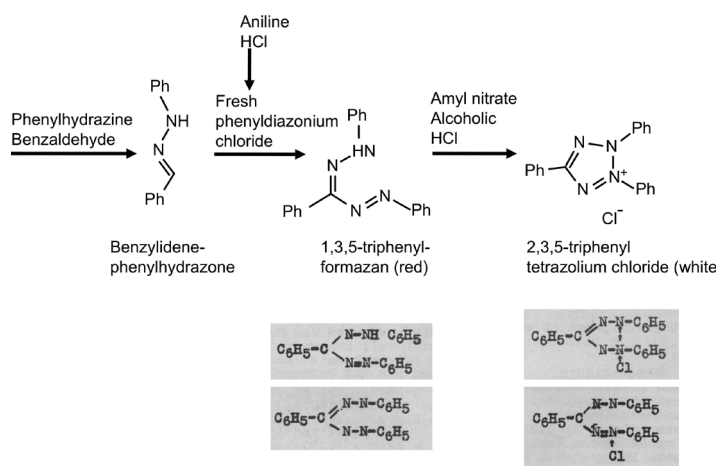


Figure 3. Summary diagram of Leuchs's process for 2,3,5-triphenyltetrazolium chloride synthesis. Tetrazolium testing of a biological system reverses the reaction and causes 2,3,5-triphenyltetrazolium chloride (white) to revert to 1,3,5-triphenylformazan (red). Structures cited in the original text (4) are copied below the relevant structure.

This low quality of interrogation product suggests the original documents were prepared hurriedly or the German scientists were perfunctory. After this stage the German text was not rigorously edited by a Germanophone. A reason for this may be the special circumstances under which the information was obtained; once released, the German scientists made themselves unavailable for further comment and revision of drafts became difficult. Some of the colleagues of Dr. Leuchs were certainly still not denazified at the time; for example a Dr. Mudrow still signed his written work *Heil Hitler* at this early post war phase (Ref. 4 and Figure 4). This is a translation of a slightly bizarre document that may be typical of a time dominated by administrators rather than scientists.

Precisely identifying the scientist responsible for the industrial process is difficult. I have assumed that, like most industrial scientists today, he was more interested in publishing patents than scholarly papers and it was common for *I.G. Farbenindustrie* to be assigned patents in the United States, even during the war years (though these may have been pre-war applications working their way through the system). This suggested to me that something may be learned from databases of patents. Hall (4) only cites the title and surname of the relevant scientist, i.e., Dr.

Leuchs. Taking into account the youngest possible scientist, i.e., a 24-year old doctoral graduate, this means Dr. Leuchs might have continued publishing patents for a further 41 years, until his retirement. This means patents published before 1993 are relevant; this screen limits the possible identities to six scientists (Figure 5). On the other hand, the oldest this scientist could be in 1945 is approximately 75, an old man working in desperate circumstances, and so searching scholarly databases for papers published from 1899 to 1945 was also considered relevant. Two organic chemists were found using these overlapping methods: Dieter Leuchs, a young man in 1945 and Friedrich Leuchs, an older man. Further evidence is clearly needed, though one may wonder whether

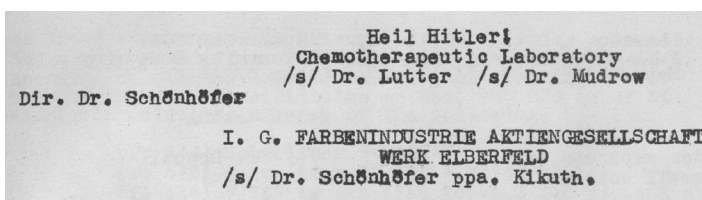


Figure 4. Transcript of signatures from Appendix 3 of Ref. 4, "Results of Testing Repellents and Mosquitocides."

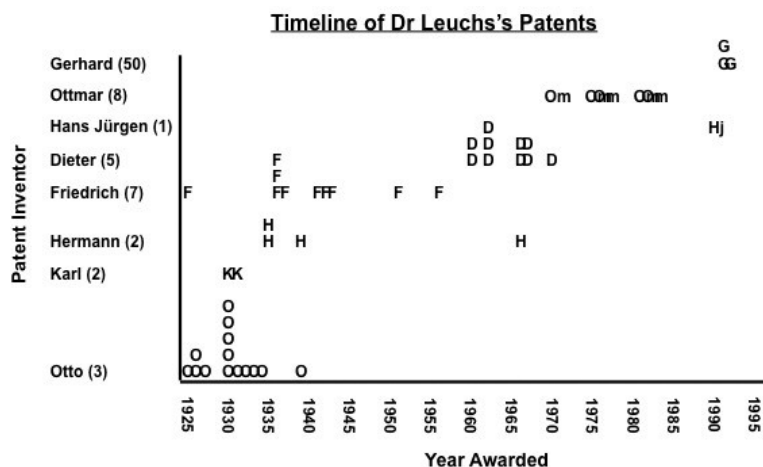


Figure 5. The following US patents were used to compose this graph: 1,558,375; 1,583,717; 1,588,758; 1,640,506; 1,694,127; 1,746,663; 1,755,657; 1,766,822; 1,767,382; 1,767,382; 1,770,412; 1,814,208; 1,853,626; 1,871,868; 1,913,478; 1,982,760; 2,004,545; 2,020,713; 2,086,585; 2,087,131; 2,087,132; 2,113,606; 2,168,167; 2,172,076; 2,229,810; 2,305,297; 2,336,179; 2,650,943; 2,856,471; 2,951,847; 2,954,370; 3,082,114; 3,086,002; 3,108,104; 3,232,928; 3,255,173; 3,297,679; 3,314,934; 3,329,710; 3,498,735; 3,698,440; 3,943,087; 4,008,368; 4,297,526; 4,345,363; 4,952,730; 5,124,994; 5,172,185; 5,177,566. The inventor's initial or two initial letters were used to define data points. The number of relevant scholarly citations for each scientist is in brackets.

the scientist interrogated in 1945 was an older man who lived to see his nation defeated a second time or a young man who managed to build a new and productive postwar career in organic chemistry.

Leuchs worked for *I.G. Farbenindustrie* at Elberfeld, a company highly influenced by the ideas of Richard Kuhn and his assistant Dietrich Jerchel. In addition to the work described above, *I.G. Farbenindustrie* was the site for nerve gas research (11), and this association may have caused workers like Leuchs to make themselves unavailable after the war. However, it is possible to trace the incorporation of Leuchs's work into the corpus of postwar chemical knowledge recorded in English by examining the papers of Reid (12) and Ninehan (13) who cite many of Kuhn and Jerchel's papers (for example 14 and 15). Kuhn and Jerchel's work was also originally published in German and only became widely circulated in English after the war.

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