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PREPARATION OF BRILLIANT CONGO R ("VITAL RED") AND THE SUITABILITY OF VARIOUS SAMPLES OF VITAL RED FOR BLOOD VOLUME WORK

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The value of Brilliant Congo R for the determination of blood volume in man was demonstrated by Dawson, Evans and Whipple.[1] Of more than sixty dyes studied but few, other than those of the Vital Red series, were suitable for this clinical test. Brilliant Congo R, although originally intended to be a direct dye for cotton, has never found favor in the trade, and at the time this research was undertaken it was therefore virtually unobtainable in the market. The little that was available was too impure to be injected into the blood. In 1918, in compliance with a request from Dr. Evans, a small quantity of the commercial dye was purified by the Bureau of Chemistry[2] in order that he might conduct further experiments in blood volume work.

Because of the very small quantity of Brilliant Congo R which may be needed for clinical tests and the difficulties involved in its preparation no manufacturer felt that the subject warranted the expenditure of time and money necessary for investigation before a satisfactory product could be expected. The Color Laboratory of this Bureau was therefore requested to coöperate in carrying out the chemical research needed to develop a satisfactory process which might be available for anyone who desired to undertake the preparation of this dye.[3] The German patents[4] describe a process for the preparation of Brilliant Congo R and similar tetrazo dyes. This process is exceedingly simple in principle, involving merely the diazotization of tolidine, followed by the successive coupling with amino R salt (2-naphthylamine-3,6-disulfonic acid) and Brönner acid (2-naphthylamine-6-sulfonic acid). All efforts to obtain a suitable product by following the patent process were futile. Not only the pharmacological tests, but the chemical behavior of the product showed clearly contamination with soluble decomposition products (brown in color) and an insoluble

[1] Dawson, Evans and Whipple, *Am. J. Physiol.*, **51**, 232 (1920).

[2] Lubs, *J. Ind. Eng. Chem.*, **11**, 456 (1919).

[3] Some time after the investigation was started by the Color Laboratory, the National Aniline and Chemical Co., which had been approached earlier, became interested and succeeded in producing a Brilliant Congo R which complied with the requirements and which is now on the market under the name of "Vital Red Evans." Their process of manufacture, however, is unknown to the authors.

[4] Ger. pats. 41,095 and 28,753.

pigment. Further purification of this product freed it from the pigment, but the resulting material did not have the properties ascribed to Vital Red.

The inventors, evidently realizing these difficulties, devised an improved process (Ger. pat. 41,362) which differs from the first in that a preliminary addition compound of tetrazo-tolidine with a diazo-amino compound, such as Fast Yellow R, is made. This addition compound is successively coupled with the two naphthylamine sulfonic acids while the diazo-amino compound is regenerated. Since the diazo-amino compound is soluble in acid, the Brilliant Congo R is removed by filtration in the form of the free acid. The product used in Expt. 22 (see Table I) was prepared in this

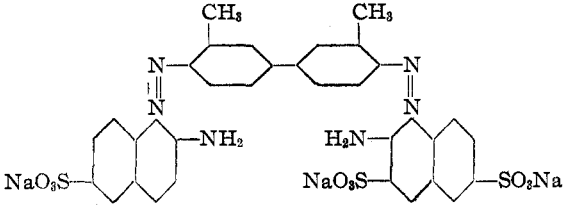
TABLE I

DATA ON VITAL RED SAMPLES SUBJECTED TO BLOOD VOLUME TESTS

Expt.	Process	Mol. ratio of Brönner acid to "other components"[c]	Preparation of "half-dye" intermediate	Use of heat in any stage of process	Dye subjected to further purification	Suitability for blood-volume work as determined by pharmacological tests
22	II[a]	1:1	No	...	Yes	Not suitable
34	I[b]	1:1	No	Yes	Yes	Not suitable
35	I	2:1	Yes	Yes	Yes	Not suitable (but better than 34)
31	I	2:1	Yes	Yes	Yes	Not suitable
28	I	2:1	Yes	No	..	Fair
32	I	2:1	Yes	No	Yes	Suitable
36	I	2:1	Yes	No	No	Excellent
25a	Old sample, manufacturer unknown.					Not suitable
25b	Old sample, manufactured in U. S.					Not suitable
German A: Made by well-known German firm.						Excellent
American B: Fresh sample, made by U. S. manufacturer						Suitable, but not as good as Sample 36 or German A

[a] Patent process (Ger. pat. 41,362), using amino-azo compound.
[b] Process described in this paper.
[c] "Other components"—1 mol. of tolidine and 1 mol. of amino R salt.

manner. Even a casual inspection of the compounds and reactions involved indicates the practical difficulties attending an orderly coupling of the two amino compounds to the tetrazo-tolidine, so that half of the molecule is satisfied by one and half by the other, in order that the resulting dye may have the formula given below.



In this paper the preparation of Vital Red is considered in two parts: (1) the preparation or purification and the quantitative estimation of the intermediates (a) tolidine; (b) amino R salt and (c) Brönner acid; (2) the preparation and purification of the dye itself, Brilliant Congo R (Vital Red).

In general it may be said that the process ultimately developed is based on the German patents 28,753 and 35,615, differing, however, in the modifications in the coupling reactions and recovery of the dye.

The following conclusions regarding the reactions and conditions involved were reached.

1. While a fair degree of purity of the intermediates is a prerequisite, quantitative analyses of these intermediates are more important to make possible the use of exact proportions. (Amino R salt runs only about 80% pure.)

2. The difficulties in the preparation of Vital Red are not traceable, as has been thought, to impurities in intermediates.

3. The coupling is sensitive to variation in conditions, particularly temperature, hydrogen-ion concentration and proportion of 2-naphthylamine-6-sulfonic acid (the second coupling agent).

4. Since it is practically impossible to avoid completely side reactions in the diazotization and coupling, some means for the elimination of the by-products during the process of preparation, aside from purification of the ultimate dye, is necessary.

5. Both heat and alkali treatment of the reaction mass (as recommended in the original patent) are detrimental.

The principal features in the modified process relate to the coupling and recovery of the dye and are essentially as follows: (1) liberation from the reacting medium of a "half-dye" addition compound of tetrazo-tolidine with amino R salt (the first coupling agent), thus affording a preliminary purification; (2) careful regulation of temperature, hydrogen-ion concentration and proportion of reacting constituents in all the steps involved, and the use of an *excess* (1 molecule excess) of Brönner acid (the second coupling agent) instead of the theoretical equivalent of one molecule;[5] (3) the elimination of the step involving heat and alkali treatment of the reaction mixture to render the dye soluble (treatment recommended in the original patent).

Experimental Part

Tolidine.—A convenient method of purifying commercial tolidine through the bisulfite was devised. The process in detail is as follows.

One hundred g. of commercial tolidine was suspended in 5 liters of water and the mixture warmed while sulfur dioxide gas was bubbled through the suspension until prac-

[5] This condition appears to be a manifestation of hydrogen-ion effect, as the excess of Brönner's salt used is recoverable almost entirely in the form of the free acid after the coupling has been complete.

tically all of the base had dissolved. The solution was then filtered through cotton and the filtrate was boiled until precipitation just began. (Passing live steam through the solution hastened the process.) It was again filtered in the same way and allowed to stand.

Most of the tarry matter was removed in the first two filtrations. After the solution had cooled and stood for some time, a precipitate formed, which was removed by filtration (filtrate, about 5 liters). A small portion of the filtrate when tested with ammonium hydroxide gave a clean, white precipitate. (If desired, the base can be precipitated with ammonia and kept as such, but it soon darkens.) For preparing the hydrochloride, about 1 liter of concd. hydrochloric acid was added to the filtrate. The whole was then allowed to stand until the dihydrochloride had separated when it was removed by filtration. This dihydrochloride remained white, even when exposed to the atmosphere for several days. Both the base and the hydrochloride were analyzed by a method published by Palkin.[6] The purified tolidine melted at 129° and was found by analysis to be 99.8% pure.

Amino R Salt.—This was prepared by the method of Cain,[7] except that the temperature was allowed to rise to 175°. It was purified by dissolving in a minimum quantity of hot water and re-precipitating with strong hydrochloric acid. A low yield was obtained.

The salt was first tested for the absence of R salt (2-naphthol-3,6-disulfonic acid) as follows. A small quantity of the substance was diazotized with the usual precautions, using a slight excess of nitrite. The solution was then made alkaline. In the presence of R salt a bright red color was produced, while in the absence of R salt the solution was yellow or yellowish-brown.

The content of amino R salt was determined by repeated evaporation with hydrochloric acid, drying each time at 125° for one-half hour and titrating the residue with 0.1 *N* alkali using methyl red as indicator. For this purpose a 2g. sample was dissolved in 100 cc. of water, and 10cc. aliquot portions were used for the analyses. Repeated determinations gave 81.6 ± 1% of amino R salt as the monosodium salt $C_{10}H_5(SO_3H).(SO_3Na)NH_2$.

The accuracy of the method was confirmed by determination of the sodium. Sodium from a "sulfate ash" determination, which is due to total sodium, gave 24.84% as sulfate. Sodium sulfate due to the constituents (a) amino R salt calculated from the titrations described above was 17.83% and (b) that due to sodium chloride as calculated from a Volhardt determination of equivalent halogen was found to be 7.08%. The sum of (a) and (b), namely, 24.91% of sodium sulfate, agreed very well with the total.

Brönner Acid.—Crude, commercial Brönner acid was purified by the method described by Green[8] and air-dried.

For analysis, to 5 g. of the sample in a 250cc. beaker were added 25 cc. of 0.1 *N* sodium hydroxide solution and 20 to 25 cc. of water. The excess of alkali was titrated back with 0.1 *N* hydrochloric acid, using methyl red as an indicator. The sample contained by analysis 99.75% of $C_{10}H_6.NH_2.SO_3H + H_2O$.

The free acid was converted into the sodium salt by means of sodium carbonate (slightly over one molecular equivalent). This salt crystallized in flakes with two molecules of water, when the hot solution cooled.

[6] Palkin, *Ind. Eng. Chem.*, **15**, 1045 (1923).

[7] Cain, "The Manufacture of Intermediate Products for Dyes," C. Griffin and Co., Ltd., London, **1916.**

[8] Green, *J. Chem. Soc.*, **55**, 36 (1889).

Analysis of the sodium salt of Brönner acid was made by precipitation of the acid with hydrochloric acid, filtration (through perforated plate and paper), washing several times with water, solution of the precipitate of Brönner acid in an excess of standard alkali and back titration as before. Five determinations averaged 99.8% of $C_{10}H_6NH_2.SO_3Na.2H_2O$.

Preparation and Purification of Brilliant Congo R or Vital Red

Diazotization of Tolidine.—In a 250cc. beaker, 2.12 g. of pure tolidine (0.01 mole) was dissolved in 20 cc. of dil. hydrochloric acid (1 part by volume of concd. hydrochloric acid and 9 parts of water). After the addition of another 2 cc. of concd. hydrochloric acid, the solution was immersed in a bath of ice and salt and diazotized with a solution of 1.4 g. of sodium nitrite (0.02 mole) in 10 cc. of water. Special precautions were taken to keep the temperature of the mixture below 5° and to add the nitrite drop by drop from a buret while stirring the contents with an electrical stirring device.

Preparation of "Half-Dye" Addition Compound.—A cooled aqueous solution containing 4 g. (0.01 mole) of amino R salt,[9] 1.7 g. of sodium bicarbonate (about 0.02 mole) and 4 g. of sodium acetate in 45–50 cc., was added from a pipet slowly so as to prevent rise of temperature above 5°, and the resulting dark red solution was kept cool and stirred for one-half hour. An approximately equal volume of 95% alcohol (previously cooled to 0°) was very slowly run into the cold reaction mixture during constant stirring and the temperature kept below 5° (care is necessary at this point, as addition to alcohol generates heat). The stirring was continued for about 10 minutes. The dark red precipitate which formed was collected on a Büchner funnel, using double paper and washing a few times with a previously cooled 50% mixture of alcohol and water.

Preparation of Complete Dye.—In a 600- or 800cc. beaker 0.02 mole of the sodium salt of Brönner acid (5.6 g.) was dissolved in 400 cc. of water and the solution was cooled to 20–25°. While the mixture was agitated the solid "half-dye" was added slowly and after it had completely dissolved the stirring was continued for about an hour. The flaky precipitate of free Brönner acid liberated in the reaction was collected on a Büchner funnel. This, when air-dried, weighed approximately 2 g. (Expt. 36). The red filtrate containing the Brilliant Congo R in solution was transferred to a 1-liter beaker, 30 to 40 cc. of saturated sodium chloride solution was added while the liquid was stirred and the mixture was centrifuged for 20 to 30 minutes. The brown, supernatant liquid was decanted off and the dye washed several times with 10% salt solution, centrifuged and the liquid decanted as before until the supernatant liquid was almost free from brown color. The dye was given a final washing with 95% alcohol and collected on a Büchner funnel. The yield of the air-dried product was

[9] Analysis of amino R salt showed it to be 81.6% pure.

6.85 g. No further purification was applied in Expt. 36 (see Table I) but in some of the other experiments the product was further purified by dissolving in water, re-precipitating with saturated salt solution, centrifuging, etc., as before.

A large number of experimental preparations of Vital Red were made, involving variations in conditions. Owing to the extensive work required in making the blood-volume tests on these samples, however, only a few which seemed the most promising were selected for the pharmacological examination. Descriptions of numerous preparations not used for blood-volume tests are therefore not included in the tabulated report. The accompanying table gives the data on the preparation that were subjected to blood-volume tests. The agreement with or deviation from the process of preparation described is indicated. The conclusions drawn by the pharmacologist as to their fitness for blood-volume test work are indicated in the column headed "suitability," etc.

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Summary

An improved process has been developed for the synthesis of Brilliant Congo R (Vital Red). This process includes (1) the preparation or purification and the quantitative estimation of the intermediates, tolidine amino R salt, and Brönner acid; and (2) the preparation and purification of the dye itself.

Some of the improved features of the revised process, which is based on the German patents, are (1) separation of a "half-dye" addition product of tetrazo-tolidine with amino R salt; (2) a more careful control of temperature, hydrogen-ion concentration and proportions of reacting constituents; (3) the use of an excess (1 molecular equivalent) of Brönner acid, instead of the theoretical equivalent; (4) elimination of heat and alkali treatments necessary in former processes to render the dye soluble.

As shown by practical clinical tests, the dye prepared by this process is suitable for blood-volume determinations.