

The following signs of intoxication appear: Within  $1/2$ -hour to 2 hours after the injection restlessness, air-hunger, formation of froth, paralysis and, in the course of four hours, stoppage of the heart. The criterion for the determination is that the stoppage is either systolic or rapidly transformed into systole.

The orientating tests are carried out as follows: Doses differing by 20 per cent per gram of frog are injected, one or two frogs being used for each dose.

The final determination can be made by the following procedure:

The mean between the smallest active and the greatest inactive dose is the first approximation. By further, more exact determination, with four to six frogs on each dose, the final value can be obtained with an accuracy of 10 per cent. The determination is completed when, of two doses differing by 10 per cent, the higher kills a majority of the frogs injected, the lower a smaller number. The value is expressed as a percentage of the standard preparation, which is tested at the same time and in the same manner. Only such leaves shall be passed for issue as differ from the standard preparation by not more than 25 per cent.

The assay of digitalis tinctures is made in the following manner:

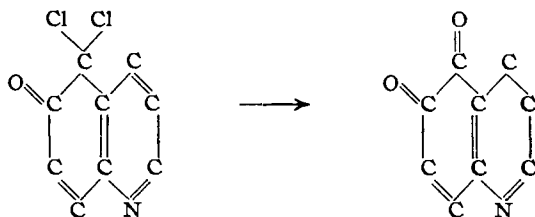
10 cc. of the official tincture (= 1 gram of leaves) are concentrated on the water-bath at temperatures not above  $60^{\circ}$  C. to 5 cc. volume, washed into a measuring flask with distilled water, and made up to 25 cc. The assay is made according to the same method as described above for digitalis leaves.

## THE PREPARATION OF QUINOLINE ORTHO QUINONE AND ITS RELATION TO THE THALLEIOQUIN REACTION.

BY GEORGE W. HARGREAVES.\*

In a previous paper (1), a study of the thalleioquin reaction, for the detection of quinine, was made and a new modification discovered which strengthened the view that a quinone is formed in the reaction. In order to shed further light upon the chemistry of this reaction, the preparation of quinoline *o*-quinone was undertaken.

Only two references were found to this body in the literature. The first was by Mathëus (2) who first prepared it, and the second by Fühner (3), who by passing chlorine into a cold solution of the hydrochloride of 6-hydroxy-quinoline obtained a 5,5-dichlor, 6-keto-quinoline, which was colored green or blue by ammonia and led him to believe that the quinoline quinone of Mathëus was formed intermediately:

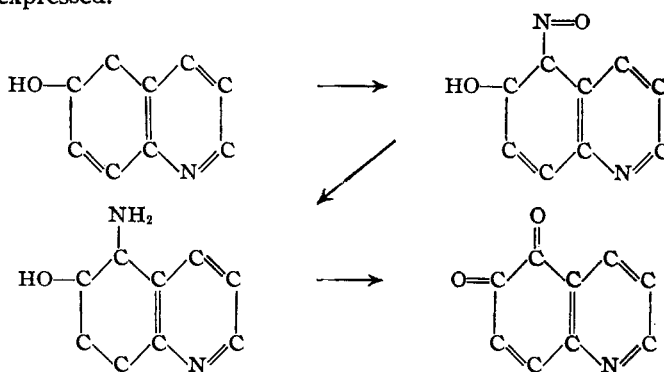


The method used in the preparation was in general similar to that used by Mathëus, but his procedures were considerably modified. The series of reactions

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used is analogous to the preparation of beta-naphthoquinone, which is the naphthalene analogue of quinoline *o*-quinone, from beta naphthol, which is the naphthalene analogue of 6-hydroxyquinoline.

First, the hydroxy-quinoline was prepared by the Skraup method. This was then converted to the 5-nitroso, 6-hydroxy-quinoline, then reduced to the 5-amino, 6-hydroxy-quinoline, which was subsequently oxidized to the 5,6-diketo-quinoline or quinoline *o*-quinone. The relation of these compounds is readily seen when graphically expressed.



#### EXPERIMENTAL.

##### *The Preparation of 6-Hydroxy-quinoline.*

Use a two-liter round-bottomed Pyrex flask equipped with a long reflux condenser. Add first 86.50 grams of glycerine, then an intimate mixture of 22 grams of para amino-phenol and 17 grams of para nitro-phenol. Finally add 40 cc. of concentrated sulphuric acid.

Warm the mixture cautiously taking care to avoid overheating. The reaction is slow in starting but it finally takes place vigorously and with enough heat liberated to cause the mixture to boil spontaneously for some time. As soon as there is any evidence of a reaction, the heat should immediately be withdrawn. If the reaction becomes too violent it should be modified by wrapping the flask with a cold towel. A long efficient condenser is necessary at the start of the reaction or some of the material may be lost. When spontaneous boiling ceases, heat should be again applied and the mixture gently boiled for five hours.

Then allow the mixture to cool and dilute it with 850 cc. of distilled water. Let it stand for three or four hours with occasional agitation. Filter and purify the mixture by fractional precipitation using 40% alkali. Enough alkali is added to cause the formation of a dark precipitate but not enough to make the solution neutral or alkaline. Repeat this procedure two or three times, taking care not to add too much alkali. Then make the solution distinctly alkaline and then immediately make it slightly acid with acetic acid. At this point a dark sticky precipitate will often result and should be discarded. Finally, carefully neutralize the solution. A turbid brownish yellow solution will result from which the hydroxy-quinoline will settle out in about an hour. Collect the precipitate which will vary in color from yellowish to brownish yellow. The yields from this reaction are poor varying from 10% to 25%. The purification is difficult to carry out and until some experience is had with the reaction, part of the compound may be lost in this pro-

cedure. Dry and recrystallize from hot water. A yellowish brown powder is obtained. Above  $140^{\circ}$  this compound darkens and melts with decomposition at  $185-6^{\circ}$ . This action seems to be characteristic of this class of compounds. 6-Hydroxy-quinoline is slightly soluble in water and dissolves readily in alkalies, acids and alcohol.

*Preparation of 5-Nitroso, 6-Hydroxy-quinoline.*

This compound is prepared by using the proportions of 6-hydroxy-quinoline one mole, hydrochloric acid two moles and sodium nitrite one mole. The hydroxy-quinoline is dissolved in water using just sufficient acid to effect solution. Then the excess of acid is added and the solution cooled with ice. The sodium nitrite dissolved in water (1:10) is slowly dropped in. The mixture is kept cool for an hour, then allowed to stand at room temperature for an hour and finally heated on a water-bath for a half-hour. It is then cooled and the nitroso-hydroxy-compound is obtained as a brownish yellow powder by carefully neutralizing the solution. Recrystallized from hot water, a golden powder results which darkens above  $140^{\circ}$  and at  $168-170^{\circ}$  melts with decomposition. It is slightly soluble in water and readily soluble in acids, alkalies and alcohol. This reaction is practically quantitative.

*Preparation of 5-Amino, 6-Hydroxy-quinoline.*

Fourteen grams of the nitroso compound were dissolved in 100 cc. of 1:1 hydrochloric acid, treated with 60 grams of stannous chloride and heated on a water-bath for one hour. The mixture was then diluted with an equal volume of water and treated with alkali until it was just slightly acid. After warming, hydrogen sulphide was passed in until all the tin was precipitated. Upon filtering, a clear dark reddish solution was obtained. The amino body was not isolated but oxidized in the next step.

*Preparation of Quinoline Ortho Quinone.*

The solution of the amino body was evaporated to about a fourth of its volume, cooled and made distinctly acid with an excess of concentrated sulphuric acid. After heating the mixture almost to the boiling point, an excess of 10% ferric chloride solution was added, and at this point the clear solution was darkened. Upon cooling and standing several hours, a crystalline precipitate of the sulphate of quinoline ortho quinone was obtained. This consisted of small, olive-green, rhombic plates. Above  $200^{\circ}$  this compound darkens and slowly decomposes between  $260^{\circ}$  and  $300^{\circ}$ . It is slightly soluble in water and acids and dissolves readily in alkalies.

In order to show the relation of this body to the thalleioquin reaction, 1:1000 solution was made and tested with ammonia, sodium hydroxide and phenylhydrazine and ammonia.

A 5-cc. portion of the 1:1000 solution was colored dark greenish blue by 10% ammonia water. When this solution was diluted, with an equal volume of water it became deep blue and with further dilution, light blue.

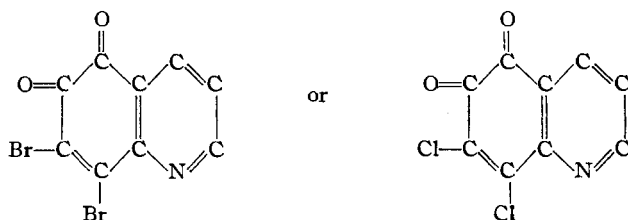
A few crystals dissolved in ammonia water gave a deep emerald-green solution which upon dilution became deep blue and then light blue.

Treatment of a 5-cc. portion of the 1:1000 solution with 10% sodium hydroxide gave a brown color. This action is analogous to a similar procedure with the thalleioquin reaction on quinine.

Treatment of a portion with phenylhydrazine and subsequently with ammonia failed to give a red color.

Later it was found that if a portion of the solution was treated with enough tenth-normal bromine solution to form the di-brom compound, the resulting solution behaved in the same manner as a quinine solution in the thalleioquin reaction. Treatment with chlorine solution gives the same results. On treatment with ammonia it gave the characteristic emerald-green thalleioquin color which did not turn blue upon dilution. On treatment with phenylhydrazine and ammonia a deep red color was produced, analogous to that given by quinine with the modified thalleioquin reaction.

These results would tend to show that the thalleioquin reaction is due to the formation of a halogenated quinoline *o*-quinone. Substitution takes place most readily in the benzene half of the quinoline ring so that the quinone probably has the structure of:



In order to get more evidence, Pollaci's (4) modification of the thalleioquin reaction was again tried. His method is to treat quinine with dilute sulphuric acid and lead peroxide, then with ammonia. It was found that the solution thus obtained was decidedly green but on further dilution became more and more blue in color. On treatment with sulphuric acid and lead peroxide, then with phenylhydrazine and ammonia, quinine did not give the characteristic red color. When the solution treated with sulphuric acid and lead peroxide was also treated with sufficient halogen, it yielded an emerald-green color with ammonia, and the characteristic red color with phenylhydrazine and ammonia.

Another strong piece of evidence was obtained by converting hydroxy-quinoline to quinoline ortho quinone by direct oxidation with sulphuric acid and lead peroxide. However, the details of this process have not been sufficiently worked out to warrant publication.

#### SUMMARY.

Quinoline *o*-quinone has been prepared and the dihalogen derivative is analogous to quinine in its behavior in the thalleioquin reaction and the phenylhydrazine modification of the thalleioquin reaction. The presence of halogen is necessary to obtain a permanent green color with ammonia or a red color with phenylhydrazine and ammonia. This behavior indicates that in the thalleioquin reaction, quinine first yields a halogenated quinoline *o*-quinone which is colored emerald-green by ammonia or red by phenylhydrazine and ammonia.

#### BIBLIOGRAPHY.

- (1) G. W. Hargreaves, *Jour. A. Ph. A.*, 15, 100-5 (1926).
- (2) Mathëus, *B.*, 21, 1887 (1888).
- (3) Fühner, *B.*, 38, 2713 (1905).
- (4) E. Pollaci, *Gazetta*, 28, 391-4 (1898); *J. C. S. Ab.*, 657 (1898).