A STUDY OF THE THALLEIOQUIN REACTION AND A NEW MODIFICATION.*

BY GEORGE W. HARGREAVES.[1]

Perhaps no reaction for the characterization or detection of an organic compound is older or more widely known than the so-called thalleioquinine or thalleioquin reaction for the detection of quinine. The earliest published record was made in 1835. Since then it has been modified by various investigators. In more recent years, the chemistry of the reaction has been investigated.

Originally, the reaction was carried out by the addition of chlorine water to the solution of quinine or its salt and the subsequent addition of an excess of ammonia water. In concentrated solutions, a green precipitate of thalleioquin results, while in more dilute solutions, an emerald color is produced. It has been found that bromine water may be substituted for and is even more efficient than the chlorine water. The directions given by the ninth revision of the United States Pharmacopœia are the following:

"Add two or three drops of bromine T.S. to 1 mil of an aqueous solution of quinine (1–100), made with the aid of just sufficient diluted sulphuric acid to effect solution, and then add 1 mil of ammonia water; the liquid acquires an emerald-green color due to the formation of thalleioquin."

Vondrasek (1) obtained the reaction by treating one centigram of quinine with 1 mil of normal hydrochloric acid and 2 mils of tenth normal potassium bromate solution, then adding ammonia water.

Another variation used by Mylius (2) consists in the treatment of about a centigram of quinine with an equal bulk of potassium chlorate and a drop of concentrated sulphuric acid, with subsequent addition of ammonia water.

Lead peroxide and sulphuric acid were used by Pollaci (3) in carrying out the reaction. A centigram of quinine is dissolved in 1 mil of water and two drops of concentrated sulphuric acid. A small piece of lead peroxide is then put in and the mixture is gradually heated to boiling. After adding 3–4 mils of water, and allowing the mixture to stand, a layer of ammonia water is poured in and an emerald green ring or disc is produced. I modified this procedure by heating the mixture for several minutes on a water-bath, filtering and adding ammonia water to the clear filtered liquid. This produced excellent results.

Hyde (4) used a clear filtered solution of calcium hypochlorite and sulphuric acid to replace the chlorine or bromine water.

Thus, it is readily seen, that the function of the chlorine or bromine water in the thalleioquin reaction is primarily that of oxidation, rather than chlorination or bromination, which Vondrasek and other investigators have supposed.

I have found that failure to obtain the thalleioquin reaction when using bromine water results if any one of three conditions are true; if insufficient time is allowed for the bromine to act, if only a small amount of bromine water is added to a relatively large volume of highly diluted quinine solution, and if an excess of bromine water is added. These conditions are readily explained on the basis of oxidation. In the first instance, bromine water being a relatively mild oxi-

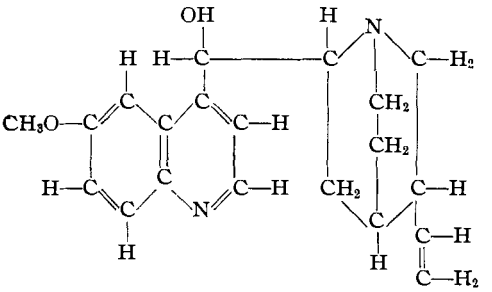
* Read before Scientific Section A. PH. A., Des Moines meeting, 1925.

[1] College of Pharmacy, University of Nebraska.

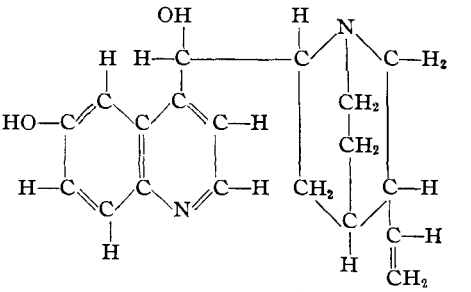
dizing agent requires a short time to react. The second instance is analagous to using an old or weakened solution of bromine water. In the third instance either oxidation is carried too far, or addition of bromine prevents the reaction in some manner.

Although in a practical way, the thalleioquin reaction is used as a test for quinine, it is also given by some of its related bases namely; quinidine, quinicine, diquinicine, hydroquinidine and cupreine; but it is not given by apoquinidine, cinchonine, cinchonidine, homocinchonidine, hydrocinchonidine, cinchonicine, dicinchonicine, quinamine, nor cinchonamine.

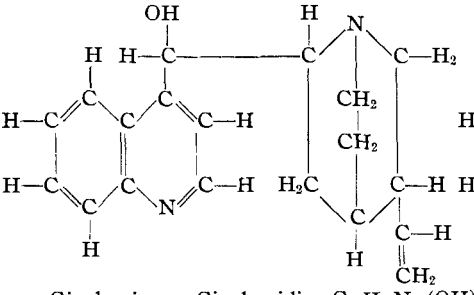
Examination of the structural formulas of some of these bases will show some interesting relationships.



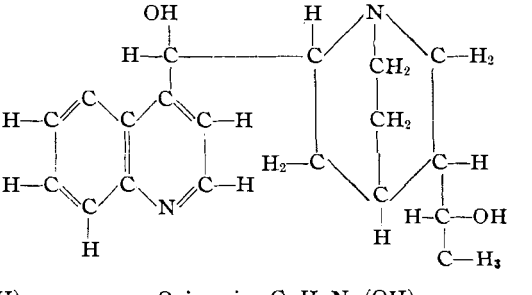
Quinine or Quinidine $C_{19}H_{20}N_2(OH)(OCH_3)$



Cupreine $C_{19}H_{20}N_2(OH)_2$



Cinchonine or Cinchonidine $C_{19}H_{21}N_2(OH)$

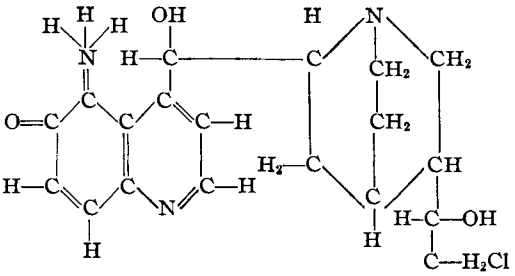


Quinamine $C_{19}H_{22}N_2(OH)_2$

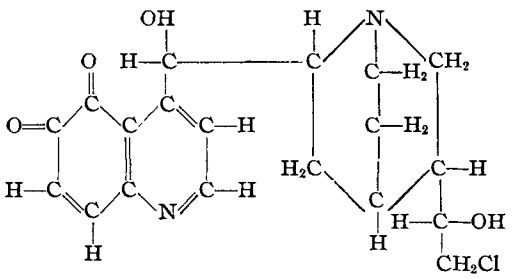
Grimaux and Arnold (5) have converted cupreine into quinine by heating it at 100°, under pressure, with metallic sodium and a solution of methyl chloride in methyl alcohol.

It is readily seen that the main point of similarity in the compounds, which give the reaction, and the main point of difference in the compounds, which do not give it, is in the 6 position of the quinoline nucleus. Those compounds which contain a more or less readily oxidizable group such as (OH) or (OCH_3), in the 6 position of the quinoline nucleus give the reaction, while those compounds which do not contain such a group fail to give it. One of the first observers of this fact was Fühner (6) who, from the fact that the reaction is given by cupreine as well as quinine, deducted that the reaction must be due to the hydroxy-quinoline group. His theory was, that in the first phase of the reaction, the chlorine converts the hydroxyquinoline group into a dichloro-ketone and in the second phase, this is converted by ammonia into a quinonimine coloring matter. He did not investigate or speculate as to the nature of the products formed where the hydroxyl group is methylated as in the case of quinine.

Perhaps the best investigation and the most conclusive evidence as to the nature of the reaction is given by A. Christensen (7). By careful treatment with ammonia water of the nitrate of the base derived from chloro-hydro-quinine he obtained a thalleioquin of the composition $C_{19}H_{21}O_3N_2ClNH_3$ with a possible structural formula of:



The molecule of ammonia is removable by keeping over phosphorous pentoxide in a vacuum, and the remaining substance probably has the structure of chloro 5:6 diketo-hydrocinchonine. The structure of the thalleioquin derived from quinine by the well-known chlorine ammonia method is therefore probably that of chloro 5:6 diketo-hydrocinchonine,



also with the addition of a loosely bound molecule of ammonia.

Evidence is adduced as to the ease with which a chlorine atom in the 5 position adjacent to a hydroxyl group in the 6 position of the quinoline nucleus of such compounds as the above is eliminated by the action of ammonia, the concurrent action of an oxidizing substance causing the formation of the .C : O.C : O. group. On this account it is possible to prepare the thalleioquin analogues from 5-chloro-6-hydroxycinchonine-hydrochloride by the action of silver sulphate or nitrate in a dilute acid medium with the subsequent addition of ammonia. In these reactions, the silver salt serves to effect the removal of the chlorine.

In an analagous manner 5-chloro-6-hydroxy-quinoline has been shown to be oxidizable to a compound which gives a green coloration with aqueous ammonia.

EXPERIMENTAL.

In experimenting with the thalleioquin reaction it was found that if after the addition of bromine water a few drops of a 2% of phenylhydrazine hydrochloride in alcohol were added and 10% ammonia water was subsequently added the mixture assumed a pink to a deep reddish wine color, varying according to the quantity of quinine used.

A careful review of the literature failed to show any record of this or a similar reaction.

After numerous trials the following technique was evolved. To 3–5 mils of an aqueous solution of quinine or its salt add a saturated solution of bromine water drop by drop until the mixture assumes a faint yellowish color. If the quinine contains 0.001 Gm. per mil or more a yellowish white precipitate will form at first but it disappears on the addition of more bromine water. Then add 3–5 mils of a 2% solution of phenylhydrazine hydrochloride in alcohol. After shaking, add a slight excess of 10% ammonia water.

It is essential that enough bromine water be added but not an excess. The best index to the fact that the optimum quantity of bromine water has been added is the asumption of a faint yellowish color by the solution.

The following is some data on the effect of bromine on the reaction.

No.	Wt. of quinine.	Amount of Br water.	Result.
1.	0.0005 Gm.	Deficiency of Br	No action
2.	0.0005 Gm.	Optimum quantity	Pink
3.	0.0005 Gm.	Excess	Greenish yellow
1.	0.001 Gm.	Deficiency	No action
2.	0.001 Gm.	Optimum	Reddish pink
3.	0.001 Gm.	Excess	Greenish yellow
1.	0.003 Gm.	Deficiency	No action
2.	0.003 Gm.	Optimum	Reddish (wine colored)
3.	0.003 Gm.	Excess	Red quickly turning to brown
1.	0.005 Gm.	Deficiency	No action
2.	0.005 Gm.	Optimum	Red
3.	0.005 Gm.	Excess	Red immediately turning to brown

An excess of phenylhydrazine also exerts an inhibiting action on the reaction. However, it was found that 3–5 drops of a 2% solution of phenylhydrazine in alcohol gives excellent results with quinine ranging from 0.0005 to 0.005 Gm.

The bromine water used was a saturated solution. The ammonia water was approximately 10% by volume. All tests were carried out with definite quantities of quinine sulphate. One gram of pure quinine sulphate was dissolved in water and the solution made up to exactly one liter. This gave a 1–1000 solution or one in which one mil represented 0.001 Gm. of quinine sulphate. From this solution other dilutions were made.

By careful manipulation, a test was obtained with 0.00001 Gm. of quinine sulphate. I failed to get a good test with the thalleioquin reaction with this quantity of alkaloid. I have found that the advantages of my reaction over the thalleioquin reaction are quite evident when working with minute quantities of alkaloid. It develops instantly and is more distinct than the thalleioquin reaction.

I also obtained the thalleioquin and my reaction by using a 10% solution of sodium carbonate in place of the ammonia water. This should prove that the primary use of the ammonia is to make the solution alkaline. However, strong alkalies inhibit or prevent the reaction.

None of the common alkaloids give or prevent these reactions except morphine and codeine, which have an inhibiting action.

It was found that if quinine is mixed with an equal amount of morphine or codeine either reaction is given quite readily, but if the proportion is greater, the

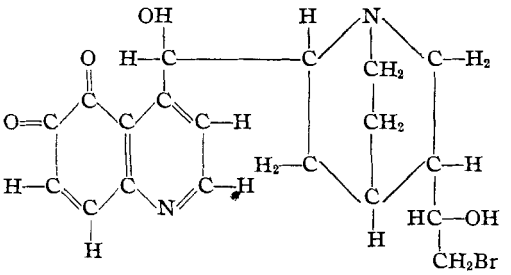
results are uncertain or the reaction may be entirely prevented. The reason for this is not known but it may be due to the reducing properties of morphine and codeine.

Since I have obtained my reaction with chlorine water and calcium hypochlorite and sulphuric acid as well as with bromine water and, since it is given by quinine and not by cinchonidine, I have concluded, that it is a modification of the thalleioquin reaction.

INTERPRETATION OF REACTION.

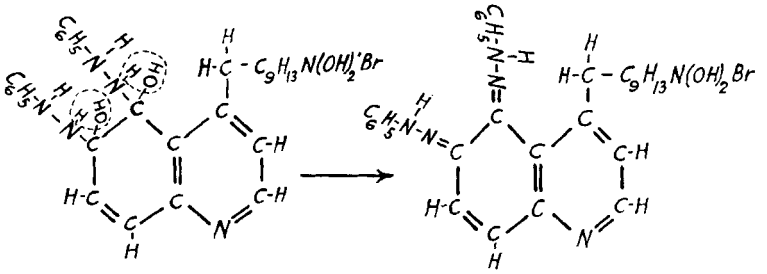
The following is the most logical explanation of my reaction from the facts known and the principles of chemistry involved.

First a diketone is formed by oxidation:



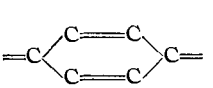
This may be caused by the addition of bromine and its removal by the ammonia or dilute alkali.

The phenylhydrazine then adds to the diketone:

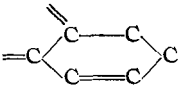


My reaction is also given with acetyl phenylhydrazine. Further research should give more facts in the mechanism of this reaction.

It is interesting to note some of the points in Witt's (8) color theory and their applications to these reactions. He attributes color to the presence of certain unsaturated groups or "chromophores" of which the most important are the following: C=C, C=O, C=N, N=N, N=O, N$\overset{O}{\underset{O}{<}}$. To these the para and orthoquinoid radicles have been added.

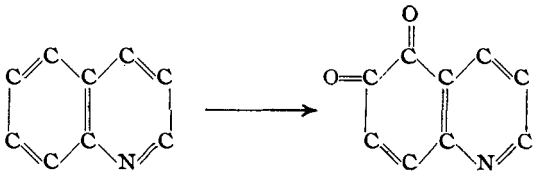


Para-quinoid



Ortho-quinoid

Thus if in the thalleioquin or my reaction, the quinoline ring is oxidized to a diketone, three chromophore groups are produced; two C=O and the ortho-quinoid group.



When phenylhydrazine is added as in my reaction, two of the chromophores are changed and in consequence the color changes.

SUMMARY.

1. The thalleioquin reaction is due primarily to oxidation.

2. A readily oxidizable group at the 6 position of the quinoline nucleus is essential.

3. A modification of the thalleioquin reaction has been found which is more efficient with smaller quantities of alkaloid.

4. The inhibiting action of morphine and codeine is quite evident if the ratio is greater than 1:1.

5. An interpretation of these reactions has been suggested.

BIBLIOGRAPHY.

(1) Vondrasek, *Pharm. Post*, 41, 57–9.
(2) E. Mylius, *Chem. Zentr.*, 602–3 (1886).
(3) E. Pollaci, *Gazetta*, 28, 391–4 (1898); *J. C. S. Ab.*, 657 (1898).
(4) Hyde, *J. Am. Chem. Soc.*, 19, 331–2 (1897).
(5) Grimaux, Arnold, *Compt. rend.*, 112, 774 (1891); 114, 672.
(6) Führer, *Arch. Pharm.*, 244, 602–22 (1906); *J. C. S. Ab.*, 1150 (1907).
(7) A. Christensen, *Ber. pharm. Ges.*, 26, 249–61 (1916). *J. C. S.*, 51 (1917A).
(8) Witt, *Berichte* 9, 522 (1876); 21, 325 (1888).

A PRELIMINARY EXAMINATION OF FOUR NORTHWESTERN PLANTS.*

BY E. V. LYNN AND PAUL Y. CHENG.

The climate of the northwest, and particularly of the state of Washington, is peculiarly adapted to the growth of a large variety of plants as well as to the health and comfort of human beings. It is said that there are more than four hundred varieties of wild flowers in Paradise Valley alone and many which are not found elsewhere in the state. The climate is mild and, at the same time, there are many sorts of meteorological conditions so that almost any plant can be grown within the state boundaries. Many medicinal plants of known value grow here naturally or can easily be cultivated, the drug garden at the University of Washington having been particularly successful for years with medicinal plant propagation.

Many of the native plants appear to possess qualities which would make them valuable medicinally. One can hardly walk along the country road or in a deep wood without being attracted by some strong aroma traceable to a shrub or herb which has never been investigated. Added to these possibilities are the hundreds

* Scientific Section, A. PH. A., Des Moines meeting, 1925.