quate with regard to the present cyclopropane market and methods of production. Any marked change in these factors would undoubtedly call for an immediate review of the method. Therefore, it is clearly indicated that further studies should be undertaken.

Detection of Quinicine and Cinchonicine*

By J. W. Millar and S. J. Dean

The presence of quinicine (quinotoxin) and cinchonicine (cinchotoxin) in preparations containing quinine and cinchonine has frequently been questioned. A number of tests have been proposed, some of which are not satisfactory. This study was undertaken to obtain satisfactory tests for these substances and to determine in each case the minimum concentration in which detection can be made in aqueous and in alcoholic solutions, also when they are present with the unchanged parent alkaloid.

EXPERIMENTAL

Preparation of Cinchonicine and Quinicine.—Employing the method used by Biddle (1), ten grams of cinchonine, ten grams of glacial acetic acid and one hundred and thirty cc. of water were heated to boiling for thirty-five hours. The cooled brownish red solution was treated with an excess of sodium hydroxide and the light brown oil which separated was extracted with ether. The ether solution was dried with calcium carbide. Any unchanged cinchonine, which is insoluble in dry ether, is precipitated when the solution is dried.

The excess of ether was evaporated and the cinchonine dissolved in alcohol or water.

Quinicine was prepared from quinine in a similar manner.

Diazo-Benzene Sulfonic Acid Reagent.—Biddle (2) makes use of this reagent for the identification of cinchonicine and quinicine.

Procedure: two cc. of a freshly prepared saturated aqueous solution of diazobenzene sulfonic acid rendered alkaline with a few drops of sodium hydroxide is added to ten cc. of a solution of quinicine. The color produced varies from a light pink to a reddish violet according to the amount of quinicine present. The color must appear within five minutes. The minimum concentration of quinicine in aqueous solutions yielding distinctive tests was found to be 1 part in 12,500.

Alcoholic solutions reduce the sensitivity of the test to 1 part in 2500.

To determine the delicacy of this test in the presence of soluble salts of quinine, saturated solutions of the hydrobromide, hydrochloride and sulfate containing known amounts of quinicine were used.

The minimum concentration of quinicine yielding satisfactory tests was 1 part in 6250.

Tests performed with cinchonicine and diazobenzene sulfonic acid in the same manner in aqueous solution, yielded distinctive tests in dilutions of 1 part in 6250.

In alcoholic solution, the sensitivity was 1 part in 3000.

With saturated solutions of salts of cinchonine, the sensitivity of the test was 1 part in 3500.

Dinitro Thiophene Reagent.—Biddle (3) also made use of this reagent for the identification of quinicine and cinchonicine.

It is prepared by dissolving dinitro thiophene in pure nitro benzene (1:200).

To two cc. of the reagent is added a few drops of alcohol and then the alcoholic or ethereal solution of the substance to be tested.

The color produced varies from a light orange to a deep purple-red according to the amount of toxin present. The solution tested must be neutral and the test is unreliable in the presence of salts of quinine or cinchonine.

The minimum concentration of quinicine yielding distinctive tests was 1 part in 2700. In the presence of quinine alkaloid the sensitivity was 1 part in 480.

With cinchonicine, tested in the same manner, the minimum concentration yielding distinctive tests was 1 part in 1740, and in the presence of cinchonine alkaloid the sensitivity was 1 part in 600.

Lipkin's Test.—Lipkin (4) describes the following test to differentiate between quinine and quinicine:

"To 5 cc. of the solution to be tested is added 2–3 drops of a 0.5% aqueous solution of Congo Red. Bromine water is added until a yellow color is obtained; ammonia added immediately and the solution extracted with chloroform. Quinine yields a green color in the chloroform extract and quinicine a red color."

Tests were made replacing Congo Red in the above test with other indicators, *viz.*, brom-phenol blue, brom-cresol green, methyl red, thymol blue, phenol red, brom-thymol blue, brom-cresol purple, and with each indicator the resulting color was the same for quinicine and no color change for cinchonicine.

The same test was made on quinicine and cinchonicine omitting the indicator; the same results were obtained.

It is suggested that this test (no indicator being used) may be used for the differentiation of quinicine and cinchonicine.

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* A contribution from the laboratories of the College of Pharmacy, University of California, Medical Center, San Francisco, California.

Ball's Test.—Ball (5) proposed the use of potassium ferrocyanide as a reagent for cinchonine.

The test is described as follows: "Potassium ferrocyanide if added to the solution of a salt of this alkaloid, produces a yellowish white curdy precipitate, which is dissolved upon the application of a gentle heat, but is again deposited, when the liquid cools, as an abundant crop of golden yellow crystals; a slight excess of ferrocyanide should be used."

This test, with some modifications was used on quinicine and cinchonicine. The test as used was: to an aqueous solution of the toxin, add an equal volume of dilute sulfuric acid (6*N*) and then an excess of a solution of potassium ferrocyanide.

Quinicine yields a brilliant yellow precipitate not dissolved by boiling.

Cinchonicine yields a salmon pink precipitate not dissolved by boiling.

Sensitivity for both toxins 1:60.

The phenylhydrazine and nitrous acid tests of Ganassini (6) were tried and discarded as unsatisfactory.

SUMMARY

1. Diazobenzene sulfonic acid reagent gives reliable tests for quinicine and cinchonicine in aqueous or alcoholic solutions and in the presence of the parent alkaloid or alkaloidal salts.

2. Dinitro thiophene reagent gives reliable tests for quinicine and cinchonicine in alcoholic or ethereal solution and in the presence of the parent alkaloid, but not in the presence of the alkaloidal salts.

3. The modified Lipkin test may be used to differentiate between quinine and quinicine and between cinchonine and cinchonicine.

4. The modified Ball test may also be used to differentiate between quinicine and cinchonicine.

5. The phenylhydrazine and nitrous acid tests are less satisfactory than others described.

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"Clearness is the ornament of profound thought"—Vauvenargues

The Stabilizing Effects of Antioxidants upon Solution of Tannic Acid*

By K. P. DuBois† and C. O. Lee‡

The decomposition of tannic acid solutions is believed to be due, in part at least, to oxidation. This belief suggested the possibility of using antioxidants as protective agents for such solutions. As a result of our study we believe that very satisfactory stabilizing agents have been found for tannic acid solutions.

In so far as we were able, we applied the information which had been obtained in various other antioxidant studies to the problem of stabilizing tannin solutions. It was realized that an antioxidant must be very active in order to be effective in stabilizing tannin solutions because they themselves act as very active antioxidants under certain conditions.

EXPERIMENTAL

Assay Method for Tannin Solutions.—The effectiveness of the various antioxidants is dependent upon their ability to prevent the loss of tannin in tannic acid solutions. This makes it necessary to determine the tannin content of such solutions at frequent intervals. A modification of the Lowenthal-Proctor method was found to be satisfactory, especially so with simple solutions of tannic acid (1).

The following tannic acid solution has been recommended for therapeutic use (2). This solution without the salicylic acid was made the subject of our stabilization study.

Tannic acid	100.00 Gm.
Sodium chloride	10.50 Gm.
Potassium chloride	0.42 Gm.
Calcium chloride	0.84 Gm.
Salicyclic acid	1.00 Gm.
Distilled water to make	1000.00 cc.

One hundred cubic centimeter portions of this solution were placed in 120-cc. prescription bottles. To each of 17 of them a different antioxidant was added. In addition one sample was stored under carbon dioxide, another under nitrogen to exclude the air, and a third served as the control.

Effectiveness of Common Antioxidants.—The antioxidants used are listed in Table I. Most of these

* An abstract of a Thesis presented to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Master of Science.

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