ALKALOIDAL REAGENTS. I. INTRODUCTION.*

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In connection with chemical and pharmacological studies of a series of alkaloids and synthetic chemicals in these laboratories, it was necessary to develop characteristic qualitative tests by which each of the alkaloids could be positively identified, especially in cases where very small amounts of material are available. It is also desired to identify these alkaloids in the presence of other alkaloids, in complex mixtures and in urine or other body fluids.

Literature contains conflicting results of tests with common alkaloidal reagents. We investigated the behavior of some of these reagents with a number of simple benzene derivatives, to determine if any relationships of the chemical nature and relative positions of the substituent groups in the ring could be determined in this manner.

The investigation has been extended to include more complex benzene derivatives and analogous derivatives of naphthalene, pyridine, quinoline or other cyclic systems. In the light of the information obtained, a study is being made of the behavior of these reagents with the well-known alkaloids and synthetic chemicals. Studies are undertaken upon compounds of known composition, to correlate their reactions with definite chemical structure. If this phase of our studies proves satisfactory, we plan to attack those alkaloids whose structure is not known at present, with the hope of shedding some light upon their chemical constitution.

STANDARD PROCEDURE.

In order to obtain comparable results, a standard procedure was adopted and used in every test except where otherwise noted.

1. PREPARATION OF REAGENTS.

The standard textbooks and references in toxicology give more or less detailed instructions for the preparation of the alkaloidal test reagents (1, 3, 7). In attempting to use these directions, we have found that close attention to a number of details, such as purity of reagents, order of mixing, and accurate weighing of the constituents, is very important in order to reproduce reagents having uniform sensitivity in successive lots. The preparation of the reagents used in this investigation is given in detail. We used U. S. P. or chemically pure chemicals throughout. Pertinent remarks and comments based upon our experience in the preparation of these reagents follow:

(1) Sulphuric Acid: concentrated sulphuric acid, U. S. P., Sp. Gr. 1.83 at 25° (corresponding to 93% of H_2SO_4).

(2) Nitric Acid: Concentrated nitric acid, U. S. P., Sp. Gr. 1.40 at 25° C. (corresponding to 67% of HNO_3).

(3) Froehde's: Ammonium molybdate in concentrated sulphuric acid (1, 2, 3, 4).

Various ratios of molybdic acid or its salts to sulphuric acid are given in the literature. We have consistently dissolved 2 Gm. of ammonium molybdate

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in 100 cc. of concentrated sulphuric acid (Reagent 1). This solution was stored in a glass-stoppered bottle at room temperature, and opened from time to time for use. We have not found any change in sensitivity over a period of a month.

(4) Marquis: Formaldehyde in concentrated sulphuric acid (1, 4).

We added 1 cc. of U. S. P. Liquor Formaldehyde (containing not less than 37% of HCHO) to 100 cc. of concentrated sulphuric acid (Reagent 1). This was stored in a glass-stoppered bottle and opened from time to time for use.

(5) Mayer: Mercuric potassium iodide (1, 4).

We dissolved 1.358 Gm. of mercuric chloride in 60 cc. of distilled water, and separately dissolved 5 Gm. of potassium iodide in 10 cc. of distilled water. These two solutions were mixed, usually by adding the mercuric chloride solution to the potassium iodide solution, and the volume made up to 100 cc. Several lots of this reagent prepared for use at different times were found to vary in sensitivity. Upon further study, we learned that the ratio of mercuric chloride to potassium iodide must be carefully maintained. Any excess of mercuric chloride produces a precipitate. Any excess of potassium iodide reduces the sensitivity of the reagent. In order to produce a reagent of the maximum sensitivity, we carefully weighed out pure chemicals on an analytical balance. A few particles of powdered mercuric chloride are added one by one, until a precipitate is obtained which fails to dissolve on shaking. This precipitate is then dissolved by the addition of the smallest possible quantity of potassium iodide. We have not observed any change in the sensitivity of this solution over a period of a month.

(6) Dragendorff: Potassium bismuthous iodide (1, 4).

We dissolved 8 Gm. of bismuth subnitrate in 20 cc. of nitric acid, Sp. Gr. 1.18 (corresponding to 30% of HNO_3), and poured this solution gradually into a solution of 22.7 Gm. of potassium iodide in 16 cc. of water. This mixture was allowed to stand until no further precipitation of potassium nitrate occurred, then decanted and made up to a volume of 100 cc. with water. The solution was stored in the dark. This was used in this concentration, and not diluted for tests.

(7) Wagner's Reagent: Iodine potassium iodide.

Following the proportions outlined in U. S. P. X, we dissolved 2 Gm. of iodine and 6 Gm. of potassium iodide in distilled water and made up to a total volume of 100 cc.

(8) Picric Acid:

We dissolved 1 Gm. of picric acid in 100 cc. of hot distilled water and allowed to cool to room temperature; with our material, complete solution was obtained, and filtration was not necessary.

(9) Ferric Chloride:

We prepared a tenth-normal solution by dissolving 0.54 Gm. of anhydrous ferric chloride in distilled water and making up to a volume of 100 cc. In case

the hydrated form of ferric chloride is used, a quantity of 0.9 Gm. of the hexahydrate is required.

(10) Potassium Dichromate:

We prepared a tenth-normal solution by dissolving 1.47 Gm. of potassium dichromate in water, and making up to a total volume of 100 cc.

(11) Potassium Ferrocyanide:

We prepared a tenth-normal solution by dissolving 1.056 Gm. of the trihydrate in distilled water and making up to a total volume of 100 cc.

(12) Potassium Permanganate:

We prepared a tenth-normal solution by dissolving 1.58 Gm. of potassium permanganate in distilled water, and making up to a total volume of 100 cc.

(13) Chlorplatinic Acid:

We prepared a tenth-normal solution by dissolving 2.59 Gm. of the hexahydrate in distilled water and making up to a volume of 100 cc. Because of the great hygroscopicity, we found it advisable to weigh out approximately this quantity from a glass-stoppered weighing bottle, determining the exact quantity from the difference in weight before and after removal, and to dilute to a proportionate volume with distilled water.

(14) Schiebler: (Folin-Wu Uric Acid); Phosphotungstic acid (6).

We shook 10 Gm. of sodium tungstate with 75 cc. of distilled water until it was dissolved. Any undissolved residue was filtered off, and the solution boiled gently under a reflux condenser with 8 cc. of phosphoric acid U. S. P. (containing 85% of H_3PO_4), Sp. Gr. 1.71, for about 2 hours. The solution was then cooled and made up to a total volume of 100 cc.

(15) Uranium Acetate.

We prepared a tenth-normal solution by dissolving 2.12 Gm. of the dihydrate in water, and making up to a total volume of 100 cc.

(16) Mecke: Selenium dioxide (SeO_2) (1, 4).

We dissolved 0.5 Gm. of selenium dioxide in 100 cc. of concentrated sulphuric acid (Reagent No. 1).

(17) Millon: Mercurous nitrate (1).

We dissolved 25 Gm. of metallic mercury in 25 Gm. of nitric acid U. S. P., Sp. Gr. 1.40 (corresponding to 67% of HNO_3), (Reagent No. 2), with gentle warming until fumes were no longer produced. This solution was then cooled and 50 cc. of distilled water added. The solution was allowed to stand for several weeks before use, then decanted from the precipitate which had formed.

(18) Erdmann's Sulphuric-Nitric acid mixture (1).

In our early work we added a trace of nitric acid (Reagent No. 2) to concen-

trated sulphuric acid (Reagent No. 1). The results obtained correspond so closely with results following the use of the individual constituents that this mixture was discarded.

2. PREPARATION OF TEST SOLUTIONS.

The compounds to be tested were prepared as 100-millimolar (tenth molar) solutions in freshly distilled water. Saturated aqueous solutions of the less soluble compounds were made at room temperature. The hydrochloride salts of the amines were used. Tests were made immediately after the preparation of the solutions to avoid any possibility of chemical change on standing.

3. STANDARD METHOD OF MAKING TESTS.

Approximately one milliliter of the test solution was introduced into a (1 x 10 cm.) test-tube. The reagent was added one drop at a time, and the test-tube shaken after each addition. If no precipitate or color change was observed when approximately 1 ml. of the reagent had been added, a negative result was recorded. When a positive result was obtained, the 100-millimolar solution was successively diluted to 10, 5, 1, 0.5 and 0.01 millimolar solutions and tested in turn. The highest of these dilutions at which the reaction was observed is recorded in millimoles per liter. In cases of compounds not soluble to the extent of 100 millimoles per liter, only the results obtained with a saturated solution are recorded. No attempts were made to determine the millimolar concentration actually tested and no tests were made upon dilutions of this saturated solution.

In cases where a precipitate was obtained, one drop of solution and one drop of reagent were mixed on a glass slide and the precipitate was examined under the microscope after several minutes (7). The precipitate is reported as amorphous or crystalline. No attempt was made to classify the type of precipitate obtained.

Because of conflicting reports in the literature upon color reactions as a means of identification of alkaloids, this type of test has fallen into disrepute. However, our experience has shown the necessity for close attention to a large number of apparently unimportant details in the proper determination of color as well as precipitate responses to these reagents and it is our belief that consistent and concordant comparative results can be obtained by the use of a uniform technic. We have compared the shades of color produced with the color standards given in Mulliken, after we learned the desirability of adopting a uniform reference standard (8).

We determine the color by holding the test-tube against a white back ground, at arms length from the observer and at eye level, with a source of diffused sunlight directly in back of the observer. The color is matched as nearly as possible with those given in Mulliken's color standard and the color is recorded in terms of the color and shade or tint designated on the standard. Where the color in the test-tube comes between two colors or comes between two shades or tints, both are recorded, the nearest one being written first. For example:

Sheet C, between RO and O.
Sheet C, YO, between tint 1 and tint 2.

CONCLUSIONS.

1. Standard methods of preparation of alkaloidal reagents and test solutions, as well as a standard technic for testing color and precipitate development, is outlined.

2. The necessity of closely following this standard technic is emphasized.

3. By this procedure, definite and characteristic tests for the benzene compounds, synthetic chemicals and alkaloids are being developed.

4. The effect of place and position isomerism upon reactivity is also being followed.

5. It is hoped that this procedure will shed some light upon the chemical composition of alkaloids whose structure is not known at the present time.

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ABSTRACTS OF PAPERS READ BEFORE SCIENTIFIC SECTION A. PH. A.

Influence of the Alkaloidal Purification Procedure in the Assay of Alkaloidal Drugs, by Geo. E. Éwe.

Incomplete alkaloidal purification procedures in the assay of alkaloidal drugs may cause inaccurate results due to inclusion in the alkaloidal residue of titratable calcium, magnesium and ammonium compounds. Sodium Carbonate or Bicarbonate cannot be used to replace ammonia in an effort to eliminate the above-mentioned interfering substances as their low efficiency in liberating alkaloids from plant tissues. The U. S. P. alkaloidal purification process ("complete" procedure) essentially eliminates these interfering substances but even residues so purified may still retain traces of calcium and magnesium, although the proportion is not significant for ordinary assay purposes.

The Pharmacognosy, Chemistry and Pharmacology of Viburnum. III. Viburnum Opulus var. Americanum. Its History, Botany and Pharmacognosy, by H. W. Youngken.

The history of past investigations of the taxonomy, chemistry and uses of this plant and its medicinal bark. The results of new observations by the author on botanically authenticated entire shrubs of this variety of *Viburnium Opulus* and its bark are described under the following captions: (1) Description of entire plant, (2) Description of root bark, (3) Histology of the root at varying levels from the tip, (4) Powdered root bark, (5) Description of the stem bark, (6) Histology of the stem at varying levels from terminal bud to base, (7) Histology of the old stem bark, (8) Powdered stem bark. Distinctions are given between the American and European varieties of *Viburnum Opulus.*