EFFECT OF HEAT ON THE MYDRIATIC ALKALOIDS APPROXIMATING THE CONDITIONS ENCOUNTERED IN THE ASSAY OF THE CRUDE DRUG.

Samples of pure *d*- and *l*-hyoscyamine hydrobromide, *l*-scopolamine and atropine sulphate were dissolved separately in water, ammonia added, and the alkaloids extracted with chloroform. The chloroform was evaporated to 5 cc., standard acid added and the solution titrated after the chloroform had been removed by evaporation. After titration each sample was made alkaline with ammonia and reëxtracted with chloroform. The chloroform was completely evaporated at water-bath temperature and the residue heated for five minutes. Three cc. of chloroform were added to redissolve the residue, and the heating continued for ten minutes after the evaporation of the chloroform. A mixture of these alkaloids was then made and the same procedure applied. The results are given in the foregoing table.

CONCLUSIONS.

1. The present official and proposed methods of assaying Hyoscyamus do not give concordant results.

2. The varying results obtained by chemists are due to volatile bases originally present, or formed during the assay, and extracted with the alkaloid, giving unusually high results.

3. Evidence was found to substantiate both the ammonia and amine contentions and proof is given, within reasonable limits of experimental error, that the alkaloids of Hyoscyamus are not affected by exposure to the heat of the water-bath for fifteen minutes.

4. It is recommended that the alkaloidal residue be heated for fifteen minutes at water-bath temperature, adding two successive portions of five cc. of chloroform during the heating.

THE ASSAY OF HYOSCYAMUS.

BY H. G. DEKAY AND C. B. JORDAN.

(*Continued from April Journal, page 322.*)

III. COLLABORATION WORK.

Samples of the Hyoscyamus which we used in our experiments were submitted to a number of collaborators. They were asked to perform the following assays:

1. Hot Extraction Process (6).—Place 25 Gm. of Hyoscyamus in No. 60 powder in a thimble, transfer to a Soxhlet apparatus and moisten with a mixture of 8 cc. of stronger ammonia water, 10 cc. of alcohol and 20 cc. of ether, mix thoroughly, and macerate over night. Extract for 3 or 4 hours on a water-bath using ether as a solvent, evaporate the extract to about 15 cc. and then add 10 cc. of approximately *N*/10 sulphuric acid and 10 cc. of water and continue the evaporation until the ether is removed. Filter into a 100-cc. graduated flask, dissolve the chlorophyll residue in chloroform, add acidulated water and evaporate until the chloroform is removed, then filter through the same filter into the graduated flask and make up to volume. Make basic with ammonia T.S. and extract the alkaloids by shaking out with chloroform. Test for complete extraction. Evaporate or distil the chloroform to low volume, then to dryness on a water-bath and keep at this temperature for 15 minutes. Dissolve the residue in chloroform, evaporate to dryness on a water-bath and continue heating for 15 minutes. Repeat this for the third time. Take up the final residue in chloroform, add 10 cc. of *N*/50th acid, remove the chloroform by evaporation and titrate the excess acid with *N*/50th base using methyl red as an indicator.

2. Method 2.—This was the U. S. P. X process with the following modifications: The

extractive was subjected to the purification process suggested. The final chloroform extract was subjected to the same heating process as given in foregoing.

The following results were obtained (7):

Worker.		Method 1.	Method 2.
1.		0.0406%	0.0409%
2.		0.0462%	0.0673%
3.	A	0.0452%	0.0272%
	B	0.0455%	0.024 %
4.	A	0.051 %	0.0505%
	B	0.0517%	0.047 %
5.	A	0.0416%	0.0576%
	B	0.0393%	0.052 %
6.	A	0.047 %	0.0867%
	B	0.041 %	0.0717%
7.	A	0.046 %	0.032 %
	B	0.044 %	0.031 %
	Average	0.0445%	0.0452%

The letters A and B designated two different assays of the drug sent a month apart.

Method 1 has been recommended for consideration to the Sub-committee on Crude Drug Assay of the U. S. P. Revision Committee.

From the results of these experiments we have come to the following conclusions: *1.* That there is a volatile basic constituent obtained in the assay processes and *this gives the isonitrile test; 2,* the present U. S. P. X process probably does not extract much of this basic constituent; *3,* if stronger ammonia water is used in this process, the basic constituent is extracted and the results are high; *4,* the process recommended by J. J. Durrett gives results too high because it extracts these volatile bases; *5,* the Watkins and Palkin purification process has decided advantages in securing sharp end-points in titration.

IV. TO DETERMINE BASIC CONSTITUENT.

The problem now confronting us was the separation and identification of the volatile basic constituent or constituents which were evidently the cause of the discrepancies in the results of the assay processes as given on page 2 and the variation of results during our experiments.

In order to obtain the volatile material, warm dry air was passed over the residues obtained upon the evaporation of the final chloroform extraction and passed through a weak acid solution. A special drying apparatus was arranged in which the air was dried by passing it through a wash bottle of sulphuric acid, then through a calcium chloride and soda lime tower and then through a "U" tube which was heated by the water-bath. The warm dry air was passed over the chloroform residue contained in a flask, which was heated at water-bath temperature, and then passed through a dilute hydrochloric acid solution. Blank determinations were made to test the apparatus. A number of experiments were then made as follows:

Experiment 1. A 50-Gm. sample of drug was placed in a thimble in a Soxhlet apparatus, macerated and extracted according to the method on page 391. The extract was purified and filtered into a 100-cc. volumetric flask and then divided into two aliquot parts (A and B). These were made basic with ammonia water, shaken out with chloroform and the assay completed.

The purpose of this experiment was to test the personal factor of the operator. Percentages are expressed in terms of alkaloids.

Sample.	(A.)	(B.)
1.	0.1225%	0.1217%
2.	0.1366%	0.1326%
3.	0.1272%	0.125 %

The personal factor can be disregarded.

Experiment 2. 50-Gm. samples of the drug were extracted as in Experiment 1 and the extract was divided into two aliquot parts. (A) was assayed in the usual manner, and (B) was made basic and extracted with chloroform, which was evaporated to about 2 cc. and placed in the drying apparatus with the temperature controlled at 40° C. and the drying continued for 1 hour, the air being passed through a standard acid solution. The final residue was taken up in chloroform, standard acid added, chloroform removed by evaporation and the assay completed. Percentages are in terms of alkaloids.

Sample.	(A.)	(B.) Dried Residue.	Volatile Portion.
1.	0.1274%	0.0901%	0.0241%
2.	0.1352%	0.0836%	0.0433%

The above results clearly indicate a volatile base.

Experiment 3. Experiment 2 was repeated and the final chloroform residue dried at 60° C. over a period of 1 hour. Percentages are in terms of alkaloids.

Sample.	(A.)	(B.) Dried Residue.	Volatile Portion.
1.	0.1352%	0.0665%	0.0125%
2.	0.146 %	0.0557%	0.0566%

In order to secure larger quantities of volatile basic material it was necessary to prepare a large Soxhlet apparatus which would extract 400 to 500 Gm. of drug. A large percolator was fixed so that a continuous extraction could be made. A number of samples were assayed according to the hot extraction process and the final chloroform residues dried in the apparatus and the volatile material collected in dilute hydrochloric acid.

The hydrochloric acid solution was evaporated to dryness and a small amount of amorphous white powder-like material was obtained having a fishy odor and giving an isonitrile reaction for primary amines. It was necessary to combine several fractions before a sufficient quantity of the material for qualitative investigations was obtained.

The following investigations were made: *1.* An organic analysis showed the presence of carbon, hydrogen, nitrogen and chlorine, the last being due, it is assumed, to the hydrochloric acid used; *2,* the white powder gave no definite melting point and the variations of the melting point led us to believe that this was a mixture. The material was made basic, extracted with chloroform and the chloroform removed below 0° C. A small amount of a volatile liquid was obtained which possessed a strong fishy odor, was basic to litmus and volatilized between 3° and 7° C. *3.* The qualitative separation for mixtures of amines given by Kamm (10) was used. A tertiary amine fraction was obtained whose hydrochloride changed at 185° C. and then decomposed at 271–275°. A chloroplatinate was made and after recrystallization it decomposed at 240–245° C. The result of these determinations led us to classify this material as trimethylamine. *4.* Attempts were now made to recrystallize the amorphous material from dilute hydro-

chloric acid and two types of material resulted, one a silky, needle-like crystal, and the other an amorphous mass. The needle-like crystals were carefully collected by mechanical separation and they gave a sharp melting point of 171° C. These crystals were made basic and extracted with chloroform, after which they were evaporated below 0° and we again obtained a small amount of liquid having a fishy odor, basic to litmus and volatilizing between 3° and 7° C. We believe that this compound is dimethylamine.

A further investigation of the presence of bases in the crude drug was made as follows: 500 Gm. of drug were macerated over night with 1/2 to 1% NaOH solution, then steam distilled and four liters of distillate were collected in dilute hydrochloric acid. The distillate was evaporated to a syrupy consistency. It became dark brown in color and gave the characteristic odor of the drug. The syrupy residue was diluted with distilled water and clarified by the use of charcoal. It was then evaporated to dryness and a copious white precipitate was obtained.

The precipitate showed the presence of chloride and ammonia and gave an isonitrile reaction. Any basic material present was separated from ammonium chloride by extraction with hot absolute alcohol. About 0.1 Gm. of alcohol-soluble material was obtained. The alcohol-insoluble material did not respond to the isonitrile test. Further tests proved this to be ammonium chloride. The alcohol-soluble residue was again treated with hot alcohol in hopes to make a further separation, by fractional precipitation, and a small amount of material was obtained which *gave the isonitrile test.* Melting-point determinations were made which were variable showing a slight change at 170°, again at 185° and apparent decomposition at 265°. A chloroplatinate was prepared which was apparently only one substance as examined under the microscope. This derivative decomposed at 236–242°. The remainder of the base was dissolved in water, made basic with ammonia T.S. and extracted with chloroform which was removed in the cold. A small amount of a viscous liquid was obtained which possessed a fishy odor and volatilized at 3° to 7° C., again leading us to believe that trimethylamine was present.

Since the W. & P. extraction process (macerating over night with stronger ammonia water followed by hot extraction) gave much higher yields of basic material than were secured in the U. S. P. X method and since a mixture of amines was obtained in these residues, we were led to believe that these amines might be due to decomposition of some basic material during the assay process. The presence of trimethylamine is not uncommon in those plants containing choline as a constituent. According to Kunz (8), Tschirch (9) and Pictet and Biddle (11), choline has been found in *Hyoscyamus niger*. We tested the drug for the presence of choline by the process outlined by Kunz (8) for the isolation of choline from extracts of Belladonna.

We isolated a product, by this procedure, which gave the following reactions: A precipitate was obtained with Mayer's reagent which melted at 110°. A chloroplatinate was prepared which decomposed at 240–242° C. The material was treated with moist silver oxide and a distinct trimethylamine odor was obtained. These same tests were performed upon known choline hydrochloride obtained from Eastman Kodak Company and the results checked those which we had obtained upon our product. We therefore believe that this verifies the presence of choline in *Hyoscyamus niger* as reported by Kunz and others.

The decomposition products of choline are usually given as trimethylamine and glycol. This led us to treat known choline hydrochloride by the hot extraction process given previously. The final chloroformic extract was divided into two equal parts. The first was evaporated to about 2 cc., placed in the drying apparatus and warm dry air passed over it for an hour, and the air passed through a weak hydrochloric acid solution. This hydrochloric acid solution gave tests for trimethylamine and a faint isonitrile reaction. The second portion was evaporated to low volume, standard acid added, chloroform removed by evaporation and excess acid titrated using $N/50$ NaOH. A basic material was definitely indicated.

SUMMARY AND CONCLUSIONS.

1. The alkaloids of Hyoscyamus are much more stable than they are usually assumed to be. When chloroform solutions of them are evaporated they can be heated at the temperature of the water-bath one or two hours without decomposition.

2. The hot extraction process recommended to the Revision Committee gives much too high results because of the fact that volatile bases other than alkaloids are extracted and assayed as alkaloids. In a smaller measure the same is true with the U. S. P. X process.

3. The presence of choline in Hyoscyamus has been reaffirmed.

4. The discrepancy between the results of the hot extraction process and the U. S. P. X process of assay is presumably explained by the fact that the former because of the presence of stronger ammonia water and heat decomposes more of the plant choline.

5. Trimethylamine and a primary amine were found in the residues secured by the hot extraction process.

6. Indications of the presence of dimethyl were obtained but not confirmed.

7. Any assay process for Hyoscyamus must take into consideration the elimination of volatile bases, other than alkaloids, that may be extracted.

We recommend the following assay process:

Place 25 Gm. of Hyoscyamus in No. 60 powder in a thimble, place the thimble in a Soxhlet extractor and moisten with a mixture of 8 cc. of stronger ammonia water, 10 cc. of alcohol and 20 cc. of ether, mix thoroughly, macerate over night, then extract for not less than 3 hours on a water-bath using ether as a solvent. Evaporate the extractive to about 15 cc. and then add 10 cc. of approximately $N/10$ sulphuric acid and 10 cc. of water. Continue the evaporation until the ether is removed. Filter into a separatory funnel, dissolve the chlorophyll residue in chloroform, add acidulated water and evaporate until the chloroform is removed and filter into the funnel through the same filter paper. Make the filtrate basic with ammonia T.S. and extract the alkaloids by "shaking out" with chloroform. Test for complete extraction of alkaloids. Evaporate or distil the chloroform to low volume, then evaporate to dryness on a water-bath and keep at this temperature for 15 minutes. Dissolve the residue in chloroform, evaporate to dryness on the water-bath and continue the heating for 15 minutes. Repeat this for the third time. Take up the residue in chloroform, add 15 cc. of fiftieth normal sulphuric acid, remove the chloroform by evaporation and titrate the excess acid with fiftieth normal base using methyl red as indicator.

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ISOLATION AND IDENTIFICATION OF SUCROSE FROM SENEGA.*[1]

BY RALPH BIENFANG.

As early as 1836, Price made mention of a sugar-like substance which he noticed in an extract of senega. In 1894 Guillaume-Gentil reported that he obtained a carbohydrate, polygalite, using a method projected by Chodat in obtaining it from the root of Polygala amara. Characters for this substance given by Guillaume-Gentil were, melting point 138°, easily soluble in water, hot alcohol and practically insoluble in ether. Fehling's solution had no immediate effect upon an aqueous solution of it, but after 24 hours, a red sediment was noticed in the bottom of the tube. In 1896, Schroeder in this country reported the presence of 5.82% of sucrose, and in 1896 Kain in Germany likewise reported sucrose as a constituent of senega.

Seven Kg. of senega in a No. 20 powder were first extracted with petroleum ether and then with alcohol. The alcoholic extracts were turbid, and so were filtered before being concentrated. The concentrated alcoholic extract was put into glass containers and allowed to stand at room temperature for two weeks. At the end of this time an appreciable crystalline deposit had formed on the sides and bottoms of the containers. This was broken up, filtered out, redissolved in diluted alcohol, and shaken with animal charcoal until a colorless syrup was obtained. In this syrup, upon standing, crystalline blocks were formed. These crystals obtained were sweet and so some of the material was tested with the Molisch reagent for carbohydrates. The result was positive. With Fehling's solution a negative result was obtained. The crystals were soluble in water and hot alcohol, but insoluble in cold alcohol and ether. The melting point was found to be 186–187° C.; specific gravity 1.5734 at 20° C. A specific rotation of +67.5° became −20.4° after boiling with HCl. An acetate was attempted with the production of a bitter syrup insoluble in water but soluble in alcohol. Glucosazone was formed when it was heated for 46 minutes with phenylhydrazine hydrochloride and sodium acetate.

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[1] From a thesis submitted in partial fulfilment of the requirements for the Ph.D. degree, University of Wisconsin, 1929.