SCIENTIFIC SECTION

THE ASSAY OF HYOSCYAMUS.*[1]

BY MARVAL D. EVANS AND EDWARD D. DAVY.

The conflicting reports which have made their appearance from time to time as to the proper procedure in the assay of mydriatic drugs, particularly *Hyoscyamus*, led to the study here reported. Likewise, results of different analysts did not agree even when the same or a similar method was employed.

A few of these differences of opinion are enumerated. Watkins and Palkin[2] attribute some of the difficulty to the presence of earthy soaps. Stikarofsky[3] suggests that since the alkaloids are sensitive to heat the operator did not observe sufficient caution while evaporating the final solvent. Hester and Davy[4] found in *Datura Innoxia* interfering ammonium compounds. Goris and Larsonneau[5] found that Belladonna leaves contain volatile bases of alkaloidal and non-alkaloidal nature. Éwe[6] called attention to these volatile bases in the assay of Hyoscyamus and offers a method for their elimination. U. S. P. X cautions against the application of heat in evaporating the solvent to avoid decomposition of the alkaloids.

EXPERIMENTAL.

Hyoscyamus samples marketed as U. S. P. were purchased with a view to finding a drug that might yield volatile bases other than ammonia when assayed. The presence of ammonia was definitely established in *Drug I*. Another lot of drug, hereinafter referred to as *Drug II*, was found which yielded volatile, chloroform-soluble material in contrast to the former which was water soluble only. The work on the crude drug was confined to these two samples.

In the results enumerated the U. S. P. X method was followed with modifications of the technique in dealing with the final chloroformic extract.

All results reported are expressed in parts per hundred.

A. U. S. P. X method, with due consideration of the precaution against the application of heat.

I. (*a*) 0.0433 (*b*) 0.0437 II. (*a*) 0.0689 (*b*) 0.0675.

B. U. S. P. X method modified slightly to determine if volatile alkaline substance was present. *Drug I*. The final chloroformic extract was evaporated on a water-bath to 5 cc., then to dryness with continued heating with a current of dry

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* Scientific Section, A. PH. A., Madison meeting, 1933.

[1] Submitted in partial fulfilment of the requirements for the degree of Master of Science at Western Reserve University.

[2] H. R. Watkins and S. Palkin, *J. A. O. A. C.*, 10 (1927), 130.

[3] Albin Stikarofsky, JOUR. A. PH. A., 16 (1927), 30.

[4] Earl E. Hester and Edward D. Davy, *Ibid.*, 22 (1933), 514.

[5] Goris and Larsonneau, *J. pharm. Belg.*; through *Pharm. Era*, 54 (1921), 247.

[6] George Éwe, JOUR. A. PH. A., 21 (1932), 870.

carbon dioxide and ammonia free air, drawn slowly through the flask the vapors being passed into standard acid. The passage of air over the residue and the heat were continued for ten minutes after the chloroform had been completely removed. The contents of both flasks were titrated and calculated as alkaloid.

I.	Alkaloid	(*a*) 0.0306	(*b*) 0.0309
	Volatile material	0.0092	0.0086
	Total	0.0398	0.0395

C. The same procedure was followed as in B except that ammonia-free water was used in the flask receiving the vapors instead of standard acid. The aqueous solutions of volatile material from two samples were Nesslerized and the presence of ammonia established qualitatively. Two additional samples were compared to a standard ammonia. The chloroform fraction showed no basic material.

		(*a*)	(*b*)		(*c*)	(*d*)
I.	Alkaloid	0.0312	0.0296		0.0302	0.0309
	Ammonia qualitative	+	+	(Quant.)	0.0081	0.0053
					0.0383	0.0362

The same procedure was followed with *Drug II* except that equal portions of chloroform and water were used in the flask through which the vapors were drawn, as previous qualitative tests showed this drug yield volatile chloroform-soluble material. The aqueous portion gave a very slight qualitative test for ammonia when Nesslerized. The chloroform portion was titratable.

		(*a*)	(*b*)
II.	Alkaloid	0.0374	0.0364
	Volatile chloroform soluble	0.0161	0.0185
	Total	0.0535	0.0549

The recovery of the volatile material was probably incomplete in both of these cases but the presence of ammonia in *Drug I* and chloroform-soluble material in *Drug II* was established.

D. U. S. P. X method, except that

1. Standard acid was added to the last 5 cc. of chloroform extract after which the chloroform was evaporated on the water-bath. Volatile bases present as ammonia or amines are titrated as alkaloid, since these are not volatilized appreciably until the chloroform is completely evaporated.

I. (*a*) 0.0509 (*b*) 0.0489 II. (*a*) 0.0732.

2. After titration of the samples above (D 1.) each was made alkaline and reëxtracted with chloroform, the chloroform evaporated to dryness and the residual alkaloidal material heated at water-bath temperature for fifteen minutes to eliminate volatile bases.

I. (*a*) 0.0301 (*b*) 0.0290 II. (*a*) 0.0352.

These results compare favorably with those obtained in C. where the heat of the water-bath was applied.

3. In order to determine whether ammonium compounds are formed during the assay each of the samples above (D 2.) was made alkaline with ammonia and reëxtracted with chloro-

form. Standard acid was then added to the last 5 cc. of chloroformic extract. In event ammonium compounds soluble in chloroform are produced during the assay the values of *Drug I* should show a corresponding increase due to reforming the ammonium compounds when heat is not applied to the dry residue. *Drug II* which yielded volatile chloroform-soluble basic material should show no increase since it was eliminated when heated in (D 2.). That is precisely what happened as shown by the results.

I. (*a*) 0.0405 (*b*) 0.0381 II. (*a*) 0.0352.

As a final check on the stability of the alkaloids of Hyoscyamus at water-bath temperature, two samples of *Drug II* were assayed by allowing the final chloroformic extract to evaporate to dryness, and the alkaloidal residue heated for ten minutes on the water-bath, during which time two successive portions of 3 cc. each of chloroform were added to the residue of each sample and evaporated. The alkaloids were determined volumetrically.

(*a*) 0.0370 (*b*) 0.0350

The titrated samples were made alkaline and reëxtracted with chloroform and the same procedure repeated.

(*a*) 0.0370 (*b*) 0.0370

This offers added proof that heat applied for short intervals at full water-bath temperature causes no loss of alkaloids.

BRITISH PHARMACOPŒIA.

While the work previously reported was in progress the Sixth Revision of the British Pharmacopœia became available. Accordingly two samples of *Drug I* were run by the British Pharmacopœial method with the following results:

(*a*) 0.0303 (*b*) 0.0296

The chief objection to the method is the direction to reduce the aqueous solution *in vacuo*. This is attended with considerable bumping and the transfer of liquid from the flask may occasion some loss. On other occasions not recorded here we have used the ether-alcohol solvent in the initial extraction and find this a distinct improvement in the elimination of emulsions which are sometimes encountered with chloroform-ether solvent.

MIXTURE OF ALKALOIDS.

		Original, No Heat Applied.	Extracted Alkaloid, Heated 15 Minutes.
d-Hyoscyamine		0.0312	0.0318
l-Hyoscyamine		0.0300	0.0318
l-Scopolamine	(*a*)	0.0286	0.0284
	(*b*)	0.0248	0.0246
Atropine	(*a*)	0.0243	0.0244
	(*b*)	0.0245	0.0242
Mixture calculated to hyoscyamine		0.0731	0.0729

The residual solutions after titration were tested for chlorides with negative results.

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