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(15) YEAR BOOK, AMERICAN PHARMACEUTICAL ASSOCIATION, 4 (1915), 95.
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THE ASSAY OF HYOSCYAMUS.*

BY H. G. DEKAY[1] AND C. B. JORDAN.

Hyoscyamus has been official in the past four revisions of the U. S. Pharmacopœia, and an assay process has been described in the last three. The changes occurring in this assay have been primarily in that part of it dealing with the extraction of the alkaloids from the crude drug. In the U. S. P. VIII (first official process) the drug was macerated for 10 minutes with a mixture of 1 part of chloroform and 3 parts of ether; then ammonia water was added and the contents agitated during 1 hour. The final extraction was made from a basic mixture by the use of chloroform.

In the U. S. P. IX, the process of extraction was changed as follows: the drug was agitated during 2 hours with 300 cc. of a mixture of 1 volume of chloroform and 3 volumes of ether to which ammonia water had been added. An aliquot part of the immiscible solvent was then decanted and the assay completed as indicated in the first process. The U. S. P. X changed this to a percolation process, the same solvent being used.

Various workers encountered many difficulties in the assay of this drug with the result that a number of processes have been presented for consideration during the past decade.

Watkins and Palkin (1), workers in the Drug Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture, described a method for the assay of Hyoscyamus "which gave a yield of from two to three times as much alkaloid as that obtained by the U. S. P. IX and X methods."

In order to check the various processes for the assay of Hyoscyamus, C. B. Jordan, Dean of School of Pharmacy, Purdue University, Chairman of Subcommittee No. 6, U. S. P. Revision Committee, submitted samples from the same lot of drug in No. 60 powder to a number of experienced chemists for collaborative work. He requested that three processes be used as follows: Process No. 1 (2), the U. S. P. X process; process No. 2, which was the same except that the drug was allowed to macerate over night; process No. 3 (3), recommended by J. J. Durrett, who was, at that time, Chief, Drug Control, the U. S. Food and Drug Administration. The last was a hot extraction process very much like that used by Watkins and Palkin (1) in their work with this drug. This process required a special apparatus.

* An abstract based upon a thesis by H. G. DeKay submitted to the Faculty of Purdue University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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The following results were obtained (4) in terms of percentage of total alkaloids:

Operator.	Process No. 1.	Process No. 2.	Process No. 3.
1	0.072	0.098	0.168
2	0.0381	0.0442	0.092
3	0.0306	0.0345	0.0898
4	0.0535	0.0606	0.1108
5	0.0858	0.080	0.152
6	0.0381	0.0462	0.0722
7	0.072	0.063	0.131
8	0.088	0.109	0.137
9	0.090	0.1014	0.1014

The collaborators pointed out that in processes No. 1 and No. 2 difficulty was encountered during the acid extraction because of coloring matter which could not be removed by filtering. They suggested that in process No. 3 the small amount of alcohol used could explain the increase in yield of the alkaloid. They also stated that it was extremely awkward to use the special apparatus for maceration and extraction and that the extractive was highly colored, making the end-point reading difficult.

These reports indicated that it was difficult to obtain concordant results by either the first or the second processes and that the agreement in the third was far from satisfactory. It is possible that the personal factor plays an important part; if so, an assay process should be devised that would reduce this factor to a minimum.

The chairman of the Revision Committee received a definite recommendation from J. J. Durrett (3) regarding this assay process and the purity rubric of this drug. It was passed on to the chairman of the Sub-committee on Crude Drug Assay. The above-quoted results indicated to him that the whole question should be carefully studied, and this explains the assignment of the problem to H. G. DeKay, who had been working on the assay of Hyoscyamus for about twelve months.

We believed that the higher results obtained by Watkins and Palkin, which we assumed was the basis for J. J. Durrett's recommendation, could be due to one or more of the following reasons: *1.* Failure of the U. S. P. X process to extract all of the alkaloid, presumably the idea of Watkins and Palkin; *2,* the presence in the drug of bases other than alkaloids; *3,* the decomposition of the alkaloids during the assay process; or *4,* the formation of some alkaloidal hydrochloride in the evaporation with chloroform which would indicate a low yield of alkaloid. Therefore, any successful study of this problem should cover these four points.

The following determinations upon the pure alkaloids had been completed previous to H. G. DeKay's special assignment to the problem.

I. TO DETERMINE THE STABILITY OF ATROPINE AND HYOSCYAMINE.

Experiments under A and B were performed by H. G. DeKay, A. C. Smith and C. N. Sprankle, graduate students at Purdue University.

A. TO DETERMINE THE EFFECT OF HEAT ON THE ALKALOIDS.

Experiment 1. Four samples of pure alkaloid were dried at 80° C. for 1 hour without loss.

Experiment 2. The pure alkaloids were dissolved in 2% sulphuric acid, the solution was made basic with ammonia T.S. and extracted with chloroform. The chloroform was evaporated to about 2 cc. on a water-bath and treated as follows, before completing the assay:

	Atropine.			Hyoscyamine.		
	Assays.	Used, Gm.	Recovered, Gm.	Assays.	Used, Gm.	Recovered, Gm.
A. Standard acid was added and the assay completed	8	0.0398	0.0472	2	0.0349	0.0354
B. Dried in a current of air at 40° C.	3	0.0486	0.0472	3	0.0328	0.0328
C. Repeat B and dry the residue 48 hours in a desiccator	2	0.1070	0.1027	2	0.0455	0.0443
D. Evaporate to dryness and heat 5 minutes	5	0.0361	0.0371	5	0.0351	0.0340
E. Repeat D except heat 30 minutes	5	0.0349	0.0349	3	0.0351	0.0300

Conclusion: From the above experiments it is evident that the heating of chloroform solutions of the pure alkaloids at water-bath temperatures does not cause any decomposition.

B. TO DETERMINE THE EFFECT OF CHLOROFORM UPON THE ALKALOIDS.

Experiment 1. The pure alkaloids were dissolved in chloroform and the solution evaporated to 2 cc. and treated as follows, before completing the assay:

	Atropine.			Hyoscyamine.		
	Assays.	Used, Gm.	Recovered, Gm.	Assays.	Used, Gm.	Recovered Gm.
A. Evaporated to dryness on a water-bath and heated (1) 1 hour	2	0.1609	0.1591	2	0.1040	0.1032
(2) over night	2	0.1609	0.1591	2	0.1040	0.1032
B. The assay completed	5	0.0422	0.0435	5	0.0301	0.0297
C. Evaporated to dryness on a water-bath, (1) heated 15 minutes				2	0.0385	0.0411
(2) heated 30 minutes				2	0.0408	0.0411
(3) heated 1 hour				2	0.0413	0.0410
D. Evaporated to dryness in a current of air, dried in a desiccator for 48 hours	3	0.0239	0.0242			

Experiment 2. The alkaloids were dissolved in chloroform and allowed to remain in the solvent (A) 12 hours and (B) 24 hours, respectively, before completing the assay.

		Gm.	Gm.		Gm.	Gm.
(A)	3	0.0419	0.0421	3	0.0301	0.0304
(B)	3	0.0431	0.0431	3	0.0200	0.0202

Conclusion: The above experiments indicate that no hydrochlorides of the alkaloids are formed when their chloroform solutions are evaporated to dryness at water-bath temperatures.

C. TO DETERMINE THE EFFECT OF ASSAY PROCESSES ON THE ALKALOIDS.

Experiment 1. (A) The U. S. P. X assay process for Hyoscyamus was followed, atropine instead of the crude drug being used. (B) Experiment A was repeated except that the final chloroform extract was dried in a current of air at 40° C. and the assay completed.

(A) Results of 2 samples: Used 0.0480 Gm. Recovered 0.0502 Gm.
(B) Results of 2 samples: Used 0.0463 Gm. Recovered 0.0451 Gm.

Experiment 2. Pure atropine was extracted with ammonia water, alcohol and ether in a Soxhlet apparatus for 2 hours, (A) the assay was completed. (B) The final chloroform extract was evaporated to dryness, placed in a desiccator over night and the assay completed.

(A) Results of 2 samples: Used 0.0481 Gm. Recovered 0.0488 Gm.
(B) Results of 2 samples: Used 0.0422 Gm. Recovered 0.0423 Gm.

Experiment 3. (A) An exhausted drug was fortified with equal parts of pure atropine and hyoscyamine, placed in a Soxhlet apparatus and assayed according to the process recommended by J. J. Durrett. (B) The above experiment was repeated, the final chloroform solution was evaporated to dryness on a water-bath, taken up in chloroform and again dried. This was repeated two more times before completing the assay.

(A) Results of 2 samples: Used 0.021 Gm. Recovered 0.0236 Gm.
(B) Results of 2 samples: Used 0.021 Gm. Recovered 0.0215 Gm.

Experiment 4. A definite weight of a mixture of equal parts of pure alkaloids was added to the crude drug and then assayed by the above method.

Results from 3 samples: Added alkaloid 0.0207 Gm. Recovered 0.0194 Gm.

Experiment 5. Experiment 4 was repeated to the point where the final chloroform solution is evaporated to low volume. This extract was evaporated to dryness on a water-bath, redissolved in chloroform and again dried; this was repeated two more times and the assay was completed.

Results from 2 samples: Added alkaloid 0.020 Gm. Recovered 0.0173 Gm.

Conclusion: A careful study of the above experiments indicates:

1. That the alkaloids of Hyoscyamus undergo no change during the regular assay process; *2*, that the alkaloids may be heated at water-bath temperatures without danger of decomposition; *3*, that no hydrochlorides of the alkaloids are formed when chloroform solutions of them are evaporated to dryness; and *4*, that the assay process recommended by J. J. Durrett extracts some volatile base.

Schou and Bjerregaard (5), while working upon sterilization of solutions, found that solutions of atropine could be heated at 120° C. over a period of 20 minutes without danger of decomposition.

Five experiments were completed to determine whether ammonia contaminates the residues from the assay processes. It is apparent that any ammonia which is carried over by the chloroform in the final extraction is eliminated in the evaporation process.

Our results corroborate the work of Watkins and Palkin on the stability of these alkaloids and verifies their conclusion that the residues after shaking out and drying are not contaminated with ammonia.

II. EXPERIMENTS ON POWDERED HYOSCYAMUS.

A 75-pound lot of Hyoscyamus in No. 60 powder was obtained from Eli Lilly & Company for this work. The drug was ground and mixed by the Company. Ash determinations were made with the following results:

Total Ash.	Acid-Insoluble Ash.
23.25%	8.71%
23.29%	8.55%
23.20%	8.67%
23.17%	8.87%

Qualitative inorganic analysis upon the ash showed the presence of potassium, sodium, ammonia and iron as the sulphates, nitrates and chlorides. There were traces of aluminum.

Assay Experiments.—Experiment 1. A sample of drug was subjected to the U. S. P. X process of extraction, stronger ammonia water being used. The extract was subjected to the purification process recommended by Watkins and Palkin as follows: The extract was evaporated to low volume, 10 cc. of 0.05*N* sulphuric acid and 5 cc. of water added and the evaporation continued until the odor of ether had disappeared. The acidified liquid was decanted into a 50-cc. volumetric flask. The residue remaining in the flask was dissolved with chloroform, 5 to 10 cc. of acidulated water added and the chloroform removed by heating. The contents of the original flask were poured into the volumetric flask, which was cooled and made up to volume. The mixture was filtered, the first few cc.'s rejected, an aliquot part collected, made basic with ammonia water, shaken out with chloroform and the assay completed with the following results:

1. 0.079%
2. 0.073%

The titrated mixtures gave positive results for primary amines by the isonitrile reaction.

Experiment 2. Samples were subjected to the U. S. P. X assay process modified by macerating over night and by the use of stronger ammonia water with results as follows:

1. 0.0663%	7. 0.073 %
2. 0.079 %	8. 0.0763%
3. 0.057 %	9. 0.066 %
4. 0.082 %	10. 0.0731%
5. 0.0834%	11. 0.0761%
6. 0.0733%	12. 0.0633%
	Average 0.073 %

The titrated mixtures gave positive results by the isonitrile test.

Experiment 3. Samples of the drug were subjected to the U. S. P. X assay process with the Watkins and Palkin purification process being used in place of shaking out with dilute sulphuric acid.

1. 0.110%	7. 0.087 %	13. 0.122%
2. 0.093%	8. 0.107 %	14. 0.114%
3. 0.092%	9. 0.083 %	15. 0.107%
4. 0.104%	10. 0.109 %	16. 0.113%
5. 0.101%	11. 0.088 %	17. 0.112%
6. 0.094%	12. 0.1225%	Average 0.103%

Positive results were obtained by the isonitrile test.

Experiment 4. Samples of the drug were subjected to Soxhlet extraction after one-hour maceration with U. S. P. solvents and then the assay completed by the W. & P. process.

1. 0.1025%
2. 0.1001%

Positive results were obtained by the isonitrile test.

Experiment 5. The above experiment was repeated except that the residues were dried, before the assay was completed, as follows: (A) On the water-bath; (B) in an oven at 80° for 1 hour.

(A.)		(B.)
1. 0.0627%	4. 0.0428%	1. 0.0319%
2. 0.0564%	5. 0.0440%	2. 0.0424%
3. 0.0553%	Average 0.0522%	Average 0.0371%

The above gave faint isonitrile tests.

Experiment 6. Lots of drug were macerated over night with stronger ammonia water and the U. S. P. solvent, and percolated according to the U. S. P. X process. Then the percolate was subjected to the W. & P. purification process. Later the marc from each sample was extracted in a Soxhlet, alcohol being used as a solvent. Additional basic material was secured.

	Assay.	Basic Constituent Extracted by Alcohol.	Total.
1.	0.122%	0.0178%	0.140%
2.	0.114%	0.021 %	0.135%
3.	0.113%	0.019 %	0.132%

The titrated mixtures gave positive isonitrile tests.

Experiment 7. Three samples were assayed by the W. & P. process, their special apparatus being used.

1. 0.100 %
2. 0.102 %
3. 0.1047%

Experiment 8. Samples of drug were assayed by the W. & P. process, the solvents recommended by them being used, were then macerated over night and were extracted in a Soxhlet apparatus.

1. 0.136%	7. 0.125 %	13. 0.146%
2. 0.132%	8. 0.109 %	14. 0.127%
3. 0.155%	9. 0.1167%	15. 0.135%
4. 0.127%	10. 0.1205%	16. 0.127%
5. 0.120%	11. 0.1025%	17. 0.125%
6. 0.125%	12. 0.135 %	Average 0.127%

All of these titrated residues gave positive results by the isonitrile test.

Experiment 9. Samples of drug were macerated over night with ether-alcohol solvent and stronger ammonia water, then percolated according to the U. S. P. X using ether and the percolate subjected to the W. & P. purification process.

1. 0.1425%	4. 0.1136%
2. 0.1437%	5. 0.132 %
3. 0.1287%	Average 0.132 %

Positive results were obtained by the isonitrile test.

Experiment 10. Four samples of the drug were macerated over night with stronger ammonia water and extracted with the W. & P. solvent in a Soxhlet apparatus. The extract was purified and made up to 100 cc. 50-cc. portions of each lot were assayed.

1. 0.1352%	3. 0.1274%
2. 0.1465%	4. 0.135 %

Positive results were obtained by the isonitrile test.

The residues of the other 50-cc. portions were treated as follows:

(A) They were dried at 40° C. in a current of dry air before the addition of the standard acid;

(B) They were dried at 60° C. in a current of dry air before the addition of the standard acid.

	(A.)	(B.)
1.	0.0901%	3. 0.0665%
2.	0.0836%	4. 0.0557%

Experiment 11. Four samples of drug were macerated over night with stronger ammonia water and extracted with the W. & P. solvents in a Soxhlet apparatus. The extractive was purified, made basic with ammonia water extracted with chloroform and evaporated to dryness on a

water-bath and the residue taken up with chloroform and again evaporated to dryness. This process was again repeated, then taken up in chloroform, standard acid added and the assay completed.

1. 0.0869%	3. 0.107 %
2. 0.0871%	4. 0.0985%
	Average 0.095 %

Positive results were obtained by the isonitrile test.

Experiment 12. Samples of the drug were assayed according to the above experiment, and the final chloroform extract was dried in an oven at 80° C. for 15 minutes.

1. 0.0676%
2. 0.0893%
3. 0.072 %

Positive isonitrile tests were obtained.

Experiment 13. Three lots of drug were macerated for 1 hour with stronger ammonia water and ether, and then extracted in a Soxhlet apparatus, ether being used as a solvent.

The marc from these assays was extracted with alcohol after the addition of small amounts of stronger ammonia water. An additional amount of basic material was obtained.

First Extraction.	Marc Extract with Alcohol.	Total.
1. 0.0894%	0.0382%	0.1276%
2. 0.096 %	0.047 %	0.143 %
3. 0.094 %	0.039 %	0.133 %

Positive isonitrile tests were obtained.

Experiment 14. Eight samples of drug were macerated over night with ether and stronger ammonia water and extracted in a Soxhlet, ether being used as a solvent.

1. 0.077 %	5. 0.089 %
2. 0.0788%	6. 0.089 %
3. 0.067 %	7. 0.096 %
4. 0.072 %	8. 0.0773%
	Average 0.0812%

Positive isonitrile tests were obtained.

Aliquot portions of the last five samples obtained after the purification process were evaporated to dryness on a water-bath and dried 1 hour. The residues were taken up in chloroform, standard acid added, and the chloroform then removed by evaporation before the assay was completed.

4. 0.0413%	7. 0.039 %
5. 0.044 %.	8. 0.036 %
6. 0.039 %	Average 0.0398%

These titrated residues gave no isonitrile test.

(To be continued.)

DI-β-BROMALLYLAMINO ETHYL *p*-AMINO BENZOATE.*

BY W. BRAKER AND W. G. CHRISTIANSEN.

During the course of an investigation of a variety of local anesthetics, we have prepared di-β-bromallylamino ethyl *p*-amino benzoate. This substance is analogous to procaine; the diethylamino group in the latter has been replaced

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