A STUDY OF THE ALKALOIDS OF DATURA INNOXIA, MILLER.*

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A review of the literature revealed that much confusion existed between *D. innoxia* classified and named by Miller in 1768 as recorded in the Eighth Edition of his "Gardener's Dictionary" and that of other *Daturas*. This confusion continued until comparatively recent date as shown by the reviews of Safford[2] and Timmerman.[3]

Datura innoxia, Miller at The Squire Valleevue.

The review, however, revealed nothing with reference to any chemical work having been done upon *Datura innoxia*, Miller, to which this paper is devoted.

The drug used in this work was grown in The Medicinal Plant Garden at The Squire Valleevue. The type of soil is a clay loam, but according to Francis H. Carr,[4] the composition of the soil, fertilizer and seasonal conditions have very little effect upon the alkaloidal content of belladonna, a closely related drug. It was grown and harvested under the direction of Dr. F. J. Bacon. The seeds

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[2] William E. Safford, *Annual Reports of The Smithsonian Institute*, 1920, pages 537–567.

[3] Helen A. Timmerman, *Pharmaceutical Journal and Pharmacist*, 118 (1927), 571.

[4] Francis H. Carr, *American Journal of Pharmacy*, 85 (1913), 487–496.

from which this plant was grown were supplied by Dr. A. F. Sievers of The Bureau of Drug Plant Industry, Washington, D. C.

The leaves were carefully selected and separated from the stems by hand and then milled. The ground leaves were passed through a Number 20 sieve. This size powder was used throughout in the assay, the report of which follows.

In the assays which follow on *D. innoxia*, Mill. the total yield was based upon the molecular weight of atropine which is the practice in Pharmacopœial standardizations for the mydriatic drugs.

Opinions differ greatly as to the proper solvent and other conditions involved in the assay of mydriatic drugs. An effort was made to learn why such discrepancies should occur when obviously it was not a question of the analyst. This led to the use of the following solvents and conditions modifying the conventional method.

GENERAL METHODS.

The U. S. P. X Method of Assay for Strammonium.

Results.

(1) 0.402% (2) 0.394% (3) 0.426%

Modifications of U. S. P. X Method.—A. The final chloroformic extract was evaporated on a water-bath to approximately 5 cc.; standard acid was added, and the remaining chloroform dissipated at water-bath temperature.[1]

Results.

(1) 0.574% (2) 0.509% (3) 0.520%

B. Standard Acid was added to final chloroform extract before any reduction in volume.

Results.

(1) 0.549% (2) 0.567%

C. Drug exhausted until negative to *p*-dimethylamino-benzaldehyde solution.[2] Standard acid added to last 5 cc. of final chloroformic extract.

Result.

0.557%

D. Solvent:

Alcohol	10 cc.
NH_4OH (28%)	5 cc.
Ether	20 cc.

After the addition of the initial solvent in which the drug was macerated, ether was used to exhaust the drug.

1. The final chloroformic extract was allowed to evaporate spontaneously, then permitted to stand three days in a covered beaker after all the chloroform had evaporated.

Results.

(1) 0.438% (2) 0.448%

2. The final chloroformic extract was evaporated on a water-bath to approximately 5 cc.; standard acid was added, and the remaining chloroform evaporated at water-bath temperature.

Results.

(1) 0.542% (2) 0.546%

[1] Suggested by Watkins and Palkin.

[2] YEAR BOOK OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, 4 (1915), 340.

3. Drug exhausted until negative to *p*-dimethylamino-benzaldehyde. Standard acid added to last 5 cc. of final chloroformic extract.

Result.
0.661%

E. Solvent. Chloroform used as the solvent throughout.

1. The final chloroform extract was allowed to evaporate to dryness spontaneously.

Result.
0.417%

2. Standard acid was added to the last 5 cc. of final chloroformic extract, after which the chloroform was evaporated from a water-bath.

Results.
(1) 0.450% (2) 0.461%

3. Drug exhausted until negative to *p*-dimethylamino-benzaldehyde. Standard acid added to last 5 cc. of final chloroformic extract.

Result.
0.713%

The objection to chloroform as a solvent is the tendency toward the formation of air pockets and channeling thereby preventing the chloroform from exerting its maximum solvent action within a limited time.

Upon comparing the results obtained, the differences prompted additional analyses in an attempt to interpret these variations.

F. U. S. P. X solvent for extraction of drug. In the assay of another portion of drug, the final chloroform extract was washed well with water to remove any ammonia that might be present and then passed through absorbent cotton. The chloroform extract was then evaporated on a water-bath to 10 cc. (a measure to insure the complete dissipation of any free ammonia). The flask containing the concentrated chloroform extract was then connected in a manner that "washed air" (air washed successively with dilute H_2SO_4 and caustic soda solution) was drawn through the flask, heated on a water-bath and the vapors passed into standard acid (5 cc.) (0.02 *N* H_2SO_4). After the residue in the flask had been reduced to dryness the heat and passage of washed air over the alkaloidal residue were continued ten minutes longer.

The flask containing the standard acid was then heated on a water-bath to volatilize the chloroform. It was cooled to room temperature and diluted to exactly 50 cc. Ten cubic centimeters were then nesslerized. A brown precipitate formed immediately. The remainder upon titration and calculating the total volatile material as alkaloid showed 0.0847%.

The alkaloidal residue remaining in the flask was titrated with the following result: 0.3743%.

0.3743% + 0.0847% = 0.459%, apparent total alkaloids.

The apparent loss upon evaporation of the chloroformic extract on a water-bath to complete dryness, leads one to suspect volatile substances, basic in character, other than alkaloids, as being present. Volatile amines have been suggested.

PROOF OF THE ABSENCE OF VOLATILE AMINES.

Method.—One hundred grams of powdered drug was treated with chloroform, followed by NH_4OH. The flask was tightly stoppered and macerated over night. Ten cubic centimeters of 28% NH_4OH was then added and the drug subjected to steam distillation. The distillate separated into chloroformic and aqueous layers. The aqueous layer was rejected; the chloroformic layer

was washed several times with water (ammonia free) and passed through absorbent cotton. It was reduced by evaporation to approximately 5 cc. and standard acid added. The residual chloroform was evaporated at water-bath temperature and the acid titrated. It showed no alkaline material whatsoever.

From this result it is concluded that volatile amines are not contributing to the variation in results.

It is not plausible to expect amines even if present in the chloroformic extract of alkaloids to nesslerize as does ammonia, but to verify this a few common amines and ammonia were nesslerized with the following results:

Methylamine HCl	(immediately)	yellow precipitate
Hexamethylenetetramine	(upon long standing)	yellow precipitate
Aniline	(upon long standing)	orange-yellow precipitate
Ammonia	(immediately)	brown precipitate
Unknown (distillate from F)	(immediately)	brown precipitate

An ammonia soap of resinous material is largely responsible for the variations noted, and is obviated only by long standing after the evaporation of the solvent or by successive additions of a solvent such as ether with spontaneous evaporation. Warming the alkaloidal residue, volatilizes the ammonia readily and does not perceptibly affect the alkaloids.

The following table is a summary of the analytical results:

U. S. P. X.

Sample.	Solvent.	Type Process.		Results.
10 Gm.	Ether-Chloroform	U. S. P. X	*a*	0.402%
			b	0.394%
			c	0.426%

MODIFIED U. S. P. X.

Sample.	Solvent.	Type Process.	Part.		Results.
				a	0.574%
		A		*b*	0.509%
				c	0.520%
10 Gm.	Ether-Chloroform				
		B		*a*	0.549%
				b	0.567%
		C			0.557%
			1	*a*	0.438%
				b	0.448%
10 Gm.	Alcohol, Ether NH_4OH (28%)	D	2	*a*	0.542%
				b	0.546%
			3		0.661%
			1		0.417%
10 Gm.	Chloroform	E	2	*a*	0.450%
				b	0.461%
			3		0.713%
10 Gm.	Ether-Chloroform	F			0.459%

ISOLATION OF ALKALOIDS.

One kilogram of drug was placed in a percolator, macerated for 18 hours with alcohol and percolated. The percolate was reduced to a viscous liquid *in vacuo*.

The semi-solid residue was extracted with water to remove the alkaloids in the form of salts as they occur in the plant. The filtered aqueous extract was reduced *in vacuo*. The residue was extracted three times with chloroform to remove any resins and chlorophyll.

After attempting crystallization of the alkaloidal extractive from ether-alcohol mixtures with negative results, the alkaloids were then removed from this solvent with diluted sulphuric acid. The alkaloids were finally recovered by shaking out with chloroform from the above solution after making alkaline with ammonia. The chloroform was evaporated spontaneously.

(*a*) To the alkaloidal residue was added 10 cc. of distilled water and gently warmed on a water-bath with continuous stirring. This aqueous extract was decanted and two other portions of water, 5-cc. and 3-cc. portions, respectively, were added to wash the residue. All three portions were combined and extracted several times with chloroform, after which the solvent was evaporated. The residue was taken up with 15 cc. of water, 2 cc. being used to rinse the container. The aqueous extract was filtered and diluted to exactly 20 cc., mixed well and polarized. A measured portion of the solution, after polarization, was evaporated and dried over calcium chloride.

$$(a)_D^{20} \quad -16.2 \text{ (water)}$$

(*b*) The portion retained by the water upon extraction with chloroform was tested qualitatively for the presence of alkaloids with positive results. It was likewise polarized as in (*a*) with the following result:

$$(a)_D^{20} \quad -16.74 \text{ (water)}$$

The specific rotations of the substance extracted by chloroform (*a*) and that remaining in the aqueous portion (*b*) indicate that they are the same, so they were combined.

A gold chloride derivative of the alkaloid was made. Large conglomerates of golden-yellow crystals formed. As far as could be detected when magnified, the crystals appeared uniform, indicating a rather pure product. Upon repeated recrystallization from hot water, long serrated crystals, together with broad yellow prisms, some having a feathery appearance under the microscope,[1] were obtained, having a melting point of 199° C. (uncor.).

There is, however, some uncertainty concerning the melting point of the aurichlorides of both the *l*- and *i*-scopolamine. Following are the results of various workers and also that recorded by two Pharmacopœias for *l*-scopolamine:[2]

E. Schmidt	212–214° C.
Kircher	208–209° C.
O. Hesse	198° C.
U. S. P. VIII	197° C.
British Pharmacopœia (1898)	198° C.

This preliminary work being done, a larger portion of drug was treated with alcohol, as in the initial extraction, using 5 Kg. of drug and the same process of purification and separation again exercised, each fraction in the process of separation being combined with the corresponding fraction of the previous lot of drug.

The portions previously polarized were below the accepted value for an aqueous solution of scopolamine, due to impurities and moisture, the latter not being com-

[1] T. A. Henry, *Plant Alkaloids*, 2nd Edition, page 88; Wilhelm Autenrieth and William H. Warren, *Laboratory Manual for the Detection of Poisons and Powerful Drugs*, 6th Edition, page 185.

[2] *American Journal of Pharmacy*, 86 (1914), 346.

pletely removed when kept in a desiccator for four days with recently fused calcium chloride. The water is probably retained by a film forming on the surface of the viscous alkaloidal residue.

To further purify the alkaloid and remove water, it was next treated with absolute ether (ether over sodium). The ethereal solution was very cloudy, indicating water, and upon the addition of anhydrous sodium sulphate the solution became clear. The ethereal solution was filtered and the ether removed by placing the solution of alkaloids in a vacuum desiccator, the vacuum being continued until the ether was removed. The fractionation with absolute ether also resulted in the separation of some resinous material which was reserved for further study.

Two separate portions were treated similarly and the optical rotation of this purified ether-soluble product resulted as follows:

1. $(a)_D^{20}$ −16.5 (alcohol) 2. $(a)_D^{20}$ −16.44 (alcohol)

The resinous substance was washed with absolute ether until the washings were negative to Mayer's Reagent and picric acid, T.S. When tested, however, with *p*-dimethylamino-benzaldehyde solution, a positive test resulted. This positive test accounts for the numerous extractions required to exhaust the drug until negative to this reagent, when the color reaction is produced not by the alkaloids, but by this resinous material, probably an alkaloidal decomposition product.

The resinous material was taken up with 0.5% H_2SO_4. The aqueous solution was rendered alkaline with NH_4OH and shaken out with $CHCl_3$ into which the brown-colored material immediately passed. Upon evaporating the chloroformic extract nearly to dryness and subsequent titration, it was found that 2.3 cc. of 0.02*N* acid was consumed. These results, then, account for the especially high results when the drug is exhausted until negative to *p*-dimethylamino-benzaldehyde and consistently higher results obtained when standard acid is added before the final chloroform is evaporated to complete dryness.

Constants obtained for this alkaloid with constants as reported by Henry, *Plant Alkaloids*, for *l*-scopolamine:

1. A thick, viscous amber-colored syrup which refused to crystallize as the base.
2. M. p. of picrate..... 188° C.
3. M. p. of aurichloride..... 199° C.
4. $(a)_D^{20}$ (*a*) −16.5 (alcohol) (*b*) −16.44 (alcohol)

Thomas A. Henry, *Plant Alkaloids.*

	Gold Chloride.	Picrate.	Specific Rotation.
Atropine	137–139	175–176	Inactive
Hyoscyamine	165	165	
Scopolamine	204–205 (uncor.)	187–188 (uncor.)	−18 in alcohol

Crystalline forms and the comparison above indicate conclusively the presence of scopolamine only in *D. innoxia*, Mill.

The usage of the term hyoscine and scopolamine has caused much confusion and controversy.[1] In Germany the names lævo- and inactive scopolamine are

[1] *American Journal of Pharmacy*, 86 (1914), 339; Allen's *Commercial Organic Analysis*, Vol. 7, 5th Edition, page 815; Merck's *Index*, 4th Edition, page 454.

employed. In England and the United States hyoscine was once used to designate the lævo- base. Preference is now given to *l*-scopolamine as the proper name.

Samples of the drug, the alkaloid, the picrate and the aurichloride have been filed for reference.

CONCLUSIONS.

1. The solvent and treatment of final chloroformic extract exert controlling influences upon the results obtained in assays.

2. The high and variable results are not attributable to volatile amines but to an ammonia soap of a resin-like material.

3. The optical rotation, crystal forms of the picrate and aurichloride and their respective melting points prove the alkaloid to be *l*-scopolamine.

4. Lævo-scopolamine was isolated, identified and was the only alkaloid found in *Datura innoxia*, Mill.

A PHYTOCHEMICAL STUDY OF GILLENIA STIPULATA.

BY L. LAVAN MANCHEY.*,[1]

Gillenia stipulata and *Gillenia trifoliata* are North American drugs indigenous to the eastern United States, known to the Indians, and used by the early colonists (1) as a substitute for Ipecac under the name American Ipecac. Of the two species the former has been generally acknowledged as the more efficacious. Although there is no botanical relation between Ipecac and American Ipecac, the roots of both plants, in being annulated, are similar; and this circumstance, no doubt, suggested to the early users of the drug a parallel in therapeutic value.

This drug received recognition in medical practice through the efforts of Barton, Schoepf and others (2, 3) and was official in the United States Pharmacopœia before the sixth revision. It was reputed to be emetic, cathartic, sudorific, expectorant and tonic (4). Ebert (5), however, states that doses of from one to twelve grains of extract prepared from drug obtained through commercial channels were entirely without effect upon himself and upon several young men. Shreeve (6), in 1835, and students (7) of the Philadelphia College of Pharmacy, in 1854, found in the root starch, gum, albumin, volatile oil, wax, fatty resin, lignin, coloring matter, gallotannic acid, lime potash and iron. Stanhope (8), in 1856, isolated a substance from the root cortex which he named "Gillenin," corroborated in 1877 by Wetherill (9). Curry (10), in 1892, reported two glucosides "Gillenin" and "Gillein," neither of which appears to be identical with "Gillenin."

EXPERIMENTAL.

For the present investigation 50-pounds of *G. stipulata*, in the form of a number twenty powder, were procured from commercial sources, and after confirming the

* From the laboratory of Glenn L. Jenkins, Professor of Pharmaceutical Chemistry, School of Pharmacy, University of Maryland. Abstracted from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science.

[1] Alumni Research Scholar.