

Chemical Studies on a Physiologically Active Substance in *Passiflora Incarnata**

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Ott (1) in 1898, DeNito (2) in 1931, and Fellows and Smith (3) in 1938 established the presence of a physiologically active substance in *Passiflora*. Ott and Fellows and Smith investigated the species *incarnata* while DeNito investigated five other species. The principal interest in the United States is in the species *incarnata* since it is this species that has been and still is in use in medicine. Its use today depends upon its supposed sedative action. Ott and DeNito reported depressant effects on the central nervous system after the administration of aqueous solutions of alcoholic extracts of the crude drug. Fellows and Smith were unable to demonstrate any such action, but they and DeNito reported the presence of some substance which produced marked lowering of the blood pressure in dogs.

DeNito was of the opinion that this substance is an alkaloid or a mixture of alkaloids but Fellows and Smith failed to confirm this observation.

The present investigation was undertaken with a view to establishing more clearly, if possible, the chemical nature of the physiologically active substance in *Passiflora incarnata*.

EXPERIMENTAL

Procedure.—Weighed portions of the crushed stems and leaves of *Passiflora incarnata* were extracted with three volumes of 85 per cent alcohol on a boiling water-bath. The extract was filtered and the alcohol was removed from the filtrate by vacuum distillation. The residue was dissolved in water and an excess of basic lead acetate was added. The resulting lead precipitate was removed by filtration. The filtrate after being freed from lead with mercuric sulfide was reduced to a syrup *in vacuo*. This syrup was mixed with plaster of Paris and allowed to set. The plaster was powdered in a mortar, then dried for six hours in a vacuum oven at 60° C. The powdered plaster was next extracted with absolute alcohol for 24 hours in a shaker and centrifuged. The alcoholic extract prepared in this

manner was treated with an excess of 20 per cent solution of mercuric chloride in absolute alcohol and a heavy white precipitate obtained. The precipitate was filtered by suction and washed with absolute alcohol and anhydrous ether. The mercury precipitate thus obtained was found to contain the physiologically active ingredient of *Passiflora*.

In attempting to purify the mercury precipitate the crude substance was dissolved in water and the solution filtered to remove a slight cloudiness. This aqueous solution was divided into three parts, A, B and C, and subjected to the following procedures.

Portion A was poured into a large (50×) excess of a mixture of equal parts of ether and acetone. The resulting precipitate was collected by decantation and centrifugation.

The water was removed from Portion B by distillation with immiscible solvents using butyl alcohol. The residue insoluble in butyl alcohol was collected.

The mercury was removed from Portion C as mercury sulfide and the mercury-free solution subjected to the plaster of Paris procedure previously described and again precipitated from absolute alcohol with mercuric chloride.

The three residues, A, B and C, resulting from these procedures were alike in appearance and behavior. They were analyzed for carbon, hydrogen, nitrogen and mercury (see Table I). Residue A was too small to provide samples for combustion analysis.

Carbon and hydrogen were determined by the combustion method using reduced copper, metallic silver and gold foil to remove nitrogen, chloride and mercury, respectively.

Mercury was determined by reduction with formaldehyde, solution of the metallic mercury in nitric acid and titration of the mercurous nitrate with standard potassium thiocyanate using ferric alum as an indicator.

Nitrogen was determined by the Kjeldahl method.

Table I.—Results of Analyses of Residues

Residue	C, %	H, %	N, %	Hg, %
A	2.64	30.05
			2.69	31.00
B	23.61	3.98	2.67	29.98
	24.01	4.11	2.71	30.16
C	25.31	4.21	2.69	31.40
	25.24	4.29	2.71	30.98

The uniformity of these results was taken as evidence that the mercury derivative was probably a pure compound.

The extraction of the crude drug was such a time-consuming procedure that a short-cut was sought. Accordingly, a sample of fluid extract of *Passiflora* was tested to see whether the fluidextract contained any appreciable amount of the active substance. Such was found to be the case and four liters of fluidextract, supplied through the courtesy of Eli Lilly and Company, were subjected to the procedure outlined at the beginning of this discussion. The mercury precipitate obtained was treated as was Portion C described above and a yield of approxi-

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mately 5 Gm. resulted. This preparation was used for the remainder of the chemical work and for the pharmacological work which is to be described in a later paper.

The mercury precipitate thus prepared was an amorphous brownish white powder. It was very hygroscopic and slowly turned brown on exposure to light and air. It therefore had to be kept in a light-proof vacuum desiccator.

The mercury derivative was very soluble in water, slightly soluble in 95 per cent alcohol and insoluble in ether, chloroform, benzene and other organic solvents. It has thus far resisted all attempts at crystallization.

Qualitative analysis revealed the presence of mercury, chloride and nitrogen but no phosphorus or sulfur. Quantitative analysis showed the following composition:

C—23.44%	N— 2.74%
H— 4.44%	Hg—31.35%
O—25.23% (by difference)	Cl—12.80%

Carbon, hydrogen, nitrogen and mercury were determined by the methods already described. Chloride was determined gravimetrically. Oxygen was determined by difference.

Molecular weight determinations were not successful. On the basis of elementary analysis and assuming that only one atom of nitrogen is present in each molecule, the molecular weight should be 511. On the same basis, an empirical formula derived from the elementary analysis would be $C_{10}H_{22}O_8N.HgCl_2$. This compound has a molecular weight of 548. A similar series of calculations could be made based on the assumption that only one atom of mercury is present. However, it will be seen in Table II that the relation based on nitrogen is quite good while that based on mercury is poor. Inherent difficulties in the volumetric determination of mercury may be responsible for low results in the table, or it is possible that the combination of the active substance with mercuric chloride is a loose one and that in the purification procedure some mercuric chloride was lost.

Table II.—Chemical Composition

Element	% Composition	% Atomic Wt.	Relation Based on N	Relation Based on Hg
C	23.44	1.952	10.0	12.51
H	4.44	4.404	22.6	28.23
O	25.23	1.577	8.0	10.1
N	2.74	.195	1.0	1.25
Hg	31.35	.156	.8	1.00
Cl	12.80	.361	1.85	2.31

The specific rotation was determined using aqueous solutions of the mercury derivative. Samples varying in amounts from 0.4 Gm. to 0.6 Gm. per 100 cc. of solution were used. The specific rotation at 20° C. varied from -18.01 to -18.21 degrees of angular rotation. The average was -18.16 degrees.

An aqueous solution of the mercury precipitate was used for qualitative tests with the following reagents:

Molisch reagent—greenish color in the sulfuric acid and a definite purple ring at the interface.

Fehling's reagent—slight greenish precipitate.

Bromine water (saturated)—decolorized.

Alkaline potassium permanganate—decolorized.
50 per cent sodium hydroxide—heavy gelatinous precipitate.

Benedict's, Fuchsin aldehyde, Tollen's, Wagner's, Nessler's and Mayer's reagents, and Hinsburg's, Simon's and Riminis tests all gave negative reactions.

It has been difficult to obtain the mercury compound in sufficient quantity to permit the preparation of derivatives. Attempts have been made to acetylate and to brominate the compound but have been unsuccessful. Oxidation with potassium permanganate has been attempted and certain substances have been obtained by extraction of the oxidized mixture with immiscible solvents. These products are crystalline but have been isolated in such very small quantities (1-2 mg.) as to preclude identification.

It is not possible at this time to draw any definite conclusions as to the nature of the active principle of *Passiflora incarnata*. It is reasonably certain that it is not an alkaloid. It may possibly be a glycoside as indicated by the positive Molisch reaction. However, this too, seems unlikely since no evidence of the presence of glycosides could be obtained by the emulsin procedure of Bourquelot (4) run on the semicrude extract following the lead precipitation.

SUMMARY

A physiologically active substance has been isolated from *Passiflora incarnata*, in the form of a mercury derivative. An empirical formula is proposed but no data are available as yet which will permit the structural identification of the compound.

REFERENCES

- (1) Ott, *Medical Bulletin*, 29 (1898), 457.
- (2) DeNito, *Rassegna di Terapia e Patologia Clinica*, 3 (1931), 193.
- (3) Fellows and Smith, *JOUR. A. PH. A.*, 28 (1938), 565.
- (4) "Handbuch der Pflanzenanalyse," Springer, Berlin, Part III (second half), page 812.

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