A CHEMICAL AND PHARMACOLOGICAL STUDY OF PHYTOLACCA AMERICANA, N. F.*

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A concise record of results obtained by previous workers on poke root (*Phytolacca Americana*, Linné) appeared in an earlier publication by Jenkins (1). The present work was carried out in an effort to clarify the uncertainties existing with reference to the chemical constituents of poke root, especially with respect to the presence of alkaloids, and the pharmacological action of the drug.

EXPERIMENTAL.

Using U. S. P. X procedures, the following constants were determined: Moisture content 9.0% and 9.3%; ash content 10.83% and 10.78%, of which 94.18% and 93.81%, respectively, was acid-soluble; crude fiber 16.10% and 16.31%; total ether-soluble extractive 0.98% and 0.985%; volatile ether-soluble extractive 0.13% and 0.15%; non-volatile ether-soluble extractive 0.85% and 0.835%; alcohol-soluble extractive 1.78% and 1.75%; diluted alcohol-soluble extractive 12.08% and 12.14%; water-soluble extractive 13.18% and 13.24%; benzin-soluble extractive 0.45% and 0.53%.

Preliminary Tests.—Tests for the presence of alkaloid gave the following results: An extract of the powdered root was obtained with Prolius fluid. The extract was shaken with 2% sulfuric acid, the aqueous layer was separated and tested with Mayer's reagent, phosphomolybdic acid, phosphotungstic acid, gold chloride and iodine test solutions. Negative results were obtained in all cases.

A chloroform extract of a dried mixture of powered root and slaked lime was evaporated and the residue was treated with 2% sulfuric acid. The aqueous solution gave amorphous precipitates with phosphomolybdic acid and phosphotungstic acid test solutions.

The procedure by which Preston (2) obtained his "phytolaccine" was repeated five times, using fresh and dried, purchased and collected poke root, without confirming his results.

The fractionation method for the separation of alkaloids as given by Thompson (3) was carried out. The tartaric acid shakings, which would certainly contain any alkaloids present in the drug gave no precipitate with Mayer's reagent and only a very slight amorphous precipitate with phosphomolybdic acid solution. The aqueous solution showed no activity when tested pharmacologically.

Separation of Starch.—The expressed juice from 200 Gm. of fresh drug deposited a white substance, which appeared to be granular when examined microscopically. The substance turned blue when treated with iodine solution, and the blue color was removed on addition of sodium thiosulfate solution. The substance gave a positive Molisch test and reduced Fehling's solution only after boiling with acid. The substance was thus identified as a starch.

Volatile Constituents.—Steam distillation of a concentrated alcoholic extract from 2.8 Kg. of powdered root yielded 1.35 Gm. of an oily substance after extraction of the distillate with ether, drying and removing the ether. The yellowish brown substance possessed a disagreeable and penetrating odor, and, when diluted, it resembled the odor of the plant. It had a sharp, pungent taste. Its specific gravity was 0.9977 23°/4° C. The high specific gravity indicated the absence of terpenes (4). A cloudy suspension was formed on mixing with 98% alcohol. This behavior is anomalous to that of the essential oils which are miscible in 98% alcohol. The oily substance may have been composed mainly of lower fatty acids and their esters.

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ALCOHOLIC EXTRACTIVES.

An alcoholic extract representing 5 Kg. of powdered root was mixed with slaked lime and extracted with a mixture of ether and chloroform (3:1) and then with chloroform. The ethereal solutions were combined, concentrated without heat and extracted with 2% sulfuric acid. The acid solution was made basic with ammonia water (10%) and then was extracted with ether. The ether was removed and the residue, which consisted of a clear yellow, semi-solid substance with crystals dispersed in it, gave amorphous precipitates with phosphomolybdic acid and phosphotungstic acid solutions. Attempts to separate the semi-solid from the crystalline matter by means of solvents and with chemical reagents were unsuccessful. The mixture was insoluble in petroleum benzin (b. p. 35–40° C.); soluble in anhydrous ether and absolute alcohol.

The results reported immediately above were inconclusive, but they were not in agreement with the other results obtained, inasmuch as they seemed to indicate the possible presence rather than the absence of alkaloid. In order to clarify this point, the following experiment was carried out. Twenty cc. of a hydroalcoholic (1:1) extract of poke root (1 cc. representing 1 Gm. of drug) was diluted with 20 cc. of distilled water, shaken with 75 cc. of chloroform in a separatory funnel and the chloroform layer was removed. Two cc. of the chloroform solution was tested in the usual manner with phosphomolybdic acid solution. The precipitate which formed appeared to be similar to the precipitates obtained in all the other experiments where positive tests were given by the same reagent. The remainder of the chloroform solution was evaporated to 5 cc., 4.596 cc. of $N/1$ sulfuric acid was added and the mixture was heated until all the chloroform had been removed. The aqueous residue required 4.708 cc. of $N/1$ sodium hydroxide for neutralization (methyl red indicator). This was 0.112 cc. more than the amount of $N/1$ sodium hydroxide necessary to neutralize the added acid.

The liquid remaining in the separatory funnel after the removal of the chloroform layer was made basic with ammonia water (10%) and was extracted several times with chloroform. The combined chloroform shakings (100 cc.) were extracted several times with approximately $N/1$ sulfuric acid. The acid solution gave a precipitate when tested with phosphomolybdic acid solution. The combined aqueous solution was made basic with ammonia water (10%) and was extracted with several portions of chloroform. The combined chloroform solution (100 cc.) was evaporated to 5 cc., 4.596 cc. of $N/1$ sulfuric acid was added and the mixture was heated until no chloroform remained. The aqueous residue required 4.563 cc. of $N/1$ sodium hydroxide for neutralization. This was 0.033 cc. less than the amount of $N/1$ sodium hydroxide required to neutralize the added acid. A blank determination required 0.012 cc. of $N/1$ sodium hydroxide less than the amount required to neutralize the added acid.

The above results show that the substance which is soluble in chloroform, 2% sulfuric acid and dilute alcohol, and which gives positive tests with phosphomolybdic acid solution, which has been found to be the most sensitive of the so-called alkaloidal precipitating agents used in this study, does not exhibit basic properties and is, almost certainly, not an alkaloid.

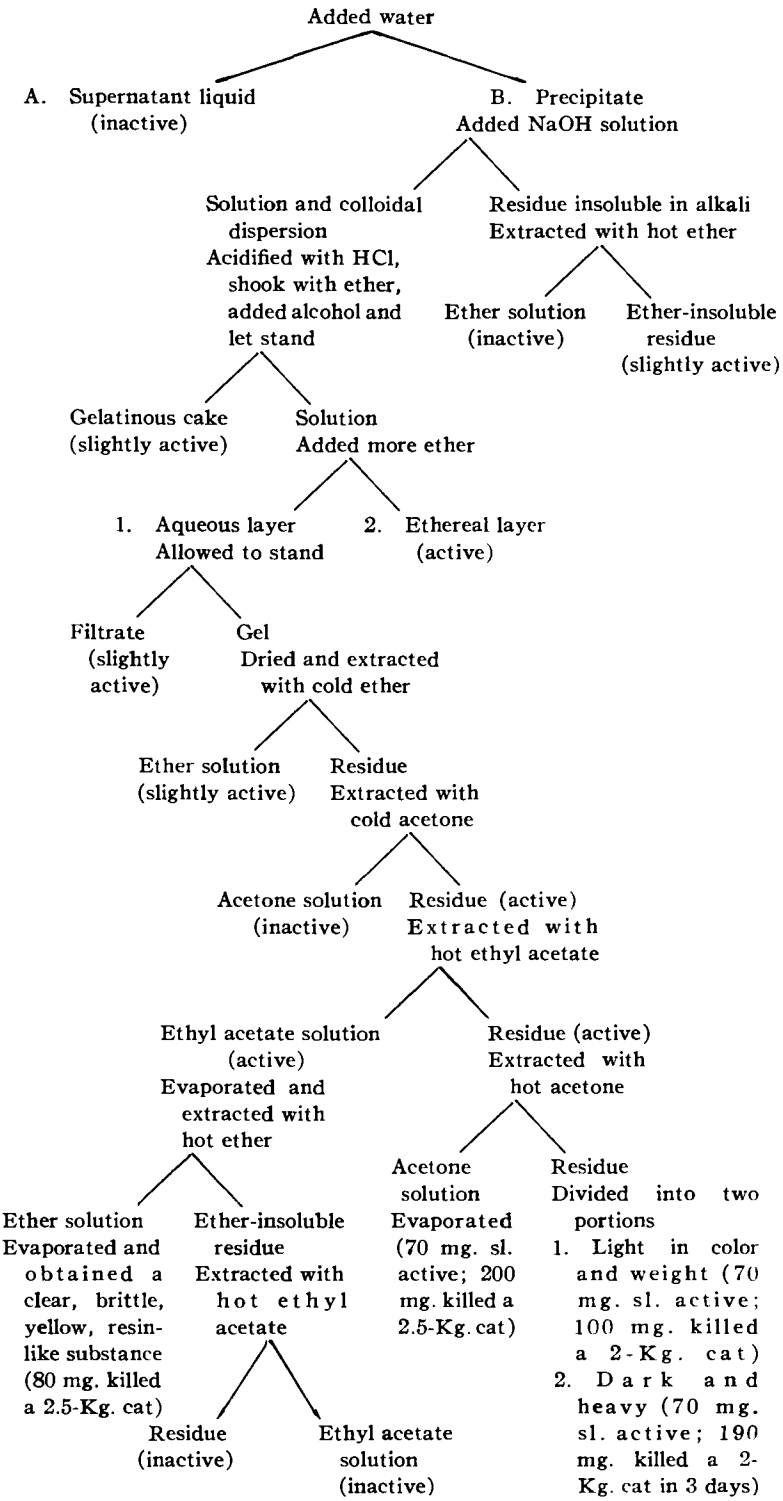
An alcoholic extract representing 11 Kg. of powdered root was concentrated without heat and the syrupy mass was mixed with water. A water-soluble fraction (A) and a water-insoluble fraction (B) were obtained.

Fraction (A), which was quite acid, was made basic with potassium bicarbonate and was extracted with ether, amyl alcohol and chloroform, respectively. The ether and amyl alcohol solution were extracted with 1% tartaric acid, the acid solutions were combined, made basic with sodium bicarbonate and extracted with ether. Removal of the ether left a pale yellowish brown, resin-like substance, part of which was dissolved in alcohol (*a*). The remainder was treated with distilled water to form solution (*b*). Both solutions formed amorphous precipitates when treated with phosphomolybdic acid and phosphotungstic acid solutions.

The chloroform solution was extracted with 1% tartaric acid until the shakings no longer gave a precipitate with phosphomolybdic acid solution. The residual chloroform solution still gave a positive test with phosphomolybdic acid solution, indicating that more than one substance in the original chloroform solution formed precipitates with the reagent. The chloroform was evaporated and the residue was dissolved in alcohol (*c*).

Solutions (*a*), (*b*) and (*c*) were tested on normal cats by injecting 5 cc., representing the water-soluble portion of the alcoholic extract of 5 Kg. of drug, of each solution intraperitoneally. In all cases the cats remained apparently normal. (*Continued on page 309.*)

Alcoholic Extractive II.



Evidently the substances obtained by extraction methods commonly used to isolate alkaloids, and which give amorphous precipitates with certain precipitating agents, are not responsible for the toxicity of poke root; especially since it was repeatedly demonstrated that suitable extracts, representing less than 5 Gm. of the same lot of drug, invariably produced death in cats weighing 2 to 3 Kg.

The aqueous solution (A), remaining after extraction with ether, amyl alcohol and chloroform, was tested by injecting 6 cc. of the solution, representing about 60 Gm. of root, intraperitoneally into a cat. The animal remained apparently normal.

Fraction (B). One-half of the water-insoluble portion of the alcoholic extract, representing 5.5 Kg. of powdered root, was treated according to the scheme on page 308.

The toxic resin-like substance, obtained according to the foregoing procedure, possessed neither alkaloidal nor glycosidal properties.

PHARMACOLOGICAL TESTS.

The pharmacological tests, by which the toxic principle was traced in the various fractionations, consisted of the parenteral administration of the extracts or fractions of the drug to cats. The intraperitoneal route was used in the majority of the tests, the end-point involving death of the animal. It was the most toxic principle of the drug, therefore, which was followed by this test in the fractionation procedure. Consequently, the entire procedure does not preclude the possibility of the existence of other undetected active but less toxic principles in the drug.

When intraperitoneal doses of 1.0 cc. per Kg. of a hydroalcoholic extract, representing 1.0 Gm. of the root per Kg., or fractions containing an equivalent amount of the toxic principle, were administered to cats, a characteristically uniform symptomatology developed. During the first half hour after receiving the drug, early symptoms of discomfort gave way to retching and, not infrequently, emesis (vomiting did not occur in all cases, especially when the purified principle was given). The animal then gradually lost the use of the hind legs and later the front legs; stupor, somnolence and a diminished perception of pain following in order. After the development of profound narcosis, the heart rate gradually became slower, the beat weaker and respiration became shallower, with ultimate death from respiratory failure.

Although these pharmacological experiments must be regarded as of a preliminary nature only, the symptomatology thus induced in normal cats by toxic doses of the resin-like principle is strongly indicative that the main site of action of this toxic substance is the cerebro-spinal axis, and that this action consists principally of an ascending depression involving both sensory and motor pathways, ultimately involving the medullary centers to cause death.

That the water-insoluble resin-like toxic principle described above is not the only constituent of the drug exhibiting pharmacological activity was shown by two noteworthy observations. Aqueous extracts of the root not containing the resin-like principle, when applied with friction to the intact human skin, produced marked irritation. Such aqueous extracts, when injected into cats, produced emesis, retching, sneezing, etc., but the cerebro-spinal depression characteristic of the resin-like principle was not produced.

Another significant observation involving humans lends itself to similar interpretation. In the milling of a portion of the root, two students, so engaged,

unwisely subjected themselves to accidental inhalation of the drug dust. Both developed very severe respiratory irritation and gastro-enteritis, one individual requiring hospitalization. More than 25 occupants of the six floors of the same building developed similar symptoms of varying degrees of severity, a considerable number being forced to discontinue their occupations for several days. The symptoms were similar to those of a severe cold associated with rhinitis and gastro-enteritis. It is important to note that no symptoms of cerebro-spinal depression developed in any case, thus indicating rather clearly that the offending principle was the water-soluble irritant substance and not the toxic, narcotic resin-like principle.

Further pharmacological study is in progress to prove or disprove the validity of the interpretations of the preliminary experiments described herein.

The fixed oil, occurring to the extent of 0.044% in the lot of drug under investigation, proved to be pharmacologically inert when administered intraperitoneally to cats in doses as high as 2.0 cc. per Kg.

The account of the chemistry and pharmacology of the drug by Solis-Cohen and Githens (5) receives considerable confirmation from the observations here reported.

AQUEOUS EXTRACTIVES.

A decoction was prepared from 300 Gm. of powdered root, and the product was treated with basic lead acetate solution, the lead precipitate being separated by filtering with suction.

Isolation of Hemicellulose.—The filtrate was treated with hydrogen sulfide gas to remove excess lead, the mixture was filtered, the precipitate was washed and the filtrate and washings were combined and allowed to evaporate without heat. The amber-colored residue consisted mainly of inorganic matter with a large amount of potassium salts. The alcoholic liquid was acidified with 2% sulfuric acid, and the white amorphous precipitate which formed was removed. The precipitated substance was insoluble in water, alcohol, ether and acetone; it was soluble in potassium hydroxide solution from which solution it was reprecipitated upon addition of acid. The substance reduced Fehling's solution, and the liquid resulting from boiling the substance with diluted sulfuric acid gave a positive test for carbohydrate with Molisch's reagent.

From the above behavior, it was concluded that the substance was a hemicellulose (6) which was liberated upon treatment with sulfuric acid. Yield: 2 Gm., representing 0.66% of the weight of the drug used.

Isolation of Isosaccharic Acid.—The basic lead acetate precipitate was treated with hydrogen sulfide and the lead sulfide and the undecomposed part of the original lead precipitate were removed by filtration. The precipitate was heated with sulfuric acid on a hot plate for 24 hours, then the supernatant liquid was treated with charcoal and filtered. Crystals separated from the filtrate on standing over night. The crystals were recrystallized from alcohol and then from acetone. They dissolved in water forming an acid solution, which, after neutralizing with ammonia water, gave precipitates with solutions of barium hydroxide, calcium hydroxide and silver nitrate. The crystals gave the color reactions given by hydroxy acids with beta-naphthol and concentrated sulfuric acid. They melted at 185° C. after drying at 100° C. The substance was thus identified as isosaccharic acid. The acid probably did not exist as such in the plant, and very likely was formed by hydrolysis and oxidation of the gum which was present.

Isolation of Gum.—The filtrate obtained after treatment of the basic lead acetate precipitate with hydrogen sulfide was mixed with alcohol. The milky-white precipitate that settled out, was purified by dissolving it in water and reprecipitating two times, and then it was allowed to dry without heat. The substance was completely but slowly soluble in cold water, first swelling and becoming very adhesive; it was readily soluble in hot water, insoluble in alcohol and other organic solvents. An aqueous solution of the substance gave a blue color with iodine solution and gave a positive test with Molisch's reagent, but did not reduce Fehling's solution until after preliminary

boiling with diluted sulfuric acid. Pentoses and galactose were absent. Tests for the presence of sulfate gave negative results. The substance was evidently the gum previously reported in phytolacca. Yield: 13.5 Gm., representing 4.5% of the weight of the drug used.

Separation of a Resin.—The hydroalcoholic filtrate, obtained after precipitation of the gum, was concentrated, the solid matter which deposited was separated, dissolved in alcohol, and the alcoholic solution was evaporated to dryness without heat. A light reddish brown, glistening, brittle, tasteless residue remained. The substance remained brittle when treated with 2% sulfuric acid, it was slightly soluble in ether, soluble in alcohol and in 10% potassium hydroxide solution, from which solution it was precipitated by sulfuric acid. Tests for nitrogen and sulfur gave negative results.

The substance very likely was an acid resin which existed as a complex salt in the root, thus allowing its extraction with water.

The liquid separated from the resin yielded, on further treatment, crystals of oxalic acid, and, on further concentration, crystals of potassium oxalate.

The second decoction was prepared using 4.6 Kg. of fresh poke root. The decoction was treated with basic lead acetate, the precipitate was separated and the excess lead was removed from the filtrate. Part of the filtrate was allowed to evaporate without heat. After two weeks, a dark reddish brown, syrupy mass interspersed with needle-like crystals was obtained. The crystals remained after treatment with alcohol and were identified as potassium nitrate.

The alcoholic solution was allowed to evaporate and the residue was dissolved in water. On addition of sodium hydroxide solution a precipitate formed and was removed by filtration.

Isolation of Saponin.—The precipitate was treated with boiling ether and then with boiling alcohol. The alcoholic solution was evaporated to dryness and the residue was again treated with hot alcohol and the mixture was filtered. The filtrate, on standing, deposited a substance which settled to the bottom of the beaker and adhered to the glass. The deposited substance was white when dried, but on exposure to air its surface appeared to become oily. On shaking with water, a very lasting foam formed. The aqueous solution caused hemolysis of blood and emulsified oil of turpentine. Slight reduction was observed with Fehling's solution, but after boiling with hydrochloric acid a copious precipitate of cuprous oxide was obtained with Fehling's solution.

The substance was thus identified as a saponin.

The solution obtained from the basic precipitate with boiling ether yielded a water-soluble substance (*a*) which gave amorphous precipitates with phosphomolybdic and phosphotungstic acids.

The remainder of the filtrate from the basic lead acetate precipitation was concentrated to a syrup and the residue was extracted with alcohol. The alcohol was allowed to evaporate, the residue was dissolved in water, the solution was made basic with sodium hydroxide and was then extracted with organic solvents. The ethereal solutions yielded several water-soluble fractions which gave amorphous precipitates with phosphomolybdic and phosphotungstic acids.

All the fractions, including (*a*), which gave precipitates with the above-mentioned reagents, were tested by intravenous injection into a male cat with intact vagi under Nembutal anesthesia. No characteristic response was observed immediately in any case with respect to blood pressure or respiration.

SUMMARY AND CONCLUSIONS.

1. The U. S. P. X constants for crude drugs have been determined for poke root.

2. The oil obtained by steam distillation from an alcoholic extract of poke root had a specific gravity of 0.9977, indicating the absence of terpenes (4). The oil was not miscible with 98% alcohol, and may have been composed mainly of lower fatty acids and their esters.

3. Results of chemical and pharmacological tests prove that poke root contains no active alkaloid, and strongly indicate the absence of any alkaloid. However, substances which form amorphous precipitates with some of the so-called

alkaloidal reagents, but which exhibit no basic properties, are present. These substances are undoubtedly responsible for the claims by others that alkaloid is present in the drug.

4. The pharmacological activity of poke root is due to at least two different principles, one or more being soluble in water, the other being soluble in alcohol and insoluble in water. The water-soluble principle or principles appear to be responsible for the strongly irritant properties of the drug, while the alcohol-soluble resin-like principle is responsible for the ascending depressant action on the cerebro-spinal axis of cats.

5. The fixed oil (7) of poke root, extracted by petroleum benzin, proved to be devoid of significant pharmacological activity.

6. Procedures by which the following substances were isolated from aqueous extracts of dried and fresh poke root are given: Hemicellulose, isosaccharic acid (m. p. 185° C.), gum, resin, oxalic acid, potassium oxalate and saponin. The isosaccharic acid probably did not exist as such in the plant, but was formed during the treatment of the lead subacetate precipitate of the gum.

7. Starch was obtained from the expressed juice of fresh poke root. The clear juice did not reduce Fehling's solution, indicating the absence of free reducing sugars.

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A NOTE ON THE STABILITY OF ERGOT.*

BY L. W. ROWE.

In connection with our recent report on the definite stability of digitalis activity in the crude drug form (1) even when the drug is not stored in air-tight and light-tight containers, it is believed that similar data concerning the stability of *ergot* as crude drug might be of interest. The opinion has prevailed even in scientific circles that these two drugs are relatively unstable and this has resulted in the inclusion in the U. S. P. XI (2) of the following requirement for the storage of ergot: "Preserve Ergot under all conditions of storage and transportation in water-proof and air-tight containers."

Undoubtedly, in the case of ergot at least, the published opinions of Rusby (3, 4, 5) have been partly responsible for the prevalent idea that the drug must be very carefully stored and it is probably true that that drug which has been allowed to mold or has been infested with insects will not be of average potency in addition to being unsuitable from the purely physical standpoint. It has been our experience that it is not necessary to store the drug in air-tight and water-proof containers, but that storage in such a way as to keep the drug reasonably dry is sufficient to

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