A PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY OF PHYTOLACCA AMERICANA LINNÉ

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HISTORICAL

The decline in the extent of use of Phytolacca Americana is undoubtedly due to its non-specificity and to lack of knowledge concerning its constituents. This study has been undertaken in an attempt to isolate some constituent or constituents of the plant to which its reported physiological action may be attributed.

Phytolacca (phytos, plant + lac or lacca, lake) is a native American plant, having been known and used by the American Indians. It is a tall, bushy, perennial herb which grows in clearings and woody pastures. The annual stems are smooth and the matured parts have a purple tinge. The leaves are long, entire ovate, smooth and green, and emit a faint light[1] in the dark. The flowers are greenish-white or purplish, growing in racemes. The fruits are purplish berries yielding a purplish-red juice. The root is thick and straight at first, later branching with the branches growing in a horizontal position, and has a yellowish-brown color.

Pickering[2] states that the plant was carried to Europe and was seen in France about 1650 where it was cultivated for its berries, which were used in coloring Bordeaux wine. In 1693 it was mentioned by Ray as a cultivated plant, but later it became naturalized and is now found in Europe, Asia and Africa; as well as in America. Lloyd[3] writes that the plant was used many years after the settling of the White men in America before it was intro-

duced into professional practice. The young shoots are similar to asparagus and are prepared and eaten in the same manner. The American settlers used the mature plant as a poultice for inflammatory conditions of the cow's udder in cases of the disease known as garget. This practice is the source of the synonym garget by which the plant is known. The plant was then used domestically in the form of a tincture, and in 1820 was made official in the United States Pharmacopoeia, where the root was mentioned in the Primary List and the berries were included in the Secondary List. Both root and berries were official in the New York edition of 1830, but were in the Secondary List of the Philadelphia edition of 1830. They appeared in the Secondary Lists of the succeeding Pharmacopoeias until 1880, when they were made wholly official. The root and berries remained official in the United States Pharmacopoeia until the revision of 1910, when they were deleted. The root was again made official in 1916, when it was included in the fourth edition of the National Formulary. The drug was official in all of the early editions of the United States Pharmacopoeia as Phytolacca decandra and this name was retained in the fourth edition of the National Formulary. Farwell[4] proposed that the official name for the plant be Phytolacca Americana in preference to the often used name Phytolacca decandra, and this name was adopted in the fifth edition of the National Formulary.

Many claims of therapeutic activity have been made for the drug, namely: Mitchell[5] treated children's itch by placing the child in a bath of a strong decoction of the root and rubbing the skin for ten minutes. He found it rarely necessary to repeat the bath more than once. The Italians used a strong decoction of the root for scrofula and syphilis. An overdose of the powdered root proves emetic and cathartic; and if still larger doses are taken, it may act as an irritant or narcotico-acrid poison. Cressler[6] reports a case of poisoning accompanied by vomiting and purging in five persons while milling the drug. Hankins and Sayre[7] mention the irritant action of the dried root on mucous surfaces, inhalation of the powder producing pain in the lungs for two weeks, due to saponinoid-like action. Hammer[8] states that phytolacca has a wonderful action on the skin and usually relaxes it so that it is able to get rid of any irritable substance. The drug has been used in the treatment of sore throats characterized by enlargement of the cervical glands and tonsils[9]. Holmes[10] writes of the root that it is a useful remedy in the treatment of cancer and ulcers. An eclectic resinoid[11] (phytolaccin), obtained by the extraction of phytolacca is still used in rheumatic and syphilitic conditions. Brundage[12] found that it produced nausea, vomiting, slowing of the heart and respiration, depression, dyspnea and palpitation. Other physiological effects attributed to the plant are emetic, purgative and

slight narcotic.

Previous work on the constituents of phytolacca has been largely of a qualitative nature. Claassen[13] worked with the seeds from which he isolated a crystalline compound which he called phytolaccin. He describes it as a tasteless, colorless compound forming acicular crystals; insoluble in water, dilute acids, concentrated acetic and hydrochloric acids, ammonia water and solution of sodium hydroxide even on heating, and in cold, concentrated nitric acid; soluble in cold, concentrated sulfuric acid, giving a brownish-yellow solution which on heating turns brownish-red, also soluble in hot, concentrated nitric acid, alcohol, ether and chloroform. It is precipitated from alcoholic or ethereal solutions upon the addition of water. Terreil[14] obtained an amorphous acid from the berries. The acid was soluble in alcohol and water. Pape[15] found, in the root; gum, starch, tannin, fixed oil, coloring matter, resin and probably an alkaloid. He also reported ash 10.73 per cent, of which 66.35 per cent was soluble in water, 24.96 per cent was soluble in hydrochloric acid, 6.71 per cent was soluble in hot sodium hydroxide solution; and potassium, calcium and iron as chlorides, sulfates and phosphates. Cramer[16] reported as constituents of the juice of the berries, gum, sugar, malic acid and coloring matter. Balland[17] working with Phytolacca dioica reported chlorophyll, wax, resin, essential oil, volatile acid, reducing sugar, non-reducing sugar, organic acid, gum,

albuminoids, pectic substances, pectose and salts. The resin he found to be soluble in ether. He also reported the presence of a potassium acid salt of an acid similar to the phytolaccic acid obtained by Terreil. The salt was soluble in water, insoluble in ether; amorphous and not precipitated by barium nitrate solution. He attempted to obtain an alkaloid but reported negative results. Preston[18] working with the fresh root in order to avoid possible decomposition of the constituents during the process of drying, reported, in addition to the constituents reported by Pape, the presence of lignin, sugar, a volatile acid characteristic of the root and a crystalline compound which was isolated from the filtrate of a strong decoction of the root after precipitation with lead subacetate. He claimed to have isolated a substance as small, nearly white crystals, which was soluble in alcohol, moderately soluble in water, nearly insoluble in ether and chloroform, and which, in aqueous solution, gave precipitates with phosphomolybdic acid, tannin, potassium iodohydrargyrate and gold chloride. The substance was thought to be an alkaloid and was given the name phytolaccine. Frankforter and Ramaley[19] prepared acid and alkaline extracts of the drug. After shaking with various immiscible solvents, they tested the dissolved residues with alkaloidal reagents, obtaining in many cases results indicating the presence of alkaloid. They failed to isolate the substance responsible for the alkaloidal reactions. Burt and

Nelson[20] state that the plant contains no alkaloid. Nagai[21] reported the isolation of phytolaccatoxin from Phytolacca acinosa, var. esculentia. The substance was a light yellow powder, stable in air, and it melted at 170°C. It was slightly soluble in alcohol and ether, difficultly soluble in glacial acetic acid, benzol and carbon disulfide. From analysis the empirical formula $C_{24}H_{30}O_8$ was assigned to the substance. Although the substance exhibited a neutral character, it was not classified as a glucoside. It was found to act as a cramp toxin similar to the action of picrotoxin, coryamyrtin and cicutoxin. Cushny[22] writes that phytolaccotoxin, which was obtained from a Japanese species of phytolacca, resembles picrotoxin in its action. Iwakawa[23] claims that Takahashi and Inoko[24], who reported the isolation of phytolaccotoxin, did not isolate the substance from phytolacca but probably worked with Cynanchum caudatum, the root of which resembles that of phytolacca, and obtained cynanchotoxin which belongs to the picrotoxin group. Molisch[25] reported anthocyan pigments. Reichert[26] reported the presence of saponin-like principles in ombu leaves (Phytolacca dioica). Jenkins[27] found moisture, 8.9 and 9.07 per cent; total ash, 9.45 and 9.32 per cent; acid-soluble ash, 71.2 and 71.4 per cent; potassium, sodium, calcium, iron, silicon, aluminum, magnesium and manganese combined as sulfate, chloride and phosphate. He extracted 100 gram portions of the drug with various solvents obtaining the following results: petroleum ether

(b.p. 40-52°C.) extracted 1.75 per cent; ether extracted 1.45 per cent; chloroform extracted 1.88 per cent; alcohol extracted 14.55 per cent; acetone extracted 3.65 per cent. He isolated an essential oil, and obtained, by a shaking-out process, a substance "which gave all the characteristic tests for alkaloids." Attempts to prepare a crystalline compound of the alkaloidal substance gave negative results. From an alcoholic extract of the root there was obtained an amount of potassium nitrate representing 0.21 per cent of the weight of the root used.

The many indefinite and conflicting reports pertaining to the physiological activity and chemical constituents of phytolacca warranted a thorough study of the plant.

EXPERIMENTAL

The poke root used in this work was obtained in number 20 and fine powders from S. B. Penick and Sons, New York City, and also was collected in fields in and around Baltimore, Maryland.

Moisture Content.--The results of previous moisture determinations are given in the following table:

Plant part	Moisture per cent	Investigator	Year
Berries	70.00	Cramer	1881
Whole plant	75.40	Balland	1881
Fresh root	80.73	Preston	1884
Air dried root	29.04	Preston	1884
Fresh root	74.90	Newcomb	1915
Air dried root	8.98	Jenkins	1929

Samples of the air dried root, when dried to constant weight in an electric oven at 100°C., lost 9.0 and 9.3 per cent of their weights.

Ash Content.--The following tabulated ash determinations have been reported:

Plant part	Total ash per cent	Acid-soluble ash per cent	Investigator	Year
Air dried root	10.73	91.31	Pape	1881
Dried berries	5.00	---	Cramer	1881
Air dried root	8.40	---	Preston	1884
Air dried root	9.38	71.30	Jenkins	1929

Determinations made according to the United States Pharmacopoeia X yielded 10.83 and 10.78 per cent of total ash of which 94.18 and 93.81 per cent was acid-soluble. Qualitative analysis showed the presence of potassium, sodium, calcium, iron, silicon, aluminum and magnesium, in addition to sulfate, chloride and phosphate ions.

Determinations of other constants for the drug according to the methods of the United States Pharmacopoeia X, yielded results as follows:

	I per cent	II per cent	Average per cent
Crude fiber	16.10	16.31	16.205
Total ether-soluble extractive	0.98	0.985	0.983
Volatile " "	0.13	0.15	0.140
Non-volatile " "	0.85	0.835	0.843
Alcohol-soluble extractive	1.78	1.75	1.765
Diluted alcohol-soluble "	12.08	12.14	12.120
Water-soluble extractive	13.18	13.24	13.210
Benzin-soluble extractive	0.45	0.53	0.497

Preliminary Extraction.--In order to determine the action of different solvents and to study the general characters of the constituents of the root, 100 g. of the drug in the form of a fine powder was successively extracted in a Soxhlet apparatus with each of the following solvents, the solvents were allowed to evaporate spontaneously at room temperature, after which the residues were weighed without heating.

	Per cent extracted
Petroleum benzin (b.p. 28-40°C.)	0.409
Ether	0.466
Chloroform	0.318
Alcohol	8.680
Ethyl acetate	2.791

The petroleum benzin extractive possessed a light tan color, formed a viscous oil when warmed and a semi-solid mass when allowed to cool to room temperature. It had a characteristic odor and taste, and resembled the waxes in its physical appearance. The extractive was quite clear, and it very likely consisted of a mixture of plant fats and waxes. It was insoluble in dilute acid but was soluble in potassium hydroxide solution.

The ether extractive was of a light amber color, having a pleasant odor and a resinous appearance. It was insoluble in dilute acid, soluble in potassium hydroxide solution and in alcohol. When the alcoholic solution was poured into a beaker containing water, acidified with dilute hydrochloric acid, a fine precipitate was formed;

indicating the presence of an acid resin.

The chloroform extractive was similar to the ether extractive in appearance and was also insoluble in dilute acid and soluble in potassium hydroxide solution and alcohol.

The alcohol extractive had a dark amber color and appeared to be resinous. It was partially soluble in acids and readily soluble in potassium hydroxide solution. The solutions, when shaken, formed fairly stable foams.

The ethyl acetate extractive was of a dark, reddish-brown color, the mass having a resinous appearance. It was slightly soluble in alcohol and was soluble in water. The aqueous solution gave positive tests with most of the alkaloidal reagents and gave negative tests for proteins and tannins.

Tests for Alkaloid.--Ten grams of the powdered root was macerated with Prolius fluid, the supernatant liquid was removed and shaken out with 2 per cent sulfuric acid solution. The aqueous layer was separated and tested with various alkaloidal reagents, all of which gave negative results.

Sixty grams of the powdered root was mixed with slaked lime, the mixture was dried in an electric oven and then was extracted in a Soxhlet apparatus with chloroform. The chloroform was removed by evaporation, and the residue was partially dissolved in 2 per cent sulfuric

acid. The acid solution gave positive tests with Mayer's reagent and other alkaloidal precipitants.

An acid extract of the powdered root was prepared by macerating the drug with 0.5 per cent sulfuric acid. After 48 hours, part of the supernatant liquid was siphoned off. The liquid was treated with Lloyd's reagent (hydrous aluminum silicate). The mixture was shaken for one-half hour in a mechanical shaker, then it was passed through a super-centrifuge to remove the suspended matter, consisting of the Lloyd's reagent in addition to the substances adsorbed by it. The solid matter was mixed with slaked lime, allowed to dry, and then extracted in a Soxhlet apparatus, using 99 per cent alcohol. After extracting with alcohol, the residue was again extracted, using ether. The ether extract, after evaporation, left a yellow residue, which, when taken up in 2 per cent sulfuric acid gave a very faint test with Mayer's reagent.

The alcoholic extract obtained from the mixture of slaked lime and Lloyd's reagent formed amorphous precipitates when tested with Mayer's reagent, phosphomolybdic acid and gold chloride solutions.

Infusion.--One hundred grams of the powdered drug was extracted with boiling water. When alcohol was added to the infusion, a mucilaginous precipitate was formed, the precipitated substance being redissolved by water. Two cubic centimeters of the infusion, diluted to 10 cc. with water, gave a positive test for carbohydrates with

Molisch's reagent, and reduced Fehling's solution.

Expressed Liquid from Fresh Root.--Two hundred grams of fresh root was expressed in a mechanical press to remove the substances present in the liquid portions of the root. On standing, a white substance settled out. This substance appeared to be granular when examined under a microscope, turned blue when treated with iodine solution, and the blue color was removed on addition of sodium thiosulfate solution. The substance was thus identified as a starch.

The supernatant liquid was tested with Fehling's solution, but no reduction was observed, indicating the absence of free reducing sugars. After boiling some of the liquid with sulfuric acid, reduction of Fehling's solution was observed. This reduction probably was caused by the sugars liberated as a result of hydrolysis of the gum which was later found to be present in the root.

Preliminary Test of Pharmacologic Activity.--A fluidextract of the dried root was prepared, using a hydroalcoholic (1:1) menstruum.

Five cubic centimeters of the fluidextract, representing 5 g. of dried root, was injected intraperitoneally into a normal cat weighing about 2 Kg. The cat first lost control of its hind legs, then its forelegs and the rest of its body became flaccid, and finally the animal died.

Five cubic centimeters of the fluidextract was injected slowly into the femoral vein of an anesthetized cat weighing about 2 Kg. No effect was observed on the carotid blood pressure or on the respiration of the animal.

In order to make a more complete study, petroleum benzin, alcoholic and aqueous extracts were prepared from larger quantities of the drug as follows.

Petroleum Benzin Extractive

Twenty-two kilograms of finely powdered poke root was extracted with petroleum benzin (b.p. 30-60°C.) in a Lloyd's extractor until the drug was exhausted. Most of the benzin was removed by distillation under reduced pressure, and a current of air was passed over the residue to remove the remaining solvent. The residue became more viscid as the benzin evaporated and finally solid particles appeared throughout the mass. On heating above 30°C., the whole mass became homogeneous and limpid, but on cooling to room temperature solid particles again separated from the oil. The solid and the oil were dissolved together in hot alcohol, and on cooling and setting overnight there was obtained a precipitate and a clear alcoholic solution.

Isolation of a Sterol-like Compound $C_{23}H_{40}O$. Small portions of the precipitate gave color reactions with the Burchard-Liebermann and Hesse reagents for sterols. In the latter case, however, the red color produced was

more pronounced in the sulfuric acid layer than in the chloroform layer. A chloroformic solution of the substance decolorized a bromine solution. The substance was readily soluble in hot ethyl acetate and hot ethyl alcohol, and was deposited from the solutions while still warm, but the coloring matter present behaved in a similar manner. A solution of the substance in hot ethyl acetate was prepared and was boiled with animal charcoal for several minutes, then filtered, while hot, through a Gooch funnel by means of a hydraulic suction pump. On cooling, crystals formed in rosettes. These crystals were removed by filtration and washed with ethyl acetate, then they were dried in an oven for thirty minutes at 95°C. The crystals melted at 107-108°C. The crystals (0.5 g.) were recrystallized from ethyl acetate, ether and alcohol respectively, and then were dried. The melting point remained unchanged at 108°C. Tests for nitrogen and sulfur gave negative results.

0.003964 g. gave 0.012062 g. CO_2 and 0.004326 g. H_2O.

C = 82.99; H = 12.21 per cent

$C_{23}H_{40}O$ requires C = 83.05; H = 12.13 per cent

0.0280 g. dissolved in 14 cc. of chloroform gave $\alpha_D^{26^\circ} = +0.14^\circ$ in a 100 mm. tube. $[\alpha]_D^{26^\circ} = +70.0^\circ$.

The compound was boiled with acetic anhydride under a reflux condenser for 2 hours. The crystals which separated out on cooling were removed by filtration and recrystallized from ether. After drying, the crystals melted

at 107-108°C., indicating that no acetylation had occurred. The compound was boiled with acetyl chloride under a reflux condenser for 2 hours. The crystals which separated out on cooling were removed by filtration, freed from acetyl chloride by means of a current of air sucked through them, then recrystallized from alcohol and again from petroleum benzin. The melting point was found to be 107-108°C. The oxygen present in the molecule apparently is not in the form of a free hydroxyl group.

The alcoholic solution obtained from the petroleum benzin extractive was freed from alcohol by evaporation and the final traces of the solvent were removed by heating the residual oil on a boiling water bath under reduced pressure. The oil obtained weighed 96.41 g., representing 0.089 per cent of the dried root used. The following constants were determined for the oil: Specific gravity $\frac{25°}{25°}$ 0.9209; Optical rotation $[\alpha]_D^{26°} = +13°$; Refractive index $N_D^{26°} = 1.4741$; Acid number 71.97; Saponification number 139.43; Ester number 67.46; Iodine number 69.14.

Isolation of Free Fatty Acids. Fifty-five grams of the oil was dissolved in 1200 cc. of ether and the solution was successively extracted with portions of 5 per cent solutions of ammonium carbonate, sodium carbonate and potassium hydroxide.

The ammonium carbonate shakings were acidified with diluted hydrochloric acid, and the mixture was extracted with ether. The ethereal solution was dried with anhydrous

sodium sulfate and then the ether was removed by distillation. The oily residue was dissolved in alcohol and the silver salt was prepared using silver nitrate solution. The silver salt was analyzed.

0.474 g. of salt gave on ignition 0.0201 g. of Ag.

Ag = 42.4 per cent

$C_8H_{15}O_2Ag$ requires Ag = 43.0 per cent.

Although the figures thus obtained are in fairly close agreement with those required for the silver salt of an octanoic acid, it is probable that the oily acid was a mixture.

The sodium carbonate shakings were obtained in two portions and these were examined separately.

Portion I. The reddish-brown aqueous solution was treated with animal charcoal, and, after cooling, was acidified with diluted sulfuric acid and extracted with ether. The ethereal solution was dried over anhydrous sodium sulfate and the ether was removed by distillation. The orange-colored residue was dissolved in alcohol and the alcoholic solution was kept at 10°C. The crystals which were deposited in the alcoholic solution were separated by filtering through a Gooch funnel with suction. The crystalline solid (1.75 g.) was dissolved in alcohol and recrystallized at 10°C. After drying in a desiccator, the melting point was found to be 72-73°C. Recrystallization from methyl alcohol and then from glacial acetic acid raised the melting point to 76-77°C. The silver salt

was prepared and analyzed.

0.0265 g. of salt gave on ignition 0.0063 g. of Ag.

Ag = 23.77 per cent

$C_{20}H_{39}O_2Ag$ requires Ag = 25.67 per cent.

The above-described substance was thus identified as arachidic acid.

The alcoholic mother-liquor from which the arachidic acid had been obtained was concentrated and kept at 5°C. overnight. A small amount (0.55 g.) of crystalline matter was deposited, and this was removed by filtration with suction. The crystals melted at 61°C. The silver salt was prepared and analyzed.

0.1229 g. of salt gave on ignition 0.0361 g. of Ag.

Ag = 29.37 per cent

$C_{16}H_{31}O_2Ag$ requires Ag = 29.7 per cent.

The crystals were thus identified as palmitic acid.

The alcoholic mother-liquor from which the palmitic acid had been obtained was further concentrated and kept at 10°C. A solid separated in small spherical bundles of crystals. The solid (0.4 g.) was removed by filtration with suction and was recrystallized from alcohol. The crystals melted at 51°C. The silver salt was prepared and analyzed.

0.1371 g. of salt gave on ignition 0.0405 g. of Ag.

Ag = 29.54 per cent

$C_{14}H_{27}O_3Ag$ requires Ag = 30.72 per cent.

The crystals were thus identified as oxymyristic acid.

The alcoholic mother-liquor from which the oxymyristic acid had been obtained was kept at 5°C., when crystals were deposited. The crystals (0.5 g.) were removed by filtration with suction, and were recrystallized from alcohol. The melting point was found to be 59-60°C. The silver salt was prepared and analyzed.

0.0632 g. of salt gave on ignition 0.0183 g. of Ag.

Ag = 28.95 per cent

$C_{17}H_{33}O_2Ag$ requires Ag = 28.6 per cent.

The crystals were thus identified as margaric acid.

Two more fractions (0.5 g. and 0.8 g.) of crystalline matter were obtained from the alcoholic mother-liquor, and, although the melting points of the crystals were 53-56°C. and 46-48°C. respectively, the analyses of the silver salts corresponded fairly well with the required results for margaric acid. Apparently some other substance had been removed along with the margaric acid to cause a lowering of the melting point.

The residual alcoholic mother-liquor decolorized a chloroformic solution of iodine. The alcohol was removed and the liquid residue was kept for a short time at 10°C., when it solidified. A silver salt was prepared and analyzed.

0.1115 g. of salt gave on ignition 0.0279 g. of Ag.

Ag = 25.02 per cent

$C_{18}H_{33}O_2Ag$ requires Ag = 27.72 per cent.

The above-described residue probably consisted of oleic

acid and some saturated acid or acids which had not been removed by fractionation.

Portion II. The second portion of the sodium carbonate shakings from the ethereal solution of the oil was treated with charcoal, acidified and extracted with ether. The ethereal solution was dried with anhydrous sodium sulfate, the ether was removed and the residue was dissolved in alcohol and the solution kept at 10°C. The crystals (0.5 g.) which were deposited were separated by filtration and recrystallized from glacial acetic acid. The crystals were found to melt at 72-73°C. The silver salt was prepared and analyzed.

0.0155 g. of salt gave on ignition 0.0037 g. of Ag.

Ag = 23.87 per cent

$C_{20}H_{39}O_2Ag$ requires Ag = 25.67 per cent.

The crystals were thus identified as arachidic acid.

The alcoholic mother-liquor was concentrated and kept at 10°C. The crystals (1.1 g.) which were deposited were removed by filtration and recrystallized from glacial acetic acid. The crystals were found to melt at 58.5°C. The silver salt was prepared and analyzed.

0.0810 g. of the salt gave on ignition 0.0239 g. of Ag.

Ag = 29.5 per cent

$C_{16}H_{31}O_2Ag$ requires Ag = 29.7 per cent.

The crystals were thus identified as palmitic acid.

The potassium hydroxide shakings from the ethereal solution of the oil had a red color which changed to yellow on acidification with diluted sulfuric acid. The

amount of the substance liberated by the sulfuric acid was too small to investigate.

Saponification of Oil. The ethereal solution of the oil which had been shaken with the alkalis was washed free of alkali by shaking with water, then dried with anhydrous sodium sulfate, and the ether was removed by distillation and evaporation. The oily residue (33.2 g.) was very limpid as compared to the original oil. The oil was saponified according to A. Boemer's method[28]. After saponification and addition of 200 cc. of water, the liquid was allowed to cool to room temperature and then was extracted repeatedly with ether. The ethereal extractions were thoroughly washed with water and the ether was removed, leaving a reddish-brown solid. The residue was dissolved in hot 98 per cent alcohol, leaving a very small amount of an amber colored, oily substance which did not dissolve.

The substance which did not dissolve in hot alcohol was soluble in hot acetone and cold chloroform. It gave negative Hesse and Liebermann-Burchard tests for sterols. The substance may possibly have been a hydrocarbon of low molecular weight.

Isolation of Hentriacontane. The hot alcohol-soluble portion (300 cc. of solution) of the residue obtained from the ethereal shakings, when allowed to cool and stand overnight, deposited a small amount of solid. The solid (0.5 g.) was removed by filtration and was crystallized from ethyl acetate. The crystals melted at 67.4°C. and, using an

ethyl acetate solution, gave faintly positive tests for sterols with the Hesse and Liebermann-Burchard reagents. The crystals were not affected by concentrated sulfuric acid, and did not react with acetic anhydride. After recrystallization from chloroform and then from alcohol, the melting point was found to remain at 67.4°C.

The above-described substance, from its manner of isolation, chemical inactivity and melting point, was identified as hentriacontane.

Isolation of a Sterol (Phytolaccasterol) $C_{30}H_{50}O$. The alcoholic solution from which the hentriacontane had been obtained, was concentrated to one half its volume (150 cc.) and allowed to stand. Crystals, in the form of platelets, formed in a few hours. The crystals (1.0 g.) were removed by filtration and recrystallized from a mixture of ethyl acetate and alcohol (1:1), and then from ether. The melting point was 168-169°C. Further recrystallization from ether raised the melting point to 169-170°C. A chloroformic solution of the substance decolorized a bromine solution. Color reactions were observed with the Liebermann-Burchard and Hesse reagents for sterols, but in the latter case the sulfuric acid layer exhibited a deeper red than the chloroform layer.

0.004191 g. gave 0.012413 g. CO_2 and 0.004467 g. H_2O.

C = 80.78; H = 11.93 per cent

$C_{30}H_{52}O_2$ requires C = 81.00; H = 11.79 per cent

0.0710 g., after heating for 4 hours at 106°C., lost 0.0029 g. of H_2O. H_2O = 4.08 per cent

1 molecule of H_2O of hydration = 4.05 per cent

$C_{30}H_{52}O_2 = C_{30}H_{50}O.H_2O$

0.0303 g. dissolved in 15 cc. of chloroform gave

$\alpha_D^{26^\circ} = +0.07^\circ$ in a 100 mm. tube.

$[\alpha]_D^{26^\circ} = +35.0^\circ$

The compound was boiled with acetic anhydride for 3 hours. On cooling, platelets with a mother of pearl lustre were formed. The crystals were separated from the solution by filtration and were recrystallized from alcohol. After heating in an oven at 110°C., the melting point was found to be 177-180°C. After recrystallizing from ether, the melting point was raised to 183-183.5°C. The acetyl derivative was recrystallized from petroleum benzin, and only perfectly clear platelets were used to determine the melting point which was found to be 183-183.5°C.

0.0518 g. reacted with 1.13 cc. of 0.1 N KOH.

Gram molecular weight = 458.4 g.

0.004008 g. gave 0.012039 g. CO_2 and 0.004326 g. H_2O.

C = 81.92; H = 11.33 per cent

$C_{32}H_{52}O_2$ requires C = 81.97; H = 11.19 per cent

Gram molecular weight = 468.4 g.

The original compound recovered from the saponification mixture and recrystallized from alcohol melted at 169-170°C.

The compound $C_{30}H_{50}O$ described above was proved to be a monohydroxy sterol isomeric with the amyrins (m.p. 170°C.)

isolated by Tschirch and Cremer[29] from the different sorts of elemi. Vesterberg[30] resolved the amyrins into alpha-amyrin (m.p. 181-181.5°C.) and beta-amyrin (m.p. 193-194°C.). He also prepared the acetyl derivative of the unresolved amyrin and found it to melt at 200°C., and on crystallizing from ligroin solution obtained two crystalline forms, i.e. leaflets and prisms, which he identified as alpha-amyryl acetate (m.p. 220°C.) and beta-amyryl acetate (m.p. 235°C.). Attempts to resolve the acetate of the compound isolated by us into more than one compound were unsuccessful, and the different melting point obtained with clear leaflets from benzin solution indicates that the compound differs from the previously reported isomers.

The mother-liquor from which the sterol had been obtained, on further concentration and standing, yielded another small crop (0.1 g.) of the same compound.

Isolation of Combined Fatty Acids. The alkaline, aqueous solution which had been extracted with ether, was acidified with diluted sulfuric acid. The solution was found to contain formic acid by testing with ammoniacal silver nitrate solution. The solution was then extracted repeatedly with ether. The aqueous solution remaining after the ether extractions was neutralized and evaporated to dryness on a water bath. The residue, after extraction with alcohol-ether and removal of the solvent, gave a faintly positive test for the presence of glycerin. The ethereal solution of the liberated fatty acids was dried

with anhydrous sodium sulfate and the solvent was removed. The residue, on cooling, solidified to a dark reddish-brown mass. The residue was dissolved in hot alcohol and kept at 10°C. The solid (1.0 g.) that separated was removed by filtration and recrystallized from alcohol. The substance melted at 73-74°C. The substance was recrystallized from ethyl acetate and then from ethyl acetate and alcohol. The melting point of the resulting granular powder was found to be 75-76°C. The silver salt was prepared and analyzed.

0.0652 g. of salt gave on ignition 0.0169 g. of Ag.

Ag = 25.6 per cent

$C_{20}H_{39}O_2Ag$ requires Ag = 25.67 per cent.

The substance was thus identified as arachidic acid.

The mother-liquor was concentrated and kept at 10°C. The solid (0.5 g.) which separated was removed by filtration. The solid seemed to have adsorbed some amber colored oily substance which caused part of the mixture to liquefy when the mixture was heated to about 27°C. The solid was dissolved in alcohol and precipitated as the colorless silver salt, which was analyzed. The colored portion remained in the alcohol.

0.0614 g. of salt gave on ignition 0.0173 g. of Ag.

Ag = 28.17 per cent

$C_{17}H_{33}O_2Ag$ requires Ag = 28.6 per cent.

The substance was very probably margaric acid with some impurity which appeared to be some of the original oil that had not been saponified.

The mother-liquor was again concentrated, and, when no solid separated on cooling, the remainder of the solvent was removed. A mixture of solid particles in oil was obtained. An attempt to separate the oil from the acids by forming the sodium salts of the free acids and removing the oil by extracting with ethereal solvents was unsuccessful. The acids were again liberated with diluted sulfuric acid and the mixture was extracted with chloroform. The chloroform was removed, leaving 13.5 g. of a reddish-brown semi-solid residue. The residue was refluxed on a water bath with 28 cc. of methyl alcohol and 0.7 cc. of concentrated sulfuric acid for 5 hours, and then the excess methyl alcohol was removed. The residue was taken up in ether, the ethereal solution was washed with water to remove the sulfuric acid, then the ethereal solution was dried and the ether removed. The remaining liquid methyl esters were distilled under a pressure of 10 mm. of mercury. A negligible amount of distillate came over between 173° and 189°C.; about 5 cc. of distillate came over at 190°C.; and a small amount of distillate came over between 191° and 215°C. The latter distillate, which was collected separately, was discarded. The distillate which had been collected between 173° and 190°C. was refluxed on a water bath with 10 per cent sodium hydroxide in 70 per cent alcohol for 2 hours. The saponified solution was shaken with ether to remove any unsaponified oil and liberated substances other than the acids. The aqueous solution was then acidified with diluted sulfuric acid and was extracted repeatedly with

ether. The ethereal solution was washed with water, dried with anhydrous sodium sulfate, the solvent removed and the solid residue was taken up in warm alcohol. The first crop of crystals, weighing 0.1 g., obtained at room temperature melted at 58.5°C. The silver salt was prepared and analyzed.

0.0413 g. of salt gave on ignition 0.0124 g. of Ag.

Ag = 30.0 per cent

$C_{16}H_{31}O_2Ag$ requires Ag = 29.7 per cent.

The compound was very likely palmitic acid with a small amount of impurity.

The mother-liquor was concentrated and kept at 10°C. The solid (2.0 g.) which separated was removed by filtration, and was found to melt at 58-59°C. Recrystallization from alcohol raised the melting point to 62-62.5°C. The silver salt was prepared and analyzed.

0.1010 g. of salt gave on ignition 0.0308 g. of Ag.

Ag = 30.0 per cent

$C_{16}H_{31}O_2Ag$ requires Ag = 29.7 per cent.

The acid melts at 62-62.5°C.

$C_{16}H_{32}O_2$ melts at 62.6°C.

The methyl ester was collected at 190°C. (10 mm.)

$C_{16}H_{31}O_2CH_3$ boils at 196°C. (15 mm.)

0.0596 g. of the acid required 2.34 cc. of 0.1 N KOH; corresponding to 220.3 mg. of KOH per g. of acid.

$C_{16}H_{32}O_2$ has a neutralization value of 219.1 mg. of KOH per g. of acid.

Molecular weight determined from the acid value is

253.9.

$C_{16}H_{32}O_2 = 256.34$.

The above-described compound was thus identified as palmitic acid.

The mother-liquor was concentrated and kept at 10°C. A crop of crystals weighing 1.0 g. was obtained. The crystals melted at 50°C., and recrystallization from ethyl acetate raised the melting point to 61-62°C. This, undoubtedly, was a further yield of palmitic acid.

The mother-liquor was found to react readily with bromine. The alcohol was removed, leaving a residue which was liquid at room temperature and which solidified when kept at 10°C. The liquefied substance gave a positive elaidin reaction when treated with concentrated nitric acid and copper wire. The silver salt was prepared and analyzed.

0.1756 g. of salt gave on ignition 0.0503 g. of Ag.

Ag = 28.6 per cent.

$C_{18}H_{33}O_2Ag$ requires Ag = 27.7 per cent.

Methyl oleate distils at about the same temperature as methyl palmitate.

The above-described residue probably consisted mainly of oleic acid together with some acid or acids which had not been removed by fractionation.

Alcoholic Extractive I.

After removal of the alcohol from the extract obtained from 7.751 Kg. of the powdered root by means of a Lloyd extractor, 1.423 Kg. of a dark reddish-brown residue re-

mained. This extract was divided into two portions, designated A and B respectively.

Examination of Volatile Constituents. Part A (510 g.) of this extract was placed in a large flask, some water was added, and steam was passed through the mixture until volatile substances were no longer carried over by the steam in the distillate. The distillate had a faintly acid reaction, which was not due to formic or acetic acid, and contained a small amount of a volatile, oily substance. The distillate was extracted many times with small portions of ether, the combined ethereal liquid was washed with distilled water, dried over anhydrous sodium sulfate, and the ether was removed. The residue was a mobile, yellowish-brown liquid which possessed a disagreeable and penetrating odor, which, when diluted, resembled the odor of the plant and had a sharp, pungent taste. The amount obtained was 1.35 g., corresponding to 0.04 per cent of the weight of the dried root used. When the distillation of a portion of the oil was attempted, decomposition took place and only a few drops of a clear, brown liquid were collected before a cloudy mixture passed over as the distillate. There remained in the distillation flask, in addition to the charred matter, a viscid, reddish-brown liquid. The specific gravity of the oil obtained from the ethereal solution was 0.9977 $23^{\circ}/4^{\circ}$C. The high specific gravity indicates the absence of terpenes.[34] The oil formed a cloudy suspension when mixed with 98 per cent alcohol. This property differentiates the oil from the essential oils, which are miscible in alcohol. The oil

may have been composed mainly of lower fatty acids and their esters.

Part B (913 g.) of the alcoholic extract was mixed with slaked lime, placed in a five gallon glass jug, and the mixture was extracted by maceration with frequent shaking, using first a mixture of ether and chloroform (3:1) and then chloroform as the menstrua. The extracts were concentrated and the solvents were recovered by distillation.

The concentrated liquids obtained by macerating the calcium hydroxide-alcoholic extract mixture with ether-chloroform (3:1) and chloroform were combined and the solution was extracted repeatedly with 2 per cent sulfuric acid. Upon the addition of the acid, the liquid assumed the appearance of a suspension and exhibited a strong tendency to emulsify. The combined acid shakings were made basic with ammonia water and the solution was extracted repeatedly with ether. Most of the ether was removed from the combined ether shakings by distillation and the remainder was allowed to evaporate spontaneously at room temperature. The residue consisted of a clear yellow, semi-solid substance with crystalline matter dispersed in it. This residue appeared to be similar to that obtained by treating an acid extract of the drug with Lloyd's reagent, mixing the Lloyd's reagent and adsorbed material with slaked lime and extracting the mixture with alcohol as previously described. Although this residue was obtained from 913 g. of alcoholic extract, representing 4,973 g. of the dried root, the amount obtained was not much greater than that obtained

from 800 g. of the dried root by the method using Lloyd's reagent. The residue obtained from the ethereal solution was found to be insoluble in petroleum benzin (b.p. 35-40°C.), soluble in anhydrous ether and absolute alcohol, and it gave positive tests with alkaloidal reagents, all the precipitates being amorphous. Attempts to separate the semi-solid from the crystalline matter by means of solvents and with chemical reagents were unsuccessful.

Alcoholic Extractive II.

An alcoholic extractive, representing 11 Kg. of dried poke root was prepared using a Lloyd extractor. Most of the alcohol was removed by distillation, and the syrupy residue was mixed with distilled water. This treatment enabled a separation into a water-soluble fraction (A) and a water-insoluble fraction (B).

A. The aqueous solution, which was quite acidic, was made basic with potassium bicarbonate and then was repeatedly extracted with ether, then with amyl alcohol and finally with chloroform.

The ether and amyl alcohol solutions were extracted with 1 per cent tartaric acid solution, the aqueous solutions were combined and made basic with sodium bicarbonate. The basic solution was then extracted repeatedly with ether. The remaining aqueous solution still gave precipitates with alkaloidal reagents; therefore it was evaporated to dryness, the residue extracted with ether, the ether removed and a solution of the water-soluble portion of the residue was prepared and labeled (U). The ethereal solution obtained

by shaking out the basic aqueous solution was evaporated and yielded a pale yellowish-brown, resin-like substance. Part of the resin-like substance was dissolved in alcohol (solution Y) and another portion was treated with distilled water (solution Z). Both solutions formed precipitates when treated with phosphomolybdic acid test solution.

The amyl alcohol solution remaining after treatment with tartaric acid was concentrated. On cooling, a solid substance separated. This solid was removed by filtration, dissolved in hot alcohol, and the alcoholic solution was allowed to evaporate to dryness. The residue obtained upon evaporation had a smooth, light yellowish-brown surface, but when the brittle substance was broken it appeared to have a crystalline structure. The substance had a bitter taste, did not reduce Fehling's solution, caused a very slight reduction of Fehling's solution after boiling with sulfuric acid, and gave no reaction on treatment with concentrated sulfuric acid or with ferric chloride solution. An aqueous solution was prepared and labeled (R).

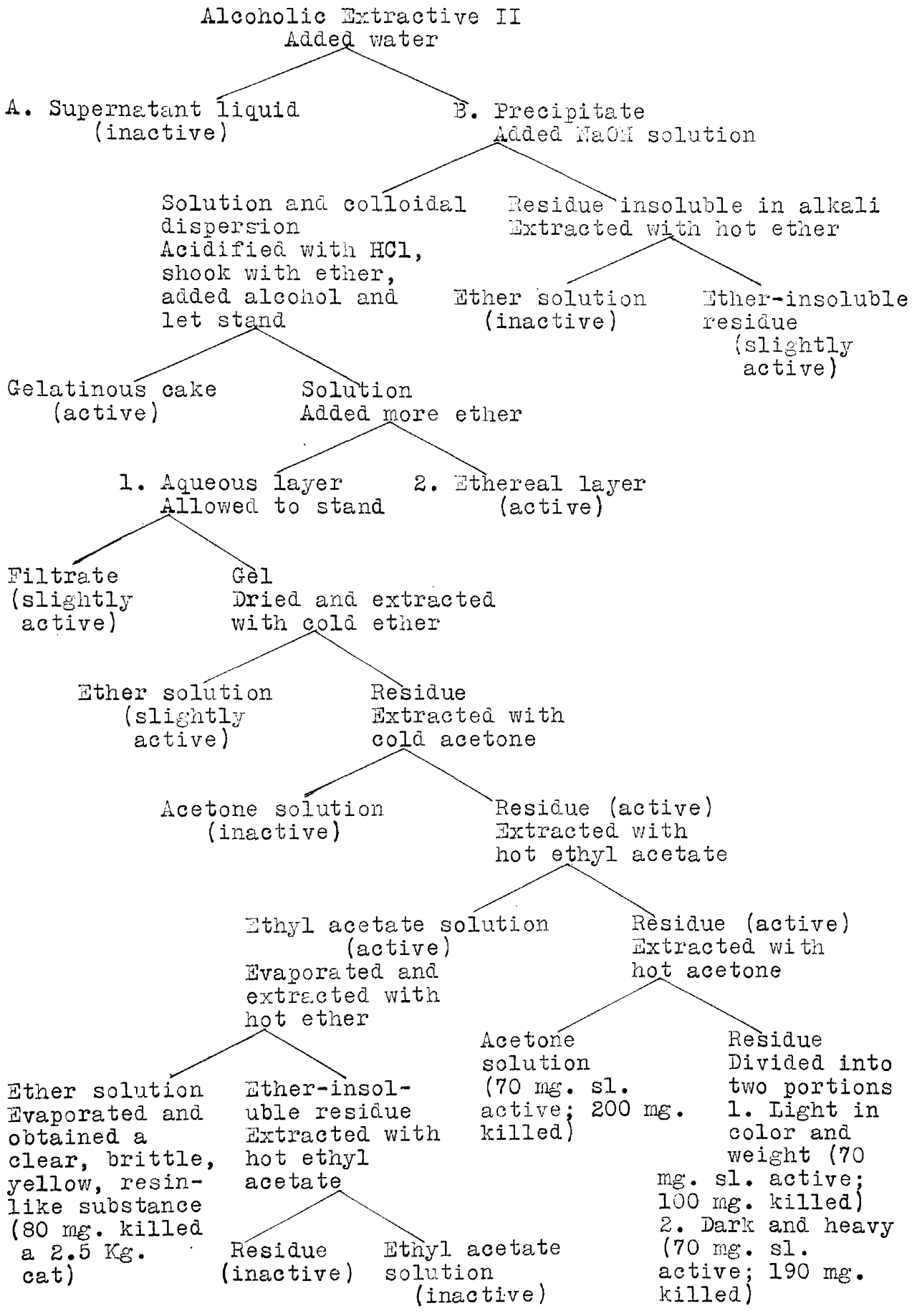
The chloroformic solution obtained from the basic aqueous solution was extracted with 1 per cent tartaric acid solution until the shakings no longer gave a precipitate when tested with phosphomolybdic acid test solution. The residual chloroformic solution still gave a positive test with phosphomolybdic acid; thus indicating that more than one substance in the original chloroformic solution formed precipitates with this reagent. The chloroform was evaporated, the residue was dissolved in alcohol and the

solution labeled (X). The tartaric acid solution was made basic with sodium bicarbonate and then was extracted with chloroform. The chloroform was removed and a solution of the water-soluble portion of the residue was prepared and labeled (B).

The solutions (U), (Y), (Z), (R), (X) and (B) were tested on normal cats by injecting 5 cc. of each solution intraperitoneally. In all cases, the cats remained apparently normal. Evidently, the substances which give amorphous precipitates with alkaloidal precipitants, but which, in other respects, do not behave as normal alkaloids, are not responsible for the toxicity of poke root.

The aqueous solution (A) remaining after extraction with ether, amyl alcohol and chloroform, was tested by injecting 6 cc. of the solution intraperitoneally into a cat. The animal remained apparently normal. Evidently, the active portion of the alcoholic extract of poke root is not soluble to an appreciable extent in water.

B. One half of the water-insoluble portion of the alcoholic extractive (scheme on page 33), representing 5.5 Kg. of dried poke root, was treated with a diluted solution of sodium hydroxide and the mixture was filtered. The colloidal filtrate was acidified with hydrochloric acid with the formation of a creamy precipitate. The mixture was shaken with ether, then alcohol was added and the mixture was allowed to stand overnight. A gelatinous cake formed at the surface of the mixture.


Alcoholic Extractive II
Added water
A. Supernatant liquid (inactive)
B. Precipitate
Added NaOH solution
Solution and colloidal dispersion
Acidified with HCl, shook with ether, added alcohol and let stand
Residue insoluble in alkali
Extracted with hot ether
Ether solution (inactive)
Ether-insoluble residue (slightly active)
Gelatinous cake (active)
Solution
Added more ether
1. Aqueous layer
Allowed to stand
2. Ethereal layer (active)
Filtrate (slightly active)
Gel
Dried and extracted with cold ether
Ether solution (slightly active)
Residue
Extracted with cold acetone
Acetone solution (inactive)
Residue (active)
Extracted with hot ethyl acetate
Ethyl acetate solution (active)
Evaporated and extracted with hot ether
Residue (active)
Extracted with hot acetone
Ether solution
Evaporated and obtained a clear, brittle, yellow, resin-like substance (80 mg. killed a 2.5 Kg. cat)
Ether-insoluble residue
Extracted with hot ethyl acetate
Acetone solution (70 mg. sl. active; 200 mg. killed)
Residue
Divided into two portions
1. Light in color and weight (70 mg. sl. active; 100 mg. killed)
2. Dark and heavy (70 mg. sl. active; 190 mg. killed)
Residue (inactive)
Ethyl acetate solution (inactive)

The gelatinous cake was removed, allowed to dry to a soft mass and then was taken up in alcohol. The alcoholic solution did not appear to have the characteristic action of the toxic portion of poke root when injected intraperitoneally into a normal cat. This may have been due to the small amount of the substance injected; because, after further treatment, an active fraction was obtained.

The solution remaining after removal of the gelatinous cake was shaken with more ether. On standing for a short time, the mixture formed two layers which were separated in a separatory funnel (aqueous layer 1, ethereal layer 2).

1. The aqueous layer, on standing, separated into a clear solution and a flocculent gel which became heavier and settled out. The mixture was separated by filtration.

The aqueous filtrate was evaporated to dryness, and a solution of the alcohol-soluble part of the residue was tested on a cat. Some activity was observed, but the cat recovered. Part of the active matter apparently was removed in the aqueous solution, very likely because of the alcohol present.

The gel was allowed to dry to a brittle mass weighing about 5 g., and then was powdered and extracted with cold ether. The ether solution was evaporated to dryness, the entire residue was dissolved in 3 cc. of alcohol and tested on a cat. The animal showed characteristic symptoms associated with the action of the drug, but it recovered.

The residual gel, after extraction with cold ether, was extracted with cold acetone. The acetone solution was evap-

orated, the residue was dissolved in alcohol and tested on a cat. The animal remained normal.

A portion of the gel remaining after extraction with acetone was dissolved in alcohol and tested on a cat. The animal died. The residual gel was extracted with hot ethyl acetate. The ethyl acetate solution was evaporated to dryness and yielded a light brown, granular residue weighing about 0.5 g. An alcoholic solution of 50 mg. of the residue showed some activity but did not kill a 2 Kg. cat. An alcoholic solution of 225 mg. of the residue did kill a 2.5 Kg. cat. The remaining 225 mg. of the granular residue was extracted with boiling ether. The ether solution was evaporated and 100 mg. of a clear, brittle, yellow, resin-like substance was obtained. An alcoholic solution of 80 mg. of the substance was tested on a 2.5 Kg. cat. The cat was affected in less than one hour and died within twelve hours.

The yellow substance gave a very faint coloration with the Molisch test for carbohydrates, indicating the absence of a glycosidal nature.

The ether-insoluble portion of the granular substance obtained from the gel with hot ethyl acetate was dissolved in alcohol and tested on a cat. The cat remained normal.

The gel remaining after extraction with hot ethyl acetate was tested and found to be active.

A portion of the gel was heated with a solution of barium hydroxide and formed a light brownish solution.

Carbon dioxide was passed into the solution, and the resulting suspension was filtered. The addition of alcohol to the almost colorless aqueous filtrate caused no turbidity to appear, and the addition of diluted sulfuric acid formed no precipitate of barium sulfate. The active substance present apparently is not a resin acid, or else it is a weaker acid than carbonic acid, which is improbable.

The remainder of the gel was extracted with hot acetone. The acetone solution was evaporated to dryness. An alcoholic solution of 70 mg. of the residue from the acetone solution did not kill a 2 Kg. cat, but an alcoholic solution of 200 mg. of the same substance killed a 2.5 Kg. cat in one day.

The residual gel was separated into two layers, a fraction (1 g.) light in color and easily suspended and a dark, heavy fraction (3 g.). An alcoholic suspension of 70 mg. of the light fraction did not kill a 2 Kg. cat, but an alcoholic suspension of 100 mg. of the same fraction killed a 2 Kg. cat in one day.

An alcoholic suspension of 70 mg. of the dark, heavy fraction of the residual gel was not fatal to a cat weighing 2 Kg. An alcoholic suspension of 190 mg. of the same fraction killed a 2 Kg. cat in 3 days.

The work previously described under (B) has resulted in the separation of an active resinous substance, and has indicated some of the properties of the pharmacologically active portion of poke root. It has also supplied information which may be used in the study of the other active fractions obtained.

Aqueous Extractive I.

A decoction was prepared according to the method given in the United States Pharmacopoeia X, using 300 g. of air dried root. The preparation was treated with 450 cc. of a freshly prepared solution of lead subacetate and filtered with the aid of a suction pump.

Examination of Filtrate. Hydrogen sulfide gas was passed into the liquid to remove excess lead, the mixture was filtered, the precipitate washed, and the filtrate and washings evaporated to about 300 cc. on a water bath.

One-half of the liquid was then mixed with an equal volume of a saturated solution of alum and the mixture was evaporated to dryness on a water bath. The residue was ground to a powder in a mortar and was extracted successively with 95 per cent alcohol, acetone and chloroform, and the extracts were allowed to evaporate spontaneously at room temperature. The chloroform extract left no appreciable residue. The acetone extract left a crystalline residue which did not char on heating, but the amount was too small to investigate. The alcoholic extract gave a reddish-brown, semi-solid mass. The crystals found by Preston[31] at this point of his investigation of the fresh root were not observed. (The work was repeated using air dried root and fresh root with the same result.) The mass was treated with 1 per cent hydrochloric acid and the mixture was filtered. The reddish-brown color turned to a deep reddish-purple on standing in acid medium. The filtrate gave positive tests with phosphomolybdic acid and Mayer's reagent. Part of the filtrate was treated with carbon to

remove the color, and the almost colorless filtrate gave positive tests with Mayer's reagent, gold chloride solution, phosphomolybdic and phosphotungstic acids; although the precipitates were lighter than before treatment with carbon. The remainder of the colored acid solution was treated with phosphomolybdic acid, filtered, and to the wet precipitate was added sodium carbonate. The pasty mixture was macerated with alcohol and then with ether. The alcoholic solution gave a precipitate with phosphomolybdic acid, but no precipitate was obtained with the other alkaloidal reagents. Upon evaporation, the alcoholic solution left a very slight amorphous residue which was too small to investigate further. The ether extract left no residue.

The other half of the filtrate from the lead subacetate precipitation was allowed to evaporate at room temperature, and the amber colored residue was extracted with hot alcohol. The remaining residue consisted mainly of inorganic matter with a large amount of potassium salts.

Isolation of Hemicellulose. The alcoholic liquid gave negative results when treated with Fehling's solution, but after boiling for one minute with diluted hydrochloric acid a strong reduction of Fehling's solution was obtained. The liquid was treated with 2 per cent sulfuric acid. A white, amorphous precipitate settled out and this was removed by filtration with the aid of a suction pump. The substance was insoluble in water, alcohol, ether, and acetone; it was soluble in potassium hydroxide solution

from which it was reprecipitated upon addition of acid. The substance reduced Fehling's solution and the liquid resulting from boiling with diluted sulfuric acid gave a positive test for carbohydrate with Molisch's reagent. From the behavior of the substance, it was concluded that the substance was a hemicellulose[32] which was liberated upon treatment with the sulfuric acid. Yield: 2 g., representing 0.66 per cent of the weight of the air dried root used.

Examination of Precipitate. The lead subacetate precipitate from the decoction was suspended in water, hydrogen sulfide gas was passed into the mixture, and the lead sulfide, together with some undecomposed lead subacetate precipitate, was removed by filtration.

Isolation of Isosaccharic Acid. The undecomposed precipitate remaining after treatment with hydrogen sulfide was mixed with sulfuric acid and the mixture was heated on a hot plate for two days. The colored supernatant liquid was treated with animal charcoal and then filtered. Crystals separated on allowing the filtrate to stand overnight. The crystals were removed by filtration and were recrystallized from alcohol and then from acetone. The crystals 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 obtained with Pinerua's test for hydroxy-acids using beta-naphthol and concentrated sulfuric acid. After

drying at 100°C., the crystals melted at 185°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 of the gum which was found to be present and the oxidation of the products of hydrolysis.

Isolation of Gum. The filtrate obtained after treatment with hydrogen sulfide was mixed with alcohol, and the milky-white suspension that was formed was allowed to settle and then was filtered. The precipitate was redissolved in water and again was precipitated with alcohol. This procedure was repeated once more. The light gray precipitate was allowed to dry spontaneously at room temperature. The substance was completely but slowly soluble in cold water, readily soluble in hot water, insoluble in alcohol and other organic solvents. When moistened, it swelled and became very adhesive. An aqueous solution of the substance gave a blue color with iodine solution and gave a positive test for carbohydrate with Molisch's reagent, but did not reduce Fehling's solution. A portion of the substance was boiled for one minute with diluted sulfuric acid, and the resulting solution gave a copious precipitate of cuprous oxide when warmed with Fehling's solution. Another hydrolyzed portion gave negative tests for pentoses using Bial's reagent. Oxidation with concentrated nitric acid yielded no mucic acid, which indicated the absence of galactose. Another portion of the substance

was hydrolyzed with diluted hydrochloric acid, and the resulting solution was tested for the presence of sulfate ions with negative results. The substance was evidently the gum reported present in phytolacca by other investigators, and, according to the above tests, it does not contain pentoses or galactose and does not exist as a double sulfate illustrated by the formula $R\begin{matrix}O\text{-}SO_2\text{-}O \\ O\text{-}SO_2\text{-}O\end{matrix}Ca$ [33]. The yield was 13.5 g., representing 4.5 per cent of the air dried root used.

The hydro-alcoholic filtrate obtained after precipitation of the gum was treated with ether by shaking the immiscible mixture in a separatory funnel and separating the two layers. The ethereal layer, on standing, deposited a small amount of light amber colored crystals. The crystals were separated by filtering through a Gooch crucible, and were found to be insoluble in water, alcohol, acetone, glacial acetic acid, diluted hydrochloric acid and 10 per cent potassium hydroxide solution; slightly soluble in ether and readily soluble in chloroform. The crystals melted at 119.5°C. Tests for nitrogen and sulfur showed both these elements to be absent. The amount of crystalline matter was too small to investigate further.

Separation of a Resin. The remaining hydro-alcoholic solution was evaporated until a concentrated aqueous solution remained. This solution deposited some solid matter which was removed by filtration and was redissolved in alcohol. Upon evaporation of the alcohol, a light reddish-

brown, glistening, brittle, tasteless film was left. The substance was insoluble in 2 per cent sulfuric acid, in which it remained brittle, it was slightly soluble in ether, soluble in alcohol and 10 per cent potassium hydroxide solution, from which it was precipitated upon the addition of sulfuric acid. Tests for nitrogen and sulfur gave negative results. The substance was very likely an acid resin which existed as a soluble salt in the root, thus allowing its extraction with water.

The aqueous filtrate from the resin was treated with alcohol, when a very slight precipitate was formed, and then with ether. On standing, a precipitate was deposited which was separated by filtration and proved to consist of inorganic salts to a large extent.

Isolation of Oxalic Acid and Potassium Oxalate. The above filtrate was allowed to stand to remove the alcohol and ether, and deposited a crystalline substance, which was separated by filtration and washed with water. The substance formed colorless plates which melted at 133°C. with decomposition, although no charring was noticed. It was slightly soluble in diluted sulfuric acid, soluble in alcohol, water and potassium hydroxide solution. An aqueous solution of a portion of the crystals gave an acid reaction with litmus paper and decolorized an acid solution of potassium permanganate. An aqueous solution of the crystals was treated with a solution of silver nitrate, when a heavy precipitate, very likely a silver salt, was formed. The precipitate was separated by filtering through

a Gooch crucible, dried in a desiccator and weighed. Upon heating the dried compound with a very small flame it decomposed with a violent action. The substance was very likely oxalic acid.

The filtrate, on further concentration, deposited colorless, rhomboid plates. The crystals were separated by filtration and the filtrate was set aside. The crystals gave a strongly positive test for potassium and decolorized an acid solution of potassium permanganate. The substance was very likely potassium oxalate.

Aqueous Extractive II.

A second decoction was prepared using 4.6 Kg. of fresh poke root. The decoction was treated with lead subacetate solution and the mixture was filtered. The filtrate was treated with hydrogen sulfide gas to remove the lead salts, and, after removing the precipitated lead sulfide, the liquid was divided into two parts.

Part 1. The solution was allowed to evaporate spontaneously at room temperature. After two weeks, a dark, reddish-brown, syrupy mass interspersed with needle-like crystals was obtained. The syrupy mass was treated with hot alcohol which dissolved most of the substance but left a residue which included the crystalline matter. The crystalline matter was separated and identified as potassium nitrate.

The alcoholic solution obtained from the syrupy mass was allowed to evaporate to a thick syrupy consistence and

the residue was then dissolved in water. On addition of sodium hydroxide solution, a precipitate formed and was removed by filtration (precipitate a, filtrate b).

a. The precipitate was treated with boiling ether on a water bath. The ethereal solution was removed and evaporated. The very small residue remaining after evaporation of the ether formed a very pale yellow solution upon treatment with cold water. The aqueous solution gave precipitates with alkaloidal reagents. The solution was labeled (S).

The residue remaining after removal of the ethereal solution was treated with boiling alcohol. The alcoholic solution was filtered and the residue remaining after evaporation was dissolved in 2 per cent hydrochloric acid. The solution gave precipitates with alkaloidal reagents. The acidic solution was neutralized with sodium hydroxide solution and labeled (A).

The alcoholic filtrate was allowed to evaporate to dryness and the residue was again treated with hot alcohol. The remaining substance appeared to be similar to the substance in solution (A). The filtrate, on standing, deposited a substance which settled to the bottom of the beaker and adhered to the glass. The supernatant liquid was removed by decantation, and was found to contain lead salts which had not been removed by hydrogen sulfide; although the gas had been passed into the solution until constant pressure was maintained in the closed system

without further generation of hydrogen sulfide.

Isolation of Saponin. The deposited substance which adhered to the beaker 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 emulsified oil of turpentine and caused hemolysis of blood. A slight reduction was observed with Fehling's solution, but after boiling with hydrochloric acid there was a copious precipitate of cuprous oxide formed on treatment with Fehling's solution. The saponaceous solution was evaporated to dryness, and the residue was found to give a negative test for nitrogen. This proved that the substance was not a saponin-alkaloid.

b. The filtrate obtained after adding sodium hydroxide solution was extracted with a mixture of chloroform and ether (3-1), and then was made acid and extracted with ether.

The chloroform-ether solution was evaporated, the residue was taken up in 2 per cent hydrochloric acid, the acid aqueous solution was made basic and was then extracted with chloroform. The chloroformic solution was evaporated to dryness, the residue was taken up in dry chloroform and hydrogen chloride gas was passed into the solution. No insoluble substance was formed.

Part 2 of the filtrate from lead subacetate precipitation. The solution was evaporated to a syrupy consistence and the residue was extracted with alcohol. The alcoholic solution was evaporated, the residue was

treated with alcohol and the alcoholic solution was evaporated again to a syrupy mass. The syrupy residue was dissolved in water, the solution was made basic with sodium hydroxide solution and successively extracted with ether, petroleum benzin, benzene, chloroform and ethyl acetate.

The ether extractive was evaporated and yielded a semi-solid residue which was treated with water. The yellow aqueous solution obtained was labeled (E).

The petroleum benzin and benzene extractives were too small to investigate.

The chloroform extractive was evaporated and the residue was taken up in 6 cc. of hot alcohol. To 1 cc. of the alcoholic solution was added 4 cc. of distilled water. The supernatant liquid of the resulting suspension was removed and labeled (C_a). The remaining 5 cc. of the alcoholic solution was evaporated to dryness and the residue was treated with distilled water. The orange colored aqueous solution was labeled (C_b).

The ethyl acetate extractive was evaporated and the residue was dissolved in 2 per cent hydrochloric acid. The aqueous solution was neutralized with diluted sodium hydroxide solution and labeled (EA).

Solutions (S) and (A) from part 1, and solutions (E), (C_b) and (EA) from part 2 gave amorphous precipitates with alkaloidal reagents and negative biuret tests. The solution (C_a) also gave a negative biuret reaction. The negative biuret tests indicated the probable absence of proteins.

The solutions were injected intravenously into a male cat with intact vagi under Nembutal anesthesia. No characteristic response was observed with respect to blood pressure or respiration.

Summary and Conclusions.

1. A condensed historical and botanical description of Phytolacca Americana (decandra), commonly known as poke, has been given, followed by reports on physiological activity and chemical studies of the plant.

2. The United States Pharmacopoeia X constants for crude drugs have been determined for poke root and compared with results obtained by previous workers.

3. The fatty oil obtained by extracting dried poke root with petroleum benzin was found to have the following constants: Specific gravity $_{25^\circ}^{25^\circ}$ = 0.9209; Optical rotation, $[\alpha]_D^{26^\circ}$ = +13°; Refractive index, $N_D^{26^\circ}$ = 1.4741; Acid number, 71.97; Saponification number, 139.43; Ester number, 67.46; Iodine number, 69.14.

4. Chemical investigation of the fatty oil proved it to be a complex mixture containing a large proportion of free fatty acids, some esters of fatty acids with glycerol, and wax-like esters of fatty acids with a sterol.

a. A sterol-like compound, $C_{23}H_{40}O$, melting at 107-108°C., and having an optical activity of $[\alpha]_D^{26^\circ}$ = +70.0°, was isolated. The oxygen present in the molecule does not behave as a part of a hydroxyl group, since boiling with acetic anhydride and with acetyl chloride had no

effect on the compound.

b. A sterol (phytolaccasterol), $C_{30}H_{50}O \cdot H_2O$, melting at 169-170°C., and having an optical activity of $[\alpha]_D^{26^\circ} = +35.0^\circ$, was isolated. It is isomeric with the amyrins (m.p. 170°C.) isolated from different sorts of elemi by Tschirch and Cremer[29]. Vesterberg[30] prepared an amyryl acetate (m.p. 200°C.) which he resolved into alpha- and beta-amyryl acetates melting at 220°C. and 235°C. respectively. Phytolaccasterol acetate was prepared and was found to melt at 183-183.5°C. The sterol acetate could not be resolved into more than one substance, using a procedure given by Vesterberg[30]. Phytolaccasterol was proved to be a monohydroxy compound by the saponification of its acetate, from which procedure a molecular weight of 458.4 was calculated as compared with 468.4 as determined by analysis.

c. A hydrocarbon, hentriacontane $C_{31}H_{64}$, was isolated and identified by its manner of separation and by its inactivity with chemical reagents, and by its melting point of 67.4°C.

d. The following fatty acids were isolated: Arachidic, 5.91 per cent; Palmitic, 8.63 per cent; Margaric, 4.19 per cent; Oxymyristic, 0.72 per cent. The presence of oleic acid, acids of low molecular weight and glycerol was proved.

5. A volatile oil, representing 0.04 per cent of the dried root, was obtained by steam distillation from an alcoholic extract of poke root. Its specific gravity of

0.9977 indicates the absence of terpenes.[34] The oil formed a turbid mixture with 98 per cent alcohol, and may have been composed mainly of lower fatty acids and their esters.

6. All attempts to isolate a pure alkaloidal substance were unsuccessful. Chemical and pharmacological tests indicated the absence of a pharmacologically active alkaloid, but the presence of substances which give amorphous precipitates with alkaloidal reagents was corroborated.

7. Five cc. of a fluidextract of poke root, when injected into the femoral vein of an anesthetized cat weighing 2 Kg., had no effect upon the blood pressure or respiration of the animal. Five cc. of the fluidextract, when injected intraperitoneally into a 2 Kg. cat, produced an ascending loss of muscle control and finally death within 12 hours. A pharmacologically active fraction was separated from an alcoholic extract of dried poke root. Death resulted within 12 hours when 32 mg. per Kg. of this fraction was injected intraperitoneally into a normal cat. A scheme indicating the method of separation of this fraction is given.

8. The following substances were isolated from aqueous extracts of dried and fresh poke root: 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.

9. Starch was obtained from the expressed juice of fresh poke root. A blue color was obtained when the starch was treated with a solution of iodine, and the blue color was removed upon the addition of sodium thiosulfate. The clear juice did not reduce Fehling's solution, indicating the absence of free reducing sugars.

References.

(1) "Gmelin's Chemie," 1 (1843), 179.

(2) "Chronological History of Plants," (1879), 750.

(3) "Origin and History of All the Pharmacopoeial Vegetable Drugs, Chemicals and Preparations," 1 (1921), 240.

(4) Drug. Circ., 61 (1917), 23.

(5) "Materia Medica and Therapeutics," (1850), 587.

(6) Am. J. Pharm., 47 (1875), 196.

(7) Drug. Circ., 36 (1902), 244.

(8) Med. World, 33 (1915), 35.

(9) Eclect. Med. J., 75 (1915), 322.

(10) Pharm. J., 105 (1920), 417.

(11) B. P. C., (1923), 841.

(12) "Manual of Pharmacology," Ed. 4 (1924), 196.

(13) Pharmacist, (1879), 466.

(14) Compt. Rend., 91, 856; through Am. J. Pharm., 53 (1881), 325.

(15) Am. J. Pharm., 53 (1881), 597.

(16) Ibid., 53 (1881), 598.

(17) J. Pharm. Chim., (5) 4 (1881), 232.

(18) Am. J. Pharm., 56 (1884), 567.

(19) Ibid., 69 (1897), 281; through Drug. Circ., 46 (1902), 244.

(20) "Introduction to the Analysis of Drugs and Medicines," (1910), 220.

(21) Ber. Jap. pharm. Ges., No. 98 (1891); through Ber. Chem. Ges., 24 (1891), 648.

(22) "Pharmacology and Therapeutic Action of Drugs," Ed. 4 (1906), 432.

(23) Arch. exp. Path. Pharm., V. 67, p. 118; through Chem. Abstracts, 6 (1912), 2136.

(24) Mitteil. med. Ges. Tokio, 6 (1893); through Chem. Abstracts, 6 (1912), 2136.

(25) Mikrochemie Der Pflanze, 3 (1923), 265.

(26) Anales asocn. quim. Argentina, 25 (1927), 221; through Chem. Abstracts, 22 (1928), 844.

(27) J. Am. Pharm. Assoc., (6) 18 (1929), 573.

(28) Ztschr. f. Untersuch. d. Nahres. u. Genussm., 1 (1898), 21.

(29) Arch. Pharm., 240 (1902), 293, 322.

(30) Ber. d. chem. Ges., 20 (1887), 1242.

(31) "Local citation," 5.

(32) Zeit. physiol. Chem., 16 (1892), 391.

(33) "The Chemistry of Plant Products," 1 (1928), 191.

(34) "Grundzüge der Chemischen Pflanzen Untersuchung," Ed. 3 (1928), 18-22.