T H E S I S

On

The Chemical Examination of

Echinopanax Horridum

(Devil's Walking Stick)

Submitted to the Faculty

of the

O R E G O N A G R I C U L T U R A L C O L L E G E

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in

Pharmaceutical Chemistry

by

Redacted for privacy

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Approved Redacted for privacy

Head of Department of Chemistry.

Appreciation

I wish to express my sincere appreciation for the advice and valued assistance rendered me, in the investigation of this plant, by Prof. C. E. Bradley and Prof. H. V. Tartar of the O. A. C. Experiment Station, Prof. J. Fulton, Head of the Chemistry Department, and Dr. E. G. Peterson, Head of the Bacteriology Department.

Chemical Examination of
Echinopanax Horridum.

Echinopanax horridum, more commonly known as Devil's Walking Stick, Devil's Cane, Devil's Club, is a plant that seems to be indigenous to the Pacific Coast mountains. In passing along the Coast, an eminent botanist became interested in the plant and suggested to Prof. C. E. Bradley,-who was then located at Pacific University,-that a chemical examination of this plant should be made, since it grew in such large quantities along this coast. It was at the instigation of Professor Bradley, Head Chemist in the Oregon Agricultural College Experiment Station, that I took up the chemical investigation of this plant.

Botanical

The United States National Herbarium, Volume XI, classifies this plant as follows:

Family.....Araliaceae (Gensing Family)
Genus.....Echinopanax

Echinopanax horridum - (Smith) 1854.
Panax horridum - (Smith) 1812.
Aralia erinacea - (Hook) 1827.
Fatsia horrida -(Benth & Hook) 1867.

Range:- Alaska to California and the Blue Mountains, Lake Superior.

It is classified as " Echinopanax horridum" by Thomas Howell in his "Flora of Northwest America" and as "Fatsia horrida" in the "Geological Survey of California

It grows all along the Coast and Cascade Ranges from California to Alaska and as far east in northern Canada as Lake Superior.

It always is found in damp or marshy places. In the deep ravines of the Coast mountains it grows along the creeks, sometimes in the "drift-wood" over the water. It is also found on the northeast hill-sides where the snow water keeps the ground wet during the early summer. It grows so thick in places that it renders the forest almost impassible, on account of its "horrible" prickly stems. It is a constant menace to "loggers" and"foresters" in these districts.

Echinopanax horridum is a perennial shrub having a stout, woody crooked stem growing from 6 to 12 feet high. It is creeping at the base, there being a large woody rhizome which follows the surface of the ground. and comparatively few small short rootlets extending down into the soil. The stem is very densely covered with slender, smooth, woody, sharp pointed thorns, and has no branches. The summit is leafy, the leaves being very large, palmately lobed, and having prickles on both sides. The leaves are sometimes as large as 12 to 14 inches long and 10 to 12 inches wide, thus rendering the plant especially well fitted for its environment in the dense forests where there is very little sunlight.

Samples

The material employed in this examination was the rhizome, roots, and stem of the plant. A sample consisting of 450 grams of the rhizome and roots and 1800 grams of the stem was obtained on the Alsea Valley Road about 8 miles above Philomath. Another fine sample consisting of one whole plant was collected by Mr. John Dallas of Nehalem, Tillamook County, and sent to me through Prof. C. E. Bradley of the Experiment Station.

Experimental

In collecting the samples it was noted that both the root and stem gave off a very penetrating and rather unpleasant odor. As this indicated the presence of a volatile oil, a small sample (230grams) of the fresh root was sliced and distilled with water. The presence of a large percent of a volatile oil was established.

On drying a portion of the root at 100° C. it was found to contain 40.74 percent of water.

About 15 grams of the dried and powdered roots were examined for the presence of an alkaloid with a negative result.

For experimental purposes, 10 grams of the dried and powdered root were successively extracted in a Soxhlet apparatus with different solvents, and the

Following amounts of extracts were obtained.

Solvent.	Dried Extract.	Percent.
Petroleum (b.p.35°-50°)	1.267 grams	12.67 %
Ether	.009 "	.09 %
Chloroform	.123 "	1.23 %
Ethyl Acetate	1.345 "	13.45 %
Alcohol 95%	.844 "	8.44 %
Water	1.560 "	15.60 %

Preparation of Samples

After being dried for about two weeks in a moderately warm place, the whole of the dried rhizomes and roots weighed 2756 grams. It was chopped up and passed through a drug mill until ground fine, and the whole (2741 grams) was placed in two large ballon flasks, and alcohol (94 per cent) was added.

Extraction

The contents of these flasks were digested for several (6) days, and the alcoholic solution thus obtained (11 liters) was filtered.

After the alcohol was expressed from the marc, it was digested with water and the aqueous solution concentrated and set aside for later investigation.

Separation of Fixed Oils.

The alcoholic solution was allowed to stand for

several days, since it was noted that what appeared to be a resinous substance was separating from the solution. This substance was separated from the solution and dissolved in chloroform. The chloroform was evaporated and a fixed oil thus obtained was purified. It was reddish brown in color and had a bland sweetish taste. It gave the following constants:

Specific Gravity at $\frac{20^{\circ}}{20^{\circ}}$ C -	.953
Refractive Index at 22° C -	1.4800
Iodine Absorption Value -(24 hours)	86.51
Saponifiction Value -	233.36

On concentrating the alcoholic solution a considerable quantity (9.5 grams) of a light yellow, bland, odorless fixed oil separated out in large globules. This oil gave the following constants:

Specific Gravity at $\frac{22^{\circ}}{22^{\circ}}$ C	.952
Refractive Index at 20.6° C	1.4772
Iodine Absorption Value	41.4

The alcoholic solution was distilled in a large flask until the alcohol was removed. There remained in the flask 302.8 grams of a dark reddish brown syrupy extract.

Distilation with Steam

Volatile Constituents

The extract was mixed with one liter of water and steam was passed through the mixture for several days. About one liter of an aromatic distillate (D) was thus obtained. It had a few globules of oil floating on the top and possessed a strong penetrating odor. The aqueous distillate was shaken with ether, the ethereal solution dried over anhydrous sodium sulphate and the solvent removed.

The residue consisted for the most part of a volatile oil, which on being purified had an odor similar to wild parsnip (Cicuta Maculata), a poisonous plant that grows along marshy streams in the West. The volatile oil gave a refractive index at 20° C of 1.4848.

The aqueous distillate (D) which was neutral in reaction, was thoroughly examined but nothing further was obtained from it.

Volatile Oil.

Distilled 889 grams of the green root, which was collected on Nov. 4, 1910, with water, and it yielded about .13 per cent of volatile oil.

Refractive Index at 20.6° C	1.4842
Specific Gravity $\frac{22^{\circ}}{22^{\circ}}$ C	.915

On April 23, 1911, I secured a large sample of both the stem and the root, (about 65 pounds in all) and distilled it for the volatile oil. 19 kilograms of the stem gave 5.9 grams of the oil, and 10 kilograms of the root gave 5.1 grams of the oil. The yield in both cases was less than .1 per cent, but considerable oil was lost during the process of distillation and purification. When pure, the oil was a pale yellow color and had the same disagreeable penetrating odor of the oils previously obtained.

Constants of Volatile Oil

Specific Gravity at $\frac{20^{\circ}}{20^{\circ}}$ C - .901

Refractive Index at 22° C - 1.4848

The Optical Rotatory Power at 18.5° C.

$a_D = + .3^{\circ}\ 53'\ 30''$ in a 25 mm. tube, or according to the formula

$[a]_D = + 0\ 10'\ 22''$.

Iodine Absorption Value

24 hours at 20° C by Hubl's Process - 269.61

It forms a clear solution with any alcohol stronger than 80° per cent.

Separation of the Contents of the Distillation Flask

After distilling off the aqueous liquid (D), the flask contained an aqueous liquid (E) with a finely divided reddish brown precipitate suspended

in it, a black oily substance (F) floating on the liquid, and a hard black brittle resin (G) mixed with the sand in the bottom of the flask.

These substances were separated and examined.

Examination of Aqueous Liquid (E)

It was noted that the aqueous liquid had a reddish brown substance suspended in it and this was separated by filtering the mixture. The filtrate was labeled (K) and set aside for future examination.

Examination of the Resin (E)

The reddish brown substance (E) was dissolved on the filters with alcohol and the solvent removed, when the residue was found to weigh 15.7 grams. It was mixed with purified sawdust by means of a small portion of alcohol and successively extracted in a Soxhlet apparatus with light petroleum, ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin

The solution by light petroleum (b.p.35°- 50°) was distilled under reduced pressure until the last trace of petroleum was removed. The residue which weighed 10.2 grams was dissolved in ether. The ethereal solution was shaken in a separatory funnel, with 5 per cent aqueous ammonium and sodium carbonate and the alkaline liquids acidified and extracted with ether,

but nothing of importance was obtained. The ethereal solution was then shaken with 1 percent sodium hydroxide, which yielded only a small quantity of an oily substance.

The ether was evaporated from the solution, the residue hydrolysed by heating with alcoholic potassium hydroxide, the most of the alcohol removed from this solution and water added. This alkaline aqueous liquid was shaken with ether, the ethereal liquid dried, the solvent removed, and the residue treated with ethyl acetate.

Crystalline Substance

A small quantity of a crystalline product separated out from the ethyl acetate solution in long needles. It was purified by suspending in 95 per cent alcohol (in which the crystals were only sparingly soluble), and quickly filtering the mixture.

On being recrystallized several times from ethyl acetate they were obtained in a pure state. The crystals were long transparent colorless needles, soluble in alcohol and insoluble in water. Tasteless and odorless.

Melting Point - 127° - 128.50° C,

Fatty Acids

After being extracted with ether the alkaline aqueous liquid was acidified with dilute sulphuric acid,

when a layer of a brown fatty product separated and rose to the top. This was separated and examined with the fatty acids from (F)

Ether Extract of Resin

The ether solution from this resin deposited a slightly bitter, reddish brown resin, insoluble in light petroleum, but soluble in the other solvents. Nothing crystalline could be isolated from it.

Chloroform and Ethyl Acetate Extracts of the Resin

The chloroform and ethyl acetate solutions each deposited small amounts of a reddish brown resin which was thoroughly examined but yielded nothing of importance.

Alcohol Extract of Resin

The alcohol extract was a sticky substance of an amber color, and had a rather sweet aerid taste. Nothing crystalline was isolated from it.

Examination of the Aqueous Filtrate (K)

This aqueous liquid was concentrated and extracted with successive portions of ether. The combined ethereal solutions were shaken with aqueous sodium carbonate, the alkaline liquid acidified with dilute sulphuric acid and extracted with ether. The ethereal liquid was washed, dried, and the solvent removed.

The residue, when treated with 95 per cent alcohol, deposited a few crystals in the form of monoclinic prisms, the quantity of which was too small for further examination.

Amyl Alcohol Separation

The aqueous liquid (K) after being extracted with ether, was shaken with successive portions of amyl alcohol. The amyl alcohol separations were united, the solvent removed and the residue dissolved in hot ethyl alcohol and poured into water. The fine brown precipitate thus produced was examined, but yielded nothing definite.

The hydro-alcoholic filtrate was evaporated to dryness and the residue divided into two parts by its solubility in ethyl acetate. The portion insoluble in ethyl acetate was a hard brittle brown product having a very penetrating nauseous odor, similar to that of Rhubarb (Rheum Officinale), and a nauseous slightly bitter taste.

The portion soluble in ethyl acetate was similar to the former in odor and taste, but was of a soft gummy consistency.

Both were soluble in hot water and in alcohol.

Further Examination of(K)

The aqueous solution which had been extracted with ether and amyl alcohol, was acid in reaction, gave a greenish precipitate with neutral ferric chloride, and a chrome yellow precipitate with basic lead acetate. The yellow precipitate was collected, suspended in water, and the lead removed with hydrogen sulphide. The aqueous solution, when evaporated to dryness gave a light brown substance from which nothing crystalline could be obtained. It contained a very little tannin.

Final Examination of (K)

After being precipitated with basic lead acetate, the excess of lead was removed from the solution with hydrogen sulphide, the aqueous solution evaporated to a dryness, and treated with hot alcohol.

The portion of the residue which was insoluble in alcohol, was very sweet in taste and readily reduced Fehling Solution. On hydrolyzing with dilute hydrochloric acid it gave evidence of a considerable quantity of saccharose.

Nothing crystalline was obtained from it.

Alcoholic Solution of Residue from (K)

The alcoholic solution by hot alcohol from this residue was allowed to cool, when on standing, a considerable quantity of the dissolved solids separated out. This precipitate was collected and found to

contain 56.5 per cent of reducing sugars, and 13.8 per cent of saccharose.

The alcoholic filtrate was evaporated to dryness and this residue was found to contain 37 per cent of reducing sugars, and 4.6 per cent of saccharose.

Examination of the Oily Black Substance (F)

The oily black substance which seemed to contain some resin weighed 65 grams. An attempt was made to separate the resins from the fats and oils by macerating with 85 per cent alcohol. Theoretically the resins would dissolve in an alcohol of this strength and the fats would remain undissolved, but the separation was not complete since part of the oils were soluble in this solvent and part of the resins apparantly insoluble. However, a considerable quantity of a brown resinous substance was separated from the mass with dilute alcohol and examined with the resin (E). The remaining black oily substance (47 grams) was mixed with purified sawdust and successively extracted in a Soxhlet apparatus with light petroleum (b.p.35°-50°) ether, chloroform, ethyl acetate, and alcohol.

Examination of Petroleum Extract of (E)

On removing the solvent from the petroleum solution, the residue weighed 33 grams. It consisted of an oily black substance, of a semi-solid consistency.

It was dissolved in ether and on concentrating the ethereal solution, a jelly like substance separated out. An attempt was made to crystallize this substance from ethyl acetate, alcohol, acetone, and pyridine, but from each of these it separated out in jelly-like lumps. It separated from pyridine as a brown tasteless odorless, jelly like mass.

The ethereal solution of the petroleum extract was shaken with aqueous ammonium carbonate, and later with aqueous sodium carbonate. These alkaline liquids were acidified and extracted with ether. The ethereal solutions were washed, dried, and evaporated, but in each case only a very small amount of an oily substance was obtained. On shaking with 1 per cent sodium hydroxide an emulsion was formed, which was separated and the oily residue examined. As it gave nothing crystalline it was added to the mixture of fats resulting from evaporating the ethereal solution of the petroleum extract. The layer of fatty acids which separated on acidifying the above sodium hydroxide solution, was also added to this mixture. It was then saponified with alcoholic potassium hydroxide, the alcohol removed and water added.

Two Crystalline Substances.

The aqueous alkaline liquid was shaken with two successive portions of ether. On removing the solvent

from the first portion a crystalline product was deposited, which was sparingly soluble in alcohol. It was purified by washing with this solvent and recrystallizing from ethyl acetate. It was finally crystallized from acetic anhydride, from which it separated as long white needles, and glistening plates, tasteless, odorless, melting at 103° -106° C.

On separating the solvent from the second ether separation, a crystalline substance mixed with fatty impurities was deposited. It was purified by washing with alcohol, and recrystallizing several times from ethyl acetate, when it separated as long white monoclinic needles.

It melted at 115° -118° C.

Fatty Acids

A mixture of the fatty acids obtained from the petroleum and ether extracts of (F) and from the petroleum extract of (E) was transferred to a Jena flask and distilled on a sand bath under reduced pressure. About 2 cubic centimeters of a yellow oil passed over between 250° - 300° C at 400 millimeters pressure. This mixture of fatty acids gave an Iodine Absorption Value of 62.66 by Hubl's Method.

After distilling off the above mixture of fatty acids, there remained in the flask, a black wax like compound which remained in a semisoild state at 290° C.

It was dissolved in ether, and was found to be complex in nature. Probably oxidation products of the fixed oils and resins.

Ether Extract of Resin.

The ether solution from (F) after standing several days deposited a considerable amount of hard resinous product which was separated and examined but yielded nothing crystalline.

The ethereal solution was shaken with aqueous solutions of the alkaline carbonates and later with 1 per cent sodium hydroxide. The latter solution separated a considerable quantity of a hard reddish brown substance, of a flat resinous taste, insoluble in petroleum ether, sparingly soluble in ether and ethyl acetate. It gave no color test for abetic acid.

The ethereal solution was evaporated nearly to a dryness and saponified with alcoholic potassium hydroxide. The most of the alcohol was evaporated, water added, and the alkaline aqueous liquid extracted with ether. The ether solution was washed, dried, and the solvent removed but only a small quantity of an oily substance was obtained.

After being extracted with ether the alkaline liquid was acidified and yielded a small quantity of a brown fatty mixture which was added to the fatty acids from the petroleum extract of (F).

Chloroform Extract of (F)

The chloroform extract was thoroughly examined but contained only a small quantity of resinous substance, containing a little fat, which was soluble in chloroform and alcohol.

Ethyl Acetate and Alcohol Extracts of (F)

The ethyl acetate and alcoholic solutions from (F) gave small quantities of a hard brittle yellowish substance, odorless, tasteless and insoluble in all other solvents. Nothing could be isolated from them.

Examination of Black Resin (G)

This hard brittle black substance was mixed with sand which had been placed in the distillation flask to prevent bumping. It weighed 13 grams, was almost insoluble in ether, and light petroleum, sparingly soluble in chloroform and cold alcohol, soluble in hot alcohol.

The solution by hot alcohol was concentrated to a syrupy consistency and poured into cold water. The resin was precipitated as a gray amorphous, tasteless, odorless product, insoluble in ether and light petroleum, sparingly soluble in alcohol and ethyl acetate. It gave no color test for abetic acid.

The solution by cold alcohol yielded a considerable quantity of a dark brown resin, having very little taste and odor. It was easily soluble in alcohol, sparingly

soluble in ethyl acetate, and insoluble in ether and chloroform. It gave no color test for abetic acid.

Examination of Aqueous Extract

After the dried and powdered rhizome and roots had been extracted with alcohol, the whole of the marc was transferred to a large container and digested with water. The aquous liquid thus obtained was evaporated to a dryness and 78 grams of a brown gummy substance was obtained which had a sickening sweetish taste. As the taste was similar to that of Extract of Glycyrrhiza (Licorice Root), I suspected the presence of Glycyrrhizin.

Ammoniated Glycyrrhizin

Accordingly the extract was disolved in water and boiled, being filtered while hot to remove certain albumens rendered insoluble by boiling, and at the same time to allow the glycyrrhizin to pass through before it gelatinized. The filtrate was treated with dilute sulphuric acid, which threw down a large quantity of a flocculent brown precipitate. The precipitate was allowed to settle, the liquor decanted, and the precipitate washed on a filter until free from sulphuric acid. It was then dissolved in water with the aid of a little ammonia water, reprecipitated from this solution by dilute sulphuric acid, redissolved in very dilute ammonia water, and evaporated to dryness on a water bath.

The residue (4 grams) corresponded in every

respect to the Glycyrrhizinum Ammoniatum of the United States Pharmacopoeia, except that it was less sweet and seemed to contain a slight amount of some ammonia soluble albumenous impurity.

Glycyrrhizic Acid

A part of the ammoniated glycyrrhizin was dissolved in boiling glacial acetic acid and filtered while hot. The filtrate deposited a crystalline substance in the form of a hard brown crust. This substance was thus recrystallized several times from glacial acetic acid, and finally from 95 per cent alcohol from which it separated in characteristic clusters of glistening white plates. It had an intensely sickening sweet taste. A few crystals dissolved in five cubic centimeters of water produced an acid solution which on boiling formed a jelly like mass. On heating with dilute sulphuric acid it reduced Fehling Solution on long continued boiling. On heating it to 100° it turned brown and melted between 170°- 185° C. It was slightly soluble in 95 per cent alcohol and very soluble in dilute alcohol and water.

From the above data, it was decided that this substance was identical with the Glycyrrhizic acid investigated by Habermann (Anallen Die Chemie und Die Pharmacie).

Beilstein's Orgonische Chemie 11 345, gives the

formula C44 H63 NO18 for Glycyrrhizic Acid and NH4 C44 H62 NO18 for the ammonium salt present in ammoniated glycyrrhizin and in the licorice root.

Ammoniated Glycyrrhizin is used in medicine as a demulcent in bronchial troubles, but more frequently to mask the taste of bitter drugs. The present market quotation on this product is $3.50 per pound.

After being precipitated with sulphuric acid which removed the glycyrrhizin, the excess of sulphuric acid was removed from the solution by means of barium carbonate and the solution evaporated to dryness.

The residue was nauseous and acrid in taste. It contained a little tannin, but consisted mostly of plant gums and pectinous substances.

Physiological

It is generally believed by "loggers" and "foresters" that this plant is very poisonous and "a thing to be avoided". This opinion evidently grew out of the fact that when the hand is pricked with one of the thorns, there is more or less local inflammation. This may be due to the very fine point breaking off in the flesh, or to the bacteria introduced by the prick of the thorn.

While distilling some of the oil in the laboratory, Prof. John Fulton, Head of the Chemistry Department, was exposed to the fumes. In a few days

a rash, similar to that produced by Rhus Toxicodendron poisoning, broke out on his face, and forehead, on two occasions. This he attributed to the volatile oil, or to some other product of the plant, probably volatile in nature.

The following physiological tests were conducted in the Bacteriological Department of the College.

Volatile Oil

Test	Result
A few drops of oil placed on tongue of a guinea pig.	No ill effects.
One half cubic centimeter injected beneath the skin of a quinea pig.	No effect.
Applied to normal skin on my own arm.	No effect.
Applied to abraded skin on my own arm.	No effect.

Conclusion

The volatile oil seems to be non poisonous and almost inert, as compared with other volatile oils.

Alcoholic Extract

Extracted 60 grams of the dried and powdered root with hot alcohol and evaporated to a dryness. Gave this

to a 467 gram guinea pig in the form of small pills.

May 17, 1911

Weight of dose	Time	Temp. F.	Remarks
1 Grain	11^{15}	98.6°	No effect.
2 "	11^{35}	98.7	" "
5 "	11^{50}	98.6	" "
10 "	2^{10}	98.8	" "

Aqueous Extract

Extracted 60 grams of the dried and powdered root with water, and gave the residue to a 448 gram guinea pig in the form of small pills.

May 22, 1911.

Dose	Time	Temp.F.	Remarks.
1 Grain	11^{20}	98.6	No effects.
2 "	11^{30}	88.6	" "
	12^{00}	100.	Rise in temperature.
5 "	3^{00}	97.	No effect.
	4^{30}	98.	" "
10 "	4^{30}	98.	" "
May 23, 1911.	9^{05}	101.	Rise in temperature.
" " "	10^{00}	101.	" " "
" " "	12^{00}	100	
" " "	4^{00}	98.8	

Three grains of the amyl alcohol separation from (K) was administered to a 448 gram guinea pig with no effect.

5 grains of the black resin (G), soluble in cold alcohol was given to the same guinea pig with no ill effects.

Antiseptic Value of Oil

In testing the antiseptic value of the oil, I used Bacillus Typhosus as typical of a vegetative disease producing form, and Bacillus Anthracis as typical of a spore bearing form of bacteria.

I took a piece of sterile silk thread and cut into short lengths. Inoculated these pieces of thread from a boullion culture of B. Thyphosus and from an agar slant culture of B. Anthracis. Dried for one hour in two sterile Petri dishes in the incubator at 35° C.

A boullion tube inoculated from each showed a growth in 24 hours proving that the drying process did not kill the organism. At 11$^{00}_{--}$ o'clock on May 17, 1911, I exposed the pieces of thread to the action of the oil by pouring the oil over the thread in the dish. Made transfers from time to time to boullion tubes with the following results:

Organism	Media Used	When Made	When Examined	Growth
B. Typhosus	Bouillion	2^{10} May 17.	9^{00} May 18	+
B. "	"	" " "	" " "	-
B. Anthracis	"	" " "	" " "	+
"	"	" " "	" " "	+
B. Typhosus	"	8^{30} May 18	10^{00} " 19	-
"	"	" " "	" " "	-
B. Anthracis	"	" " "	" " "	+
"	"	" " "	" " "	-
B. Typhosus	"	3^{30} " "	" " "	-
"	"	" " "	" " "	-
B. Anthracis	"	" " "	" " "	-
"	"	" " "	" " "	-
B. Typhosus	"	8^{30} " 19	9^{00} " 22	-
"	"	" " "	" " "	-
B. Anthracis	"	" " "	" " "	-
"	"	" " "	" " "	-

Kills B. Typhosus in about 4 hours.

Kills B. Anthracis in 20 to 27 hours.

Summary

The results of the investigation may be summarized as follows:

1. The material employed was the rhizome, roots, and stem of Echinopanax horridum.

2. A volatile oil was obtained from the green rhizome, roots, and stem, which in the spring represents about .1 per cent of the plant, and in the autumn about .13 per cent. The oil has a specific gravity of .901 at $\frac{20^{\circ}}{20^{\circ}}$ C., a refractive index of 1. 4848 at 22° C, an optical rotatory power of +3° 53' 30" in a 25 millimeter tube, hence $[\alpha]_D$ = + 0 10' 22"; and iodine absorption value 269.6, and forms a clear solution with any alcohol above 80 per cent. Two deposits of fixed oils separated out from the alcoholic solution of the constituents. The first had a specific gravity of .952 at $\frac{20^{\circ}}{20^{\circ}}$ C, a refractive index at 22° C of 1.4800, an iodine absorption value of 86.5, a saponification value of 233.36.

3. The second deposit of fixed oil had a specific gravity of .953 at $\frac{22^{\circ}}{22^{\circ}}$ C, a refractive index of 1.4777 at 20.6°, and an iodine value of 41.4.

4. A crystalline substance was separated from the saponified petroleum extract of the brown resin that melted at 127° - 128° C.

5. Two gummy nauseous substances were separated from the aqueous solution by amyl alcohol.

6. The aqueous solution from the alcoholic extract contained a large percent of reducing sugars and saccharose.

7. The saponified petroleum extract from the black oily substance deposited two crystalline products, one melting at 115°- 118° and the other at 103°-106° C.

8. A mixture of fatty acids was distilled from the combined fatty products which gave the iodine value of 62.66.

9. The plant yielded two black resins. One soluble in cold alcohol and the other in hot alcohol. There was also a reddish brown resin (E).

10. The aqueous extract, when precipitated with dilute sulphuric acid and the precipitate dissolved in very dilute ammonia, produces the Ammoniated Glycyrrhizin of the U. S. P.

11. From this residue was able to crystallize out Glycyrrhizic Acid which has the formula C44 H63 NO18. In addition the plant contained tannin, gums, starch, and pectin.

12. No where in the investigation was there any indication of an alkaloidal substance, and no glucoside could be isolated.

13. The physiological tests would indicate that the plant was not poisonous and that the volatile oil had no injurious effects on the body of some people.

14. It was found that the volatile oil would kill B. Typhosus in 3 to 5 hours and B. Anthracis spores in 20 to 28 hours.

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By, Horace Fennimore.

Respectfully submitted:

[redacted]

May 31, 1911.