1939

Chemical investigation of devil's club

Hubert William Murphy

The University of Montana

Let us know how access to this document benefits you.
Follow this and additional works at: https://scholarworks.umt.edu/etd

Recommended Citation
Murphy, Hubert William, "Chemical investigation of devil's club" (1939). Graduate Student Theses, Dissertations, & Professional Papers. 6264.
https://scholarworks.umt.edu/etd/6264

This Thesis is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.
A CHEMICAL INVESTIGATION

of

DEVIL'S CLUB

by

Hubert William Murphy

B.S., State University of Montana, 1937

Presented in partial fulfillment of the requirement for the degree of Master of Science

State University of Montana

1939

Approved:

Chairman of Board of Examiners.

Chairman of Committee on Graduate Study

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
PREFACE

The author wishes to thank all those persons who have given generously of their time and knowledge in assisting and helping develop this problem.
**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Description of the Plant</td>
<td>5</td>
</tr>
<tr>
<td>Medicinal Use</td>
<td>8</td>
</tr>
<tr>
<td>Review of the Literature</td>
<td>9</td>
</tr>
<tr>
<td>Experimental Work</td>
<td>10</td>
</tr>
<tr>
<td>Table of Extractives</td>
<td>11</td>
</tr>
<tr>
<td>Purified Petroleum Benzin Extracts</td>
<td>12</td>
</tr>
<tr>
<td>Constants of the Fixed Oils</td>
<td>14</td>
</tr>
<tr>
<td>Anhydrous Ether Extracts</td>
<td>16</td>
</tr>
<tr>
<td>Acetone Extracts</td>
<td>17</td>
</tr>
<tr>
<td>Alcoholic Extracts</td>
<td>17</td>
</tr>
<tr>
<td>Volatile Oil</td>
<td>19</td>
</tr>
<tr>
<td>Examination for Alkaloids</td>
<td>20</td>
</tr>
<tr>
<td>Tannins</td>
<td>21</td>
</tr>
<tr>
<td>The Aqueous Extract</td>
<td>22</td>
</tr>
<tr>
<td>Table of Extractives Obtained by the Successive Extraction</td>
<td>24</td>
</tr>
<tr>
<td>The Hypoglycaemic Substance</td>
<td>24</td>
</tr>
<tr>
<td>Skin Reactions</td>
<td>27</td>
</tr>
<tr>
<td>Summary</td>
<td>28</td>
</tr>
<tr>
<td>Bibliography</td>
<td>29</td>
</tr>
</tbody>
</table>
A CHEMICAL INVESTIGATION

of

DEVIL'S CLUB

- INTRODUCTION -

This investigation was suggested by the work of Drs. Large and Brooklesby(19) who have reported that the decoction prepared by boiling Devil's Club root bark was capable of reducing blood sugar and also of preventing alimentary hyperglycaemia when fed orally or injected intraperitoneally into normal fasting rabbits. They succeeded in precipitating the hypoglycaemic principle from the aqueous solution by the addition of 40- to 50 percent of acetone, but isolation, purification and chemical identity were not established. The present research was undertaken for the purpose of a general investigation of this plant from a chemical standpoint, with a particular search for alkaloids, glucosides and other plant principles which might possess this hypoglycaemic activity. An attempt was also to be made to separate the acetone-precipitable-fraction, obtained by these Canadian doctors, into its components, or to purify the main constituent present so that an insight into its chemical nature might be obtained. Lastly, a general survey of the use of Devil's Club in Indian medical practices was undertaken and a description of the plant has been included.

A reliable insulin substitute is desirable for several reasons, the most important of which are:

(1). Insulin as prepared today from the pancreas of animals
always contains some extraneous protein material as impurities. Although this protein is present in only minute quantities, it is often sufficient to cause a protein reaction in a number of individuals, thus complicating the parenteral use of insulin.

(2). The method of subcutaneous administration is also undesirable because of the constant danger of infection and that considerable knowledge and technic are required in securing aseptic conditions and regulated dosaged. This method of administration is not only painful but quite troublesome.

(3). Insulin secreted by the pancreas passes into the blood stream and is collected by the splenic and superior mesenteric veins. These flow into the portal vein and insulin is thus transported quickly to the liver where it does a large share of its work in the body. When insulin is administered subcutaneously, however, it is carried back through the extensive vascular system which supplies the surface of the body. This blood is not returned directly to the liver so that considerable time may elapse before the insulin collects in the liver and undoubtedly a not unnoticeable amount is adsorbed or perhaps even destroyed in the other portions of the body. For this reason oral administration of insulin or a satisfactory substitute, would appear to be more effective, since after absorption from the intestine it would pass by the way of the portal vein directly to the liver.

It is a recognized fact that insulin is destroyed when subjected to the action of the digestive juices so, as yet, administration by mouth has not been satisfactory.

If the exact chemical structure of insulin were known, perhaps a synthetic substitute could be prepared that would be entirely satisfactory. Likewise, the exact mechanism of insulin action in the body is not com-
pletely understood, but it is known that it is necessary for the storage of glycogen for the rapid combustion of dextrose and that its main action takes place in the liver. Any insulin substitute should, therefore, possess these properties, as well as being non-toxic when used continuously, to be of any value from a therapeutic standpoint.

Several substances have already been isolated from various plants which have the property of reducing blood sugar. Some of these are alka-loidal in nature such as galagine, which is a guanidine derivative, obtained from Goat’s Rue (Galega officinalis). It has been recommended for the treatment of mild diabetes since it effects an increase in glucose utilization as well as an activation of fat and protein metabolism. This alkaloid is also effective when administered by mouth, but possesses a disadvantage in the narrow margin between hypoglycaemic and toxic doses. Lupenine, an alkaloid obtained from the seeds of Lupinus albus, when administered by mouth or parenterally is capable of exerting a hypoglycaemic action on normal animals, but due to the uncertainty and to the slight transitory action, it is not important from a therapeutic standpoint.

Edgars has succeeded in isolating a glucoside in a highly purified state from blueberry leaves, which he describes as a methoxy gallyl glucose. This substance effects a marked reduction of blood sugar in hyperglycaemia without lowering the renal threshold when administered by mouth or parenterally and is now marketed in capsules under the trade name of "Neo-my-tillin" for the treatment of diabetes.

Aqueous extracts of barley malt dust yield a precipitate with alcohol which, when purified and dried, causes a marked hypoglycaemia when injected into rabbits. Collip and others have obtained this hypoglycaemic action with aqueous extracts of barley roots, and barley sprouts. An insulin-like substance has also been isolated from onion tops, onion roots, green wheat
leaves, bean tops, lettuce and lawn grass. Active extracts have been prepared from potatoes, rice, wheat, beet roots, peas, celery, etc. An amorphous yellow fraction obtained from the petroleum ether extract of dried carrots was prepared by M. Franke et al. which, when dissolved in almond oil and injected into rabbits and dogs caused considerable reduction in blood sugar. An extract of Mulberry leaves and the alcoholic extract of *Rhizoma polygonata* were found to effect a reduction in blood sugar, the latter after an initial temporary increase.

This would suggest that the occurrence of insulin-like substances is not infrequent in the plant kingdom.

Since insulin is definitely associated with the power of the liver to form glycogen, Collip has hypothesized that wherever glycogen is found, a hormone like insulin, if not identical with it, will be found. To this hormone he has given the name glucokinin and has also suggested that the sugar metabolism may not differ greatly in the plants from that in the animals. Although all of these substances have not been thoroughly studied, it would appear that these plant principles with hypoglycaemic action differ greatly in their chemical nature.

The introduction of synthetic guanidine derivatives as substitutes for insulin resulted from studies on the effects of parathyroidectomy on dogs. Guanidine itself is rather toxic, but by the continual research of Frank, Nothman, and Wagner an active compound known as synthalin (decamethylene diguanidine) was prepared. Synthalin, however, is not devoid of toxic action and has not been generally accepted as a reliable insulin substitute.

Collip observed that the blood serum of an animal in hypoglycaemia, resulting from the injection of synthalin sulfate, was capable of producing hypoglycaemia in a second rabbit and so on without any apparent decrease in
the effect, thus differing greatly from the action of insulin in this respect.

Anticoman (decamethylene diguanidine tartrate) is less toxic than synthalin and is capable of reducing blood sugar and assisting in glycogen formation much like insulin. Monias believes that because it is synthesized by a different method than that used for synthalin, that it does not contain the toxic by-products that are present in synthalin.

- DESCRIPTION OF THE PLANT -

Devil’s Club is a shrub well-known throughout the Pacific Northwest by its appropriate name. It is also called Devil’s Walking Stick. Comanche states that, “To know the Devil’s Club you have to live with it in the woods winter and summer, go fishing with it, fall into a thicket of it, grab it with the bare hands and camp beside it, and the better you know it, the less you like it.”

It has been given various names by different botanists: Echinopanax horridum (J. E. Smith) Decne & Planch. (Cooper. Pac. R. R. Rep. 12: 31, 1860). Echinopanax <L. echinoza> a hedgehog; spiny or bearing spines + <panax <Gr. panax> a healing herb and <horridum <L. horridus = bristly or rough; Panax horridum in Rees Cyclop. 26: No 10, 1819; Oplopanax horridum (Miq. Ann. Mus. Bot. Lugd. Bat. 1: 116, 1863) and Fatsia (a Japanese word) horrida, Bentham and Hooker (S. Wats. Bot. Cal. 1:273, 1876). Youken has pointed out that its valid name according to the rules of the International Code is Oplopanax horridum. However, this shrub has been generally reported as Fatsia horrida in the literature.

It belongs to the Aralia (Araliaceae) family and is closely related to Fatsia papyrifera which produces the famous rice paper of the Chinese. Ginseng, which has been used by the Chinese in medicinal practices for hundreds of years, is also a rather closely related member of this family.
Oplopanax horridum is a deciduous shrub with stems erect from a decumbent base. These grow to a height of 12- to 13 feet and are often bent to the ground by their own weight or by snow, the ends springing up again in an upright position. The stems are woody, with a pithy center, tough and springy, are densely covered with sharp spines and bear large alternate, long petioled leaves near the top of the stem. The leaves are nearly oblong in outline, 9- to 12 inches across, cordate at the base with a rather narrow sinus, 3- to 11 palmately lobed, with scattered prickles, puberulent underneath, lobes acute, sharply irregular serrate. The plant bears racemose or panicled umbels of small greenish-white flowers, inflorescence wooly, terminal 4- to 12 inches, calyx-teeth obsolete, petals 5, valvate, stamens 5; filaments filiform; anthers oblong or ovate; ovary 2- to 3 celled, styles 2; stigma terminal. The peduncles are subtended by a narrow laciniate bract; pedicels filiform; stamens about twice as long as the ovate petals. It blooms in June and green berry-like fruits are then developed. These ripen in late July and August into bright red plume-like clusters that resemble sumac berries. The fruit is from 1/5- to 5/24 of an inch in length.

The spines are very sharp, but not barbed, and cannot be extracted from the flesh without considerable laceration of tissue. They also appear to contain a poison.

Devil's Club grows where there is a constant supply of fresh water preferably on the spring water bogs of side hills, along mountain streams and is sometimes found in the thick, black "muck" soil situated along trout streams or lake margins. It seems to prefer to grow in rocky places and is infrequently found in swampy areas where stagnant water is present. The roots often extend from the stream bank down through the cold water into the rocky bed below. The plant likes shade and full sunlight for an hour or two in the morning is sufficient. Shelter is also necessary to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
prevent the wind from whipping its large foliage. It grows extensively in the fir forests of British Columbia where it is said to form an undergrowth with a jungle-like aspect. At a distance it resembles the castor bean plant with all but the uppermost leaves stripped off, but being a lighter green in color. It has been found growing at very high altitudes under rather adverse conditions along mountain streams. Doughty claims to have seen it within a few yards of the summit of the Coast Range in northern Oregon.

There is always a faint, penetrating disagreeable odor about a thicket of Devil's Club that becomes rank and very noticeable whenever the plants are disturbed.

Its range is Isle Royale in Lake Superior, from the Coast Ranges in northern Oregon eastward to the main range of the Rockies in Montana and north through British Columbia to Alaska and Japan. It does not frequently occur east of the main range of the Rockies, and was first reported in Montana as growing near Columbia Falls by R. S. Williams in 1892. We have also found it growing abundantly along Mud Creek in the Mission Mountains. Whitford has previously described it as forming part of the vegetation of the Flathead National Forest, Montana.

The possibility that Devil's Club might have originated during the period in which the glacial flora covered most of North America is suggested in the work of Harshberger. He points out that the climatic conditions of this age resembled those of Greenland and Alaska at the present time. Forests occur on the stagnant ice margin and extend up over the moraine and even over the surface of the glacier covered with morainic material on the Malaspina Glacier at the base of Mt. St. Elias in Alaska. Oplopex horridum (Fatsia horrida) forms a part of the vegetation thus found on the glacier itself.
The use of Devil's Club for medicinal purposes is not new. Doughty states that he remembers the coast Indians making a tea from it - the exact method of preparation is unknown - which was used to treat various ailments from lumbago to a head cold.

Mitchell claims to have seen a Chippewa Indian use the dried roots to make a tea which was drunk for its curative properties. The drug had to be obtained from the western side of the Rockies by expedition or by purchase from other tribes. The dried roots were first boiled with water. This yielded a dark yellowish-brown solution which was discarded and a second boiling with water yielded a light wine-colored decoction. The latter was freely drunk as a medicine, and described by this Chippewa Indian as, "Good medicine cure all trouble inside."

Devil's Club has also been used by the Flathead Indians as a medicine being known by the name "Huh huh weh" (spelling has not been confirmed). Its principal use is in the treatment of bronchitis, being employed in the form of a tea which is made by boiling the stems with water. In some cases sugar is added to sweeten the tea.

Other plants of this family have been extensively used in medicinal practices for many hundreds of years. Ginseng has already been mentioned. Aralia racemosa, the medicinal use of which we learned through the Chippewa Indians by whom it was called O'kadak or aya leidjidji bekugisen, has been used in treating diabetes. Likewise, these Indians knew the medicinal use of Aralia nudicaulis (Wabos' odji tik) and Fatsia japonica has been used in Chinese medicine for over 2,000 years. The Japanese have done considerable work with Aralia chinensis variety grabresesens as a common diabetic drug.
A complete review of the literature revealed that, except for the work of Drs. Large and Brocklesby, no chemical investigations had been made on Devil's Club. Younken has recently published an article on the pharmacognosy of Devil's Club bark. We quote his description of the barks:

Root bark: "In transversely curved pieces and in single and double quills up to 10.5 cm. in length, from 0.5 cm. to 3 cm. in width and up to 2 mm. in thickness; outer surface varying from grayish-brown to dark brown, irregularly and longitudinally wrinkled, some of the pieces exhibiting circular scars or stumps of rootlets, others transverse or oblique ridges, fracture short, the fractured surface exhibiting a brown cork and a spongy, yellowish-white middle and inner bark through which are scattered numerous minute brownish areas (oleoresin reservoirs); inner surface yellowish to yellowish-white, streaked with brown, and longitudinally striate, some of the pieces with adherent whitish wood; odor characteristically aromatic, peppery; taste distinctly aromatic, slightly bitter and pungent." It seems noteworthy to mention that the odor is very penetrating, characteristic and disagreeable producing a rather dull pain in the forehead of most individuals.

Stem bark: "This bark varies from grayish to brown on the outer surface and differs chiefly from the root bark by exhibiting a greenish phelloderm where cork is abraded and by showing closely set circular scars from the bases of the prickles."

Pammel states that "...aralin occurs in the roots of Fatsia horrida....", but it is doubtful whether he refers to Devil's Club, but probably to Aralia spinosa or Hercules Club which was examined by Lilly.

Drs. Large and Brocklesby have demonstrated the presence of a hypo-
glycaemic principle in the decoction prepared from the root bark of Devil's Club, which can be precipitated by acetone. Toxicity tests on rabbits indicated that this extract, when administered daily for a period of five months, had very little if any deleterious effects and furthermore, they gave experimental proof that it was as effective when fed orally as when injected intraperitoneally. Because the acetone precipitate seemed to be rather insoluble in water and alkalies but readily soluble in dilute acids, these Canadian doctors believed it to be of a basic nature.

- EXPERIMENTAL WORK -

The first material secured was that supplied by S. B. Penick & Co. of New York. This consisted of five pounds of air-dried root bark which was collected either in northern Washington or in British Columbia. An additional collection of roots and stems was made during December near Seattle, Washington and this supply, supplemented by that from Penick & Co., was used for the experimental work. We have also made a collection of Devil's Club growing along Mud Creek in the Mission Mountains in Montana and noticed that its growth is not as rank and that the plants do attain the size that they do on the Pacific Coast.

The bark was cut from the woody portions of both the roots and stems and allowed to dry by exposing to the air at room temperature for about two weeks.

Both barks were then reduced to coarse powders by grinding in a mechanical grinder (salvaged sausage grinder) and the powdered barks passed through a #20 standard mesh sieve and the powders stored in brown wide-mouthed bottles.

The powdered drug is yellowish-brown in color being somewhat darker.
when freshly ground and acquiring a lighter shade on standing. This change is especially noticeable in the case of the stem bark.

Microscopic examination revealed that the bulk of the powder consists chiefly of parenchyma; numerous starch grains are present, that stain blue with iodine. These vary from simple to 2- to 4 compound, the individual grains having a spheroidal, oval or elliptical form; the larger grains having a circular or cleft hilum. Rossette aggregate crystals of calcium oxalate are quite numerous. Brownish fragments of the walls of secretin reservoirs and particles of adhering wood are scattered throughout the powder.

The root wood, which is yellowish white, rather light, tough and springy with a slight characteristic odor, was cut into shavings. These were mechanically ground and passed through a #20 sieve and stored in the same manner as the others.

These powders were used for the determinations of the various values as listed below. All determinations were made with original samples of the air-dried powders and calculations were based on the constant weight obtained by drying at 105°C.

<table>
<thead>
<tr>
<th></th>
<th>Root Bark</th>
<th>Stem Bark</th>
<th>Root Wood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9.65%</td>
<td>7.47%</td>
<td>4.25%</td>
</tr>
<tr>
<td>Ash</td>
<td>8.48%</td>
<td>6.29%</td>
<td>0.82%</td>
</tr>
<tr>
<td>Water - insoluble Ash</td>
<td>5.66%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water - soluble</td>
<td>2.82%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid - insoluble</td>
<td>0.60%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid - soluble</td>
<td>7.89%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purified Petroleum Benzin Extractive</td>
<td>10.71%</td>
<td>12.89%</td>
<td>2.09%</td>
</tr>
<tr>
<td>Anhydrous Ether - Extract, volatile</td>
<td>2.00%</td>
<td>2.02%</td>
<td>0.54%</td>
</tr>
</tbody>
</table>
The purified petroleum benzine extracts of both the root bark and the stem bark, 10.71% and 12.89% respectively, consist chiefly of dark reddish-brown fixed oils, which are brownish yellow in thin films with characteristic earthy odors, not unlike that of young carrots and bland tastes.

In order to secure quantities of these oils for examination, an improvised soxhlet extractor was made and continuous percolation with purified petroleum benzine carried to exhaustion on 1,500 Gm. of root bark and 500 Gm. of stem bark.

The petroleum benzine extracts were filtered, the petroleum benzine allowed to evaporate off at room temperature, and the oils were finally heated to about 70°C for 12 hours. Although the oils are soluble in petroleum benzine, in dilute solutions they produce a turbidity resembling that produced by castor oil. The oils are very soluble in ether, chloroform, benzene, carbon tetrachloride, carbon disulfide and partially soluble in cold alcohol and almost completely soluble in hot alcohol.

When the oil of the root bark was treated with nitrous acid it solidified after about two minutes forming a yellowish-orange solid; an elastic transformation suggesting the presence of oleic acid. A deep
reddish coloration was produced when a dilute etherial solution of this oil was superimposed on concentrated sulfuric or concentrated nitric acids which might be due to oxidation. A portion of this oil was mixed with 1/5 its volume (5 cc: 1 cc.) of concentrated sulfuric acid. A violent reaction occurred with the evolution of sulfur dioxide and other gases, the temperature rising from 20°C to 145°C. This rise in temperature, indicating unsaturation, corresponds closely to that produced by raw linseed oil when treated similarly. On cooling a black pliable, resinous mass was formed that had a peculiar, aromatic somewhat peppery odor.

An attempt was made to distill the oil of the root bark under atmospheric pressure, but the oil began to decompose at about 200°C with the formation of acraldehyde. By steam distillation it is possible to separate a small amount of volatile oil from this fixed oil which amounts to about 1% of the petroleum benzin extract. A large part, but not all, of the odor of the petroleum benzin extract is due to this volatile oil. Thin films of the oils were exposed to the air and they slowly resinified. This resinification is more like that of cottonseed oil despite the high iodine number and reaction with sulfuric acid which indicates an unsaturation like that of linseed oil.

A portion of this oil was treated with lead monoxide and saponified by heating on a water bath. The reaction mixture was then extracted successively with two portions of ether and the washings combined. This etherial solution gave a positive test for lead indicating the presence of an ether-soluble lead soap. Because the oil formed this ether-soluble lead soap, and because of the elastic transformation which it undergoes when treated with nitrous acid, it was concluded that oleic acid esters form a portion of the oil.
The various values as determined on the fixed oils from the stem and root barks respectively have been arranged in the following table:

**Constants of the Fixed Oils**

<table>
<thead>
<tr>
<th></th>
<th>Root Bark</th>
<th>Stem Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity 25°C</td>
<td>0.9684</td>
<td>0.9541</td>
</tr>
<tr>
<td>Refractive Index 20°C</td>
<td>1.492</td>
<td>1.495</td>
</tr>
<tr>
<td>Optical Rotation</td>
<td>([\alpha]^{20^\circ}D = + 36.86^\circ)</td>
<td>([\alpha]^{20^\circ}D = + 41.8^\circ)</td>
</tr>
<tr>
<td>Saponification Number</td>
<td>173-190</td>
<td>132-150</td>
</tr>
<tr>
<td>Ester Number</td>
<td>162-178</td>
<td>119-137</td>
</tr>
<tr>
<td>Acid Number</td>
<td>11-14</td>
<td>12-14</td>
</tr>
<tr>
<td>Iodine Number (Hanus)</td>
<td>160-175</td>
<td>123-138</td>
</tr>
<tr>
<td>Unsaponifiable Matter</td>
<td>23.4%</td>
<td>27.18%</td>
</tr>
<tr>
<td>Congealing Point</td>
<td>-5 °C (viscus)</td>
<td>-5 °C</td>
</tr>
<tr>
<td>Titer</td>
<td>3.8 °C</td>
<td>-</td>
</tr>
</tbody>
</table>

In order to determine the optical rotations of the oils, it was necessary to dissolve them in chloroform and observe in a 100 mm. tube. The opacities of the oils are so great that only dilute solutions could be used in the determinations. 6.3019 Gms. of the oil from the root bark was dissolved in sufficient chloroform to make 100 cc. and from the observed average rotation of +5.333, the rotation of the original oil was calculated according to the formula: 

\[ [\alpha]^{20^\circ}D = \frac{A}{l} \times \frac{100}{c} \]

where "a" equals the observed rotation of the solution in degrees, "l" the length of the tube in decimeters and "c" the concentration of the solution in Gm. per 100 cc. 5.4050 Gm. of the oil from the stem bark was used for the determination of optical rotation and from the observed rotation of +2.26, the optical rotation of the original oil was computed in like manner.

As shown in the table, both oils contain a relatively large unsaponifi-
fiable portion; this being slightly greater in the oil from the stem bark than that of the root bark. It is a mobile reddish-brown liquid with a very characteristic earthy, carrot-like odor. It has a refractive index of 1.530 at 20^\circ C, which would indicate the presence of unsaturation and this portion is probably partly responsible for the high iodine numbers of the fixed oils.

When the unsaponifiable portion of the root bark oil was treated with concentrated sulfuric acid considerable heat was produced, gases were evolved and on cooling a tenaceous, black resinous substance remained. This substance had a peculiar aromatic, somewhat peppery or minty odor, is practically tasteless, very pliable and can be drawn into fine threads.

The unsaponifiable matter does not react with acetyl chloride and no precipitate was obtained when it was subjected to the Schotten-Bauman reaction, therefore, it does not contain any substance with an alcohol group. Further investigations in this line were discontinued.

The soap, which is soft, dark brown in color, and has good lathering properties, formed in the determination of the unsaponifiable matter was decomposed by adding dilute sulfuric in excess thus liberating the free fatty acids. These were extracted with ether, the ethereal solution dried over anhydrous calcium chloride, then filtered off, the ether allowed to evaporate, the acids dried in a desiccator over sulfuric acid and then used for the determination of titer. They do not congeal at the temperature recorded as titer, 3.8^\circ C, but the temperature merely remains constant at this point for a short time. The fatty acids are liquid, deep reddish-brown in color and have a characteristic rancid odor.

The acid aqueous solution remaining after removal of the fatty acids, was evaporated almost to dryness on a water bath and extracted with a mix-
ture of two volumes of alcohol and one volume of ether. The ether and alcohol were then evaporated off by heating on a water bath and a sweet tasting residue, almost completely soluble in water remained. A portion of this was intimately mixed with 0.5 Gm. of potassium bisulfate and heated in a test tube. The characteristic odor of acetaldehyde was readily detected thus proving the presence of glyceryl esters in the oil.

The recorded congealing point of the oil is not a point of definite solidification, but the oil greatly increases in viscosity at this temperature which remains constant for a short time and then falls slowly. Even at a temperature of -23°C, the oil does not become definitely solid, but displays a very high viscosity and a great increase in adhesiveness.

- ANHYDROUS ETHER EXTRACTS -

The powdered root and stem barks were then freed from petroleum benzine by exposure to the air and finally heated to 60°C. for two hours. These were then each extracted continuously to exhaustion with anhydrous ether, the etherial extracts filtered, the ether allowed to evaporate off slowly at room temperature. No crystalline precipitate was observed to form and after all of the ether was removed, viscous, resinous masses remained.

The etherial extract so obtained of the root bark was treated with hot water, and hot acidulated water but did not yield any appreciable water soluble extractive. It was then treated with successive portions of alcohol and the alcoholic solution decanted from the alcohol insoluble portion.

The alcohol insoluble portion solidified into a waxy-like material, brown in color with practically no odor and taste. The alcohol soluble portion was obtained as a viscous reddish-brown oleoresin-like liquid after evaporation of the alcohol. It has a specific gravity of 1.025.
as determined in salt solution and a refractive index of 1.521 at 20°C. It had a slight odor and a very persistent, bitter taste. When exposed to the air it was not noticeably affected — concentrated sulfuric acid produces a dark coloration indicating the presence of a readily carbonizable substance and concentrated nitric acid changes the color to a light orange. 21 Gms. of this fraction was obtained from 1500 Gms. of root bark corresponding to a yield of 1.55%.

- ACETONE EXTRACTS -

The ether extracted powders were then freed of ether by exposure to air and finally heated to 60°C. for two hours. Each was then extracted with acetone to exhaustion, the extract filtered and the acetone allowed to evaporate off slowly. A few crystals separated from the acetone extract of the root bark, which because of their sweet taste were regarded as sucrose which was subsequently identified in the alcoholic extract.

The acetone extract of the root bark freed of solvent was treated with hot water which dissolves a large portion of it forming a milky solution that is acid to litmus paper and has a persistent bitter, nauseating taste. The solution clarifies on the addition of alcohol indicating that it must contain resinous bodies held in suspension by some emulsifying agent. This agent probably is a saponin or saponins because considerable frothing occurred in the determination of the water-soluble extractive. Further treatments, however, failed to yield any insight as to the nature of the substances present and the extract was reserved for future reference.

- ALCOHOLIC EXTRACTS -

The acetone extracted powders were then freed of solvent in a similar manner and each extracted continuously with alcohol to exhaustion. These extracts were filtered and allowed to stand. On cooling large crystals
The alcoholic extract of the root bark was allowed to stand for several weeks, at a temperature of about 30°C. The alcohol slowly evaporated off and a copious deposit of colorless monoclinic crystals was obtained. The solution was then decanted and the crystals washed twice with a little alcohol. The crystals had a very sweet taste and were soluble in water, and it was suspected that they were a sugar. They were transferred from the walls of the beaker to a casserole, dissolved in hot water with slight heating and the solution treated with Merck's adsorptive charcoal. The solution was filtered and the filtrate concentrated to a syrup on a water bath. A small amount of alcohol was then added and the solution set aside to promote crystallization and the crystals finally dried at 105°C. The sugar has a tendency to crystallize in large crystals like those obtained from sugar cane.

A few of the crystals were transferred to a test tube dissolved in water and 5 cc. of Fehling's solution added, but no reduction occurred on heating. The sugar was then hydrolyzed by treating a similar solution with three drops of concentrated hydrochloric acid and heating to 70°C. for 5 minutes. The excess acid was neutralized with Fehling's solution B. and Fehling's solution added. A very active reduction occurred when this solution was heated. The original sugar solution did not form an osazone but after hydrolysis, as described above, a typical glucosazone was produced. The hydrolyzed sugar solution also gave a positive test with Saliwanoff's reagent and reduces ammoniacal silver nitrate solution.

From the fact that this was a nonreducing sugar which hydrolyzed to give monosaccharides which yield only the one typical glucosazone and that both an aldose and ketose monosaccharide are produced on hydrolysis, it was concluded that the sugar was sucrose. Measurements of optical rotation and inversion also confirmed this conclusion.
It is rather difficult to make an accurate quantitative determination of the sucrose present in the powdered barks because of the presence of other dextrorotatory compounds. The alcoholic method of extraction and calculation of sucrose by the optical rotation produced, furnishes results which are far too high, even though the oil has been removed by previous extraction with anhydrous ether. The method of warm aqueous digestion\(^{22}\) was found to give the most accurate results. 13 Gms. of each of the dried root bark and dried stem bark were digested with 100 cc. of diluted basic lead acetate solution for one-half of an hour, allowed to cool, sufficient distilled water added to bring to the required volumes. The solutions were then filtered and the per cent of sucrose determined by observation in a 200 m.m. tube in a Schmidt and Haensch polarimeter. These readings when doubled gave the amount of sucrose in the root bark as 6.2% and 4.6% for the stem bark.

Further work on the alcoholic extracts, by separation into water soluble, water insoluble and chloroform soluble fractions, and treating these with acidic and basic solutions, did not yield definite results. The water and chloroform insoluble fraction seemed to consist chiefly of resinous matter, sugar was present in the water soluble portion as well as a bitter principle, but no definite compound could be isolated. The fractions were reserved for further reference.

- VOLATILE OIL -

The strong penetrating odor of the plant suggested the presence of a volatile oil. A preliminary test was made for the presence of volatile oil by macerating 100 Gms. of powdered root bark supplied by Fenick & Co. in distilled water for 6 hours. The macerated powder was then subjected to steam distillation and the distillate cohabated once. A few globules of light yellow oil collected on the surface of the water. The oil had
a peculiar carrot-like odor which is characteristic of the Aralias. The aqueous portion of the distillate was neutral to litmus indicating the absence of volatile organic acids and produced no coloration with ferric chloride solution indicating the absence of phenols.

The oil was extracted from the aqueous distillate with two successive portions of 15 cc. each of chloroform, the chloroformic solution dried with a few granules of anhydrous calcium chloride, decanted and the chloroform allowed to evaporate off at room temperature. 0.08 Gm. of oil was thus obtained corresponding to a yield of 0.08% for the root bark. This yield was too small for the determination of any constants. The oil appeared to be very volatile.

An attempt was made to secure a quantity of the oil for experimental study by the steam distillation of a large quantity of root bark which was gathered near Seattle, Washington in December. The distillation was carried out in the large steam still but only a few globules of oil were obtained. It seems probable, therefore, that the volatile oil content may vary greatly with seasonal changes and perhaps with the locality in which Devil's Club grows.

- EXAMINATION FOR ALKALOIDS -

An examination for alkaloids was made by extracting 40 Gms. of powdered root bark to exhaustion with 90% alcohol. The alcoholic extract was evaporated to a pilular consistency on a water bath. The residue was then mixed with purified siliceous earth and thoroughly triturated in a mortar to obtain a uniform powder. This was treated with two successive portions of warm 1% hydrochloric acid solution. The acid solutions were decanted off, filtered, combined and made alkaline with ammonia water. It was first extracted with 40 cc. of ether in two successive portions of 20 cc. each and then with 40 cc. of chloroform in the same manner. Both the ethereal
and chloroformic extracts were then each extracted with 15 cc. of 1% hydrochloric acid. 1 cc. portions of each of these dilute acidic solutions were then placed in test tubes and 3- to- 4 drops of each of gold chloride T.S., Lugol's solution, platinic chloride solution, Mayer's reagent, picric acid T.S., tannic acid T.S., and phosphomolybdic acid 1% added respectively. No precipitation was observed even after twenty-four hours standing indicating the absence of alkaloids.

The alcohol extracted powder was allowed to dry by exposure to air and then moistened and macerated with 1% hydrochloric acid solution for 48 hours. Percolation was allowed to proceed slowly until about 200 cc. of percolate was obtained. The percolate was then made alkaline with ammonia water. A precipitation of an amorphous, bluish-green substance occurred, but this was not dissolved on subsequent extraction with ether or chloroform but tended to produce an emulsion rendering separation difficult. The etherial and chloroformic extracts were evaporated to dryness on a water bath and the residue extracted with 1% hydrochloric acid. 1 cc. portions of these acidic extracts were then treated as in the manner already described, but no observable precipitation occurred even after 24 hours. In view of these facts, we do not believe that there is any substance of a true alkaloidal nature present in the root bark.

- TANNINS -

An aqueous extract of the powdered root bark produced a light bluish-green coloration with ferric chloride solution and no color with ferrous sulfate solution. The blue-green color produced by ferric chloride solution disappears on the addition of dilute sulfuric acid, which is characteristic of the inks produced by tannic acid. The aqueous extract does not form any observable precipitate with 20% gelatin, however, and produces only a slight turbidity with dilute quinine and strychnine sulfate solutions.
The etheral extract when shaken with ferric chloride solution does not produce any coloration. It is probably that there is a very small amount of tannic acid or tannin present as indicated by these results, but the quantity is too small to be determined by the general methods in use.

**THE AQUEOUS EXTRACT**

The powdered barks were freed of alcohol by exposure to air at room temperature and finally heated to 70°C to completely dissipate the alcohol. These were then extracted to exhaustion with chloroform water, as a very active fermentation with the formation of butyric acid soon commences if pure distilled water is used. The aqueous extracts were concentrated on a steam bath to a volume equivalent to the original weights of the powdered barks used.

300 cc. of the aqueous extract of the root bark was treated with 50 cc. of 1% sodium carbonate solution. An amorphous almost white flocculent material separated. This was collected by filtration and washed with warm distilled water and then alcohol.

It dissolves completely in dilute acids and forms a precipitate with neutral lead acetate solution in dilute acetic acid solution and also with ferric chloride test solution. However, it does not give a positive Molish reaction indicating that it is not a gum and it does not give the common protein color and precipitation reactions. This alkali-insoluble substance was not obtained in a large enough quantity for further investigation.

The remainder of the aqueous extract of the root bark was then treated with an equal volume of acetone. The flocculent precipitate was allowed to settle, collected by centrifugilization, dried, and redissolved in water.
and reprecipitated, collected in like manner and the process repeated. In this way it was possible to free the acetone precipitate of most of the coloring matter and an almost pure white amorphous, somewhat sticky, gum-like substance was obtained. This was washed with alcohol, then with ether and finally dried under carbon dioxide. On drying it formed scales which readily darkened in color due either to the action of light or perhaps to oxidation.

The dried acetone precipitate prepared in this manner is insoluble in all of the common organic solvents, slowly soluble in cold water, more readily soluble in hot water and very soluble in acidulated water. On hydrolysis with 1.25% sulfuric acid it forms a large quantity of sugar that reduces Fehling's solution and forms a characteristic osazon.

The alkali precipitated fraction is quite different chemically from the acetone precipitated fraction since the former does not form a sugar on hydrolysis.

Similar treatments were carried out with the aqueous extract of the stem bark and it was determined that both the alkali precipitable-fraction and the acetone-precipitable fraction are present. The values of the various extracts obtained in the method of successive extraction with the solvents used and the approximate yield of acetone precipitable substance as determined gravimetrically have been arranged in the following table:
### TABLE OF EXTRACTIVES OBTAINED BY SUCCESSIVE EXTRACTION

<table>
<thead>
<tr>
<th>Extractive</th>
<th>Root Bark</th>
<th>Stem Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Petroleum Benzin Extractive</td>
<td>10.71%</td>
<td>12.89%</td>
</tr>
<tr>
<td>Anhydrous Ether Soluble-Extractive</td>
<td>1.68%</td>
<td>1.55%</td>
</tr>
<tr>
<td>Acetone Soluble-Extractive</td>
<td>1.60%</td>
<td>1.67%</td>
</tr>
<tr>
<td>Alcohol Soluble-Extractive</td>
<td>10.65%</td>
<td>10.58%</td>
</tr>
<tr>
<td>Water Soluble-Extractive</td>
<td>20.33%</td>
<td>15.00%</td>
</tr>
<tr>
<td>Acetone Precipitable-Fraction</td>
<td>2.12%</td>
<td>1.27%</td>
</tr>
<tr>
<td>Ash of Acetone Precipitable-Fraction</td>
<td>52.06%</td>
<td>53.62%</td>
</tr>
</tbody>
</table>

In determining the acetone precipitable-fraction the aqueous extract obtained by percolation with chloroform water was first treated with neutral lead acetate solution, the precipitate filtered off, the excess of lead removed by treatment with hydrogen sulfide. The insoluble lead sulfide was filtered off and the filtrate concentrated by evaporation on a water bath to a volume corresponding to three times the weight of the original drug used. This solution was then cooled and combined with an equal volume of acetone and the precipitate collected and weighed in an alundum crucible. The ash is stated as percentage of the acetone-precipitable fraction; the other values on the constant weight of the powders obtained by drying at 105°C.

### THE HYPOGLYCAEMIC SUBSTANCE

The dried acetone-precipitated material was redissolved in water in a concentration of about 2%; solution being facilitated by adding 5 cc. of diluted acetic acid per 100 cc. This solution was then treated with a slight excess of a solution of neutral lead acetate and the flocculent precipitate removed by filtration. The excess of lead was then removed by treatment with hydrogen sulfide and the lead sulfide removed by filtration. The filtrate was then concentrated to a volume of about 50 cc. by evaporation on a water bath, cooled and combined with an equal volume of acetone.
The precipitate which formed was allowed to collect on the bottom of the vessel, the supernatant liquid decanted, the precipitate washed twice with 95% alcohol, decanting the washings, then washed with anhydrous ether and finally dried at 105°C for one hour.

This method of separation varies from that used by Large and Brocklesby in that the aqueous extract was treated with acetone first to precipitate the material, which was further purified by reprecipitating and then finally treated with neutral lead acetate and again precipitated by acetone. It is doubtful whether this method possesses any distinct advantage over the method used by Large and Brocklesby who treated their extract with neutral lead acetate first and then precipitated the active material with acetone.

A small quantity of pale yellow powder was obtained which on microscopical examination appeared to be composed of small rounded particles resembling red blood cells in general appearance. They are circular in outline and when observed along the plane of their diameters appear biconvex. It is possible that they may be rosette crystal aggregates because of the uniformity of size and shape observed but definite plane surfaces were not discernible. When treated with a small amount of water they slowly disintegrate and on evaporation, definite prismatic crystals of a benzene-ringlike appearance are deposited.

The powder is not very soluble in water, practically insoluble in all of the common organic solvents, but readily dissolves in dilute acetic acid and dilute hydrochloric acid. The acetic acid which remains in solution after treatment with lead acetate and hydrogen sulfide keeps the material in solution until precipitated by acetone. On treatment with diluted sulfuric acid (1.25%) a copious precipitate of calcium sulfamate was obtained which was also formed when the original acetone pre-
precipitate was hydrolyzed by treatment with sulfuric acid. The other fraction of the molecule has not yet been identified.

This acetone-precipitable substance forms definite prismatic crystals when dissolved in dilute acetic acid and the solution allowed to evaporate to dryness.

The acetone-precipitate does not melt below 350°C, but on higher heating it liquefies and decomposes. The temperature of this change could not be determined due to the limitations of the mercury thermometer.

At this time it is impossible to state definitely the chemical nature of the hypoglycaemic substance, but experimental data indicates that it is probably an organo-calcium compound. Sodium decompositions indicate the absence of nitrogen, therefore, rendering doubtful the assumption that this substance is of a basic nature. Nor is it probable that it contains a carbohydrate grouping, as the Molisch test is negative and hydrolytic treatment with sulfuric acid does not yield a reducing substance. The possibility that the hypoglycaemic substance, if very active, might be present in minute traces contaminated with a calcium salt also exists.

The close resemblance between this acetone-precipitate obtained from Devil's Club and the alcohol precipitate obtained from an aqueous infusion of barley malt dust by Donard and Labbe is rather striking. The acetone-precipitate of Devil's Club is also precipitated from an aqueous solution by the addition of alcohol. Donard and Labbe found that the hypoglycaemic action of their precipitate varied considerably in different rabbits and the effects lasted for several hours while Large and Brocklesby also found considerable variation in response but the effects were not as prolonged.
SKIN REACTIONS

The poisonous nature of the spines of Devil's Club has been mentioned by several who have described this plant. We have also found that one who works with these powdered root and stem barks is apt to get a skin reaction or allergic response similar to that produced by poison ivy. Although we have not definitely proved that previous sensitization is necessary for this reaction to occur, we believe that because this urticaria occurred in one individual who had been previously exposed to Devil's Club on several occasions, several weeks before it occurred in another, who had not been exposed to it, is of sufficient evidence to conclude that it is probably of an allergic nature.
SUMMARY

1. A study of the use of Devil's Club in Indian medical practices has been made.

2. A general examination of the stem and root barks has been made and various extracts determined.

3. The fixed oils contained in the root and stem barks have been extracted and studied.

4. Sucrose has been isolated, identified and quantitatively determined.

5. The presence of a volatile oil has been established and a quantitative determination made.

6. The acetone-precipitable fraction has been studied and a crystalline substance has been obtained from it.
BIBLIOGRAPHY

1. Anderson, James R., Trees and Shrubs, Food, Medicinal and Poisonous Plants of British Columbia; Published by Dept. of Education, Victoria, 1925, p. 89.


19. Large, R. G. and Brocklesby, H. N., A Hypoglycaemic Sub-
stance from the Roots of the Devil's Club (Fatsia horrida);
32-35.
20. Lemery, Louis, Secretary, Flathead Tribal Council, Flat-
head Indian Agency, Dixon, Montana; personal communica-
tion.
21. Lilly, Josiah Kirby, Aralia Spinosa; American Journal of
22. Lunge, G., Technical Methods of Chemical Analysis, English
translation by C. A. Keane, Ph.D., Vol. III, Part II; Gurney
23. Mitchell, Sam Pierre, An Indian on the Flathead Indian
Agency, personal communication.
24. Monias, Bruno L., The Effect of the Peroral Antidiabetic
Anticoman on Blood Sugar, Wein. Med. Wochschr. 84: 584-8,
25. Pammel, L. H., Manual of Poisonous Plants; The Torch Press,
Cedar Rapids, Iowa, 1911, p. 647.
26. Rydberg, Per Axel, Catalogue of the Flora of Montana and the
Yellowstone National Park; New York Press of the New Era
Printing Co., Lancaster, Pa., 1900 p. 284.
27. Whitford, Harry N., The Forests of Flathead Valley, Montana,
Botanical Gazette XXXIX, 194: 218, Mr', 1905.
28. Younken, Herber W., The Pharmacognosy of Devil's Club Bark;
Druggists Circular, Vol. LXXXII, No. 12, Dec. 1938, p. 20,
52.