POLYPODIUM OCCIDENTALE.*

BY LOUIS FISCHER AND F. J. GOODRICH.

Licorice fern, a plant named as early as 1781 by Linnæus and later by Kellog, Eaton, Maxon and Jepson, appears never to have been subjected to an examination. Its early use as a flavoring in tobacco, as an alterative and as an antisyphilitic, and its characteristic licorice taste led us to conduct experimentations upon the drug. Its characteristic flavor, although somewhat masked by a slight and bitter taste, is similar to that of *Glycyrrhiza glabra*. Since the official licorice is universally used in prescriptions and proprietaries it seemed that there would be some advantage in substituting for it the licorice fern, which grows on the Pacific Coast, and the latter was studied with this idea in mind.

The licorice fern used in this analysis has had varied names. Hooker (15) in 1840 named it after the European species, calling it *Polypodium vulgare occidentale*. In 1854, Dr. A. Kellog (1) described and named it *Polypodium falcatum*. D. C. Eaton (2), in 1856, described a specimen of the fern, sent from Southwestern Oregon, and designated it as *Polypodium glycyrrhiza*. Maxon (6) gave it the name *Polypodium occidentale* in 1904. Jepson (20) termed it *Polypodium vulgare* L. **var.** *occidentale* Hook.

The fern is most abundantly found along the Pacific Coast, ranging from Alaska to California. Swan (3) believes that he saw it in Massachusetts and Alabama. It grows on old logs and trees, and is most commonly found vegetating in moss on animate maples, sometimes on rocks and stumps, in damp shady places.

Licorice fern has been used by some persons to flavor tobacco, and has been said to be an excellent alterative. The belief of Swan (3) is that its medicinal properties are equal to those of sarsaparilla. It has a sweetish bitter taste and a decoction is not unpleasant. The natives (1) highly esteemed it as a medicine, and thought it to be an antisyphilitic. The rootstocks are often gathered by children for chewing because of the strong licorice taste. The roots (20) are also roasted as confection. The ancients (3) used a polypody growing upon oak trees for the cure of melancholy and madness; this may have been the licorice fern.

The fern has a creeping, tuberculate rhizome varying greatly in length according to the conditions of growth and age. The longest rhizome noted in the present collection of the fern was 51 inches, with numerous branches from 12 to 18 inches in length. The width also varies from $^1/_8$ to $^1/_2$ inch. The rhizomes are often covered with scales, which are oblong to ovate in shape. The rootstock is yellow, the underside being smooth, while on the upper side are numerous radicles.

The fronds of the fern are 6 to 18 inches long and $1^1/_2$ to 4 inches wide. They are oblong to linear in shape, very acuminate, and membraneous in texture. The leaf segments are numerous and alternate; they taper from a wide base to an acute or acuminate tip and are serrate. The stipes are usually much shorter than the blades; they are naked and straw-colored. The veins are free, with two to four veinlets, and are somewhat translucent. The sori have a roundish oval shape and are in two rows, one on each side of the midrib. They vary in number from eight to twenty-four according to size and shape of the leaf segments.

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Polypodium hesperium Maxon (16) is very similar to *Polypodium occidentale* but the fronds are not as large. It is found from Yukon to California, in South Dakota, New Mexico and Arizona, commonly on dry, rocky hillsides (9). The rootstocks are sweet and have a licorice taste.

Polypodium vulgare is the European variety, found throughout North America, Europe and Asia. The rootstocks are covered with cinnamon-colored scales. The fronds are four to ten inches high and are simple and deeply pinnatified. It is used as a remedy for catarrh and asthma (8) and is said to be an expectorant and diuretic. An infusion of *Polypodium vulgare* is said to be a cure for dyspepsia (14). Guignet (4) reports the presence of glycyrrhizin in this plant and states that it grows near Paris, Brest and in Vosges. It is said (4) to be present in rhizomes of *Polypodium semi-pennatifidum*, **var.** *indursum*, a native of the temperate region of Andes. Both plants are stated to be used as a substitute for licorice. *Polypodium Scouleri*, Hook and Grov. (9), also called leather-leaf polypod has a broadly ovate, leathery blade, 3–12 inches long, and rounded teeth. It grows near sea water from British Columbia to California.

CULTIVATION.

The collection of licorice fern in its natural habitat involves such great difficulties that for any economical production one must consider other methods of cultivating and harvesting. Attempts were made to find conditions suitable to its growth other than the natural one on logs and moss-covered trees.

The entire rhizome and fronds were planted in various types of soil and with varying amounts of light excluded, others were planted in heavy beds of moss with considerable moisture and shade, and still others placed in soil but the rhizomes covered with a heavy layer of moss.

From the results of the experiments, the fern seemed to grow best when the rhizomes were covered with moss and in the absence of any beneath them. This is probably due to the necessity for direct contact of the rhizomes with the soil. (Under further investigation.)

COLLECTION AND EXAMINATION.

Collections of the rhizomes were made at varying periods during 1927 and 1928, freed from adhering materials, washed and placed in a dryer at 75° C. for ten to fourteen days, then ground and placed in closed containers.

The samples were thoroughly cleaned and dried to constant weight at room temperature, then placed in an oven at 110° C. and again reduced to constancy. These and the ash determinations were made according to the methods of analysis of the Assoc. of Off. Agr. Chem.

VOLATILE MATTER AND ASH.

	No. 1.	No. 2.
Loss in air	75.44%	75.81%
Loss at 110° C.	3.48	3.60
Total volatile	78.92	79.41
Total ash	2.68	2.69
Acid-insoluble ash	0.26	0.28
Acid-soluble ash	2.42	2.41
Water-insoluble ash	2.06	2.09
Water-soluble ash	0.62	0.60

EXTRACTIONS.

The following tables give the percentage of extract soluble in organic solvents. In the first analysis the rhizome was used as such, in the second it was previously macerated with 10 per cent ammonium hydroxide for 12 hours and then dried at 85° C. All analyses were made by Soxhlet extractions, each sample being extracted for 18 hours, the percolate evaporated at room temperature and dried to constant weight in a desiccator. The volatile extract was determined by reducing the extract to constant weight at 110° C.

	Extracts from Pure Drug.			Extracts from Ammoniacal Drug.		
	Total.	Non-volatile.	Volatile.	Total.	Non-volatile.	Volatile.
		Alcohol.			Alcohol.	
1	37.49%	33.40%	4.09%	35.84%	32.12%	3.72%
2	41.05	38.23	2.82	35.56	32.12	3.44
3	31.44	28.21	3.23	...	...	..
4	33.25	31.11	2.14	...	...	..
Average	35.81	32.73	3.07	35.70	32.12	3.58
		Ethyl Acetate.			Ethyl Acetate.	
1	15.61	14.18	1.43	12.87	10.75	2.12
2	15.58	13.99	1.59	13.06	10.81	2.25
Average	15.59	14.08	1.51	12.96	10.78	2.18
		Chloroform.			Chloroform.	
1	7.73	7.68	0.05	9.01	8.60	0.41
2	7.69	7.61	0.08	8.91	8.47	0.44
Average	7.71	7.64	0.06	8.96	8.53	0.42
		Ether.			Ether.	
1	7.57	7.38	0.19	7.23	6.95	0.28
2	7.08	6.90	0.18	7.20	6.81	0.39
Average	7.32	7.14	0.18	7.21	6.88	0.33
		Toluene.			Toluene.	
1	6.82	6.81	0.01	8.29	7.93	0.36
2	7.11	6.91	0.20	8.45	7.73	0.72
Average	6.96	6.86	0.10	8.37	7.83	0.54

Aqueous Extract.—The aqueous extract was determined by a method given in the New Dutch Pharmacopœia (21) for extract value of licorice root. The method was as follows: The gross weight of a flask containing 2 Gm. of the drug and 100 cc. of water was noted. It was then heated under a reflux condenser for fifteen minutes, cooled, adjusted to the original weight and filtered. Fifty grams of the filtrate was evaporated in a tared dish and the residue dried to constancy in a desiccator.

The results as obtained from the licorice fern were 41.34 and 41.04 per cent; an average of 41.19 per cent.

Selective Extraction.—The dried powdered sample was successively extracted with various solvents in a Soxhlet apparatus, the extracts being dried to constant weight at room temperature and then at 110° C.

	Total.	Non-volatile.	Volatile.
		Petroleum Ether.	
1	5.92	5.89	0.03
2	6.22	6.19	0.03
Average	6.07	6.04	0.03
		Ether.	
1	2.58	1.86	0.72
2	2.61	2.50	0.11
Average	2.59	2.18	0.41
		Chloroform.	
1	1.03	0.63	0.40
2	1.14	0.97	0.17
Average	1.08	0.80	0.28
		Ethyl Acetate.	
1	2.49	2.24	0.25
2	2.06	1.85	0.21
Average	2.27	2.04	0.23
		Alcohol.	
1	20.03	15.95	4.08
2	22.70	17.19	5.51
Average	21.36	16.57	4.79

Sugars.—The rhizome has a very sweet taste, which is masked somewhat by a distinct and prolonged bitterness. Qualitative tests based on the formation of characteristic osazones, and the reduction (15) of heavy metals in alkaline solution showed the presence of glucose or levulose or both. Quantitative determination gave a yield of 13.45 and 13.72 per cent reducing sugars. The presence of sucrose was demonstrated by isolation and determination (7) of melting point and by formation of the glucosazone upon hydrolysis.

Starch.—A two-gram sample (15) of ground drug was freed from sugars by washing with cold water. The sugar free drug was boiled five minutes to gelatinize the starch. After cooling, 3 cc. of a saturated solution of Taka-diastase was added. A control test was made using 3 cc. of Taka-diastase. Both solutions were digested at 50° for twenty-four hours, filtered, treated with HCl and refluxed for $2^1/_2$ hours. The solutions were cooled, neutralized, clarified with lead acetate and made up to a definite volume. From the weight of dextrose found the original amount of starch was calculated and gave 1.30 and 1.22 per cent.

Pentosans.—The dried ground sample (13) (2 Gm.) was hydrolyzed with 8 cc. of concentrated HCl and 150.0 cc. of water. After neutralizing, 2 cc. of a uniform suspension of yeast were added. The sample was allowed to stand 12 hours at 37°, filtered and the residue washed thoroughly with water. The filtrate was boiled to remove any alcohol formed, and transferred to a 500-cc. volumetric flask. Lead acetate was added and made to volume and the whole filtered. The excess of lead was removed from an aliquot part and amount of sugars determined by the Munson and Walker method. From the amount of dextrose determined the pentosans

calculated were 6.39 and 6.05 per cent. Blanks using exact amounts of yeast suspensions were made.

Tannin.—Since the aqueous extract from the licorice fern had a somewhat astringent taste and an acid reaction, qualitative tests were made for tannic acid. Various test reagents gave positive indications of considerable amounts. The determination of total tannin was made by Lowenthal's gelatin method and gave an average of 3.45 and 3.89 per cent. This was found to be chiefly of the catechol variety.

Alkaloids.—Fifty grams of the drug were macerated for 48 hours with 150 cc. of alcohol, with occasional shakings. The drug was filtered off and the filtrate evaporated. The residue was macerated for 24 hours in 25 cc. of 1 per cent tartaric acid solution and filtered. The filtrate was then tested with Mayer's reagent, tannic acid, picric acid and Lugol's solution, one cc. of the filtrate being used for each. At the end of 24 hours no precipitate or turbidity was noticed in any case. It would appear, therefore, that the licorice fern does not contain appreciable amounts of alkaloids.

Volatile Oil.—Upon steam distillation of the licorice fern, 0.005 per cent of volatile oil was obtained. The oil was a reddish brown, viscid liquid, with a characteristic, disagreeable, pungent odor. The taste is first burning and a numbness of the tongue is noted, accompanied by a characteristically bitter and bland taste, which is very persistent and lasting.

Isolation of Color.—The yellow coloring matter occurring in the rhizomes was obtained by a method used by Houseman (10) in extracting a dye from licorice. One hundred grams of dried, ground licorice fern was exhausted with hot water and evaporated to dryness. The resulting extract was exhausted with 200 cc. of absolute alcohol and the solvent evaporated. The dye was then extracted with hot water.

Cotton, wool and silk were boiled with an aqueous solution of the dye, dried and then thoroughly washed in water. The coloring substance had no effect upon cotton or wool, but dyed silk a fast, pale yellow.

Glycyrrhizin.—Five grams of an extract of the drug was warmed with water according to a method by Linz (11). On cooling it was treated with 100 cc. of alcohol, allowed to stand, but no sediment was formed. The solution was filtered and concentrated to 30 cc. and made up to 50 cc. Then 5 cc. of sulphuric acid was added and allowed to stand, first one hour at room temperature and then twenty-four hours on ice. The precipitated glycyrrhizic acid-like compound was washed with 15 cc. of 2 per cent sulphuric acid at 0°. The precipitate was dried over sulphuric acid and extracted with successive volumes of hot 95 per cent alcohol and filtered into a tared dish, evaporated and weighed.

The mother liquid above, together with the washings, was saturated with ammonia and evaporated to a thick syrup. The residue was washed into a cylinder and made up to 18 cc. with water and 2 cc. of H_2SO_4 added and allowed to stand twenty-four hours. The precipitate was transferred to a filter, washed with 5 cc. of 2 per cent H_2SO_4 and 10 cc. of ether-saturated water. The precipitate was dried over H_2SO_4, extracted with 95 per cent alcohol, filtered and evaporated. The weight of this residue with that obtained above gives the glycyrrhizin content in 5 Gm. of the extract. A yield of 2.36 per cent was obtained, based upon the dried, powdered drug.

No crystallizable calcium or barium salts of glycyrrhizic acid could be obtained.

Ammoniated Glycyrrhizin.—Five hundred grams of powdered licorice fern was macerated with a mixture of 475 cc. of water and 25 cc. of ammonia water for twenty-four hours, then packed in a percolator and extracted with water until 500 cc. of the percolate was obtained. Sulphuric acid was added as long as a precipitate formed. The precipitate was collected on a strainer, washed with water until the washings no longer had an acid reaction and the residue dissolved in water with the aid of ammonium hydroxide. The solution was filtered, again precipitated with sulphuric acid, and the precipitate was collected and redissolved in ammonium hydroxide previously diluted with an equal volume of water. The clear solution was spread on plates of glass to dry.

The finished product was in black, shiny scales, inodorous and lacked the characteristic sweet taste. The solubility of the scales was the same as ammoniated glycyrrhizin prepared from the official licorice root. Both were insoluble in strong alcohol and ether, but were soluble in water, more so if warm, and in water containing a few drops of ammonium hydroxide.

The black shining scales and ammoniated glycyrrhizin prepared from glycyrrhiza were compared by tests given by Gawalowski (5). A cold, saturated solution of ammonium molybdate gave no change in either. No changes were noted when sesame oil and a solution of alpha-naphthol were added separately to the substances under analysis, but both showed a reduction with Fehling's solution by a quantitative analysis. Reactions given by Plenderleith (12) were carried out and gave the same results. Strychnine sulphate (Plenderleith used the hydrochloride) and quinine hydrochloride each gave a precipitate with solutions of the black crystals and of the ammoniated glycyrrhizin. Tinctures of opium and digitalis each gave a slight cloudiness with both substances.

From tests the crystals are identical with ammoniated glycyrrhizin in the reactions and solubilities, but vary in taste and color.

COMPARATIVE GALENICALS.

In order to compare the taste and color of the substance under analysis and as a possible substitute for the official licorice, preparations were made using the United States Pharmacopœia and the National Formulary procedure for glycyrrhiza products, and the characteristics of each noted. The drug under analysis was previously cleaned, dried and ground to the stated degree of fineness, while the official licorice was of the Pharmacopœia standard.

Fluidextract.—The percolate of the official drug was darker and, on prolonged percolation, the liquid contained a green coloration, while the percolate from the licorice fern was yellow. The finished product of both drugs on standing developed a small amount of brown sediment. The color of both fluidextracts was reddish brown, but the fern was a shade lighter. The taste of the two was remarkably different, the official had a sweet, pleasant aromatic taste, while the fern was unpleasant, decidedly bitter and lasting. The "licorice" taste was noted in both, but was much more pronounced in the official drug.

Extract.—The difference in appearance of the finished product was readily noticeable. The official preparation was of a brownish black color while that from

the fern was reddish brown. Both extracts were sweet and contained the characteristic licorice taste, stronger, however, in the extract of glycyrrhiza. A bitter taste develops in both products, but in the official preparation it was only slight, while in the fern extract it was more noticeable and prevailed a greater length of time.

Compound Mixture.—The finished products on standing contained a brown sediment, which was easily mixed. Both preparations were reddish brown in color. The official compound mixture was sweet and possessed a lasting aromatic taste. In comparison, the finished fern product was also sweet but it was masked by a lasting bitter taste.

Compound Powder.—The finished products were very similar in appearance, both were grayish brown in color, while the licorice root preparation contained a yellow tint which made it an outstanding feature. Compound licorice powder was sweet and aromatic, while the sugary taste in the fern product was veiled with a slight characteristic bitter taste. The licorice taste was noticeable in both products.

Elixir.—Both preparations had the characteristic reddish brown color, with the elixir of licorice perceptibly darker. The taste was very similar, except that the official elixir was somewhat sweeter. In the fern product the bitter taste was absent.

Aqueous Elixir.—The finished preparations were both clear and reddish brown in color, but the official one was somewhat darker. Both were sweet and aromatic in taste, with the dulcet taste prevailing in the official preparation. The fern product obtained showed the absence of its peculiar bitter taste.

Fluidglycerate.—The characteristic reddish brown color was present with a darker shade in the official licorice product. Both were sweet in taste, the official product being more palatable and sweeter, while the noted bitter taste was absent in the fern preparation.

Syrup.—Both were clear, reddish brown preparations, that of the fern being a shade lighter. The products had a sweet, pleasing and licorice taste, the latter being more predominant in the official preparation.

Acid and Alkaline Preparations.—A fluidextract was made of the licorice fern using the United States Pharmacopœia IX Method containing ammonia water. Another preparation was made of the same drug using 2 cc. of concentrated hydrochloric acid and boiling water as a menstruum with which to macerate the drug, and then exhausting with boiling water. The percolate was concentrated to $^3/_4$ of the desired amount and alcohol added to the required volume.

Both preparations were reddish brown in color, the ammonia product being darker and more viscid. The licorice taste was present in both but not as prominent in the acid preparation. The acid product possessed a strong, puckering taste. According to Berg (18), ammonia water added to the menstrum in percolating licorice produces a gelatinous and otherwise undesirable product. He used varying amounts of ammonia water on the drug without any indication of increased sweetness. It was also noted that the amount of precipitate in the preparation on standing increased with the amount of alkali added.

Starch-Splitting Enzymes.—Fifty grams of powdered root and a like quantity of leaves were ground separately with 50 Gm. of purified sand and macerated for 48 hours with 250 cc. of distilled water. The percolate was filtered, put into measured quantities of starch solution and incubated (19). Control analyses were conducted

using the enzyme and starch each as blanks. The amount of cuprous oxide corresponding to the hydrolyzed starch is as follows:

	Blank No. 1.	Blank No. 2.	Total blank.	Blanks and enzyme.	Difference due to enzyme.
	7 cc. starch	3 cc. enzyme sol.		7 cc. starch	
	3 cc. water	7 cc. water		3 cc. enzyme sol.	
Root enzyme	0.0055	0.0080	0.0135	0.0884	0.0749
Leaf enzyme	0.0090	0.0029	0.0119	0.0201	0.0082

On the determination of diastase, the controls made with the starch and enzyme would take care of any intervening substances. Therefore, the difference in reduction between the controls and between the action of the plant juices on the starch must be due to enzymes.

Toxicity.—In order to compare the relative toxicity of licorice fern and glycyrrhiza, tests were made upon white rats. For this purpose a fluidextract of each plant was prepared and one cc. of this solution administered orally to separate animals. A third rat was retained as control. Since the average adult dose given by the Pharmacopœia is 2 cc. the amount used in this case was more than 300 times a usual dose. In the case of neither extract, however, was there any appreciable evidence of effect, except that none of the animals appeared as active as before. It would seem safe to conclude that extracts of the licorice fern are not harmful in large amounts.

SUMMARY.

1. The rhizome of the licorice fern, *Polypodium occidentale*, which grows prolifically in the Northwest, may be a substitute for licorice, *Glycyrrhiza glabra.*

2. It develops favorably and may be cultivated by growing in sphagnum moss covered with soil.

3. A proximate analysis of the plant was made including moisture, ash and selective extractions.

4. There were identified in the rhizome: reducing sugars, sucrose, starch, pentosans and tannic acid, presumably of the catechol variety. There were also present some volatile oil, a yellowing coloring matter and a starch-splitting enzyme. The plant is apparently free from alkaloids.

5. A product similar in properties to ammoniated glycyrrhizin was found in the rhizomes, the yield being 2.36 per cent based on the dried material.

6. Experiments in which the rhizome was fed to white rats indicated that it is non-toxic even in large amounts.

7. A comparison of galenical preparations made from licorice and from the licorice fern shows that, although those from the latter are somewhat less sweet and have a slightly bitter taste, these disadvantages might possibly be remedied.

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THE LEAF OILS OF WASHINGTON CONIFERS: II. JUNIPERUS SCOPULORUM.*

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In a previous paper, a review was made of work which has been done on conifers of Washington. The present paper deals with the Rocky Mountain juniper. It does not occur to a great extent, being limited to certain regions, but it happened to be easily available to the writers and forms the basis of this investigation.

This species of juniper is a tree, ten to forty feet in height, with a short stout trunk, two feet or more in diameter, and often divided near the ground into a number of slightly spreading stems. The stout, spreading branches, which are covered with a scale-like bark, give the tree a rounded appearance. The bark of the trunk is reddish brown in color. The slender branches are covered with a thin, scaly bark. The leaves are opposite in pairs, acute, glandular and dark green. The fruit is about one-sixteenth of an inch in length and blue or rose color, ripening at the end of the second season, when it is from one-fourth to one-third of an inch in diameter, bright blue, covered with a glaucous bloom and usually two-seeded. The ripe seed is ovate, acute, light chestnut-brown, lustrous and about three-sixteenths of an inch in length.

Juniperus scopulorum, found ordinarily in arid regions, is common enough east of the Cascades and in the Rocky Mountains, whence its name, Rocky Mountain juniper. Strangely enough it has crossed its natural barrier, the Cascades, to reappear west of these mountains in only a few isolated localities in the northern Puget Sound region where there is an abundance of soil moisture. The material for this work was obtained from trees growing in bottom land having a sandy and clayey composition. This section receives an annual rainfall of about forty inches and, from the general character of the land, the indications are that this area is covered with water during certain times of the year, especially after heavy rains.

EXPERIMENTAL.

The material was gathered in the spring from a grove of trees near Everett, Washington. The leaves were separated as far as possible from the twigs and im-

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