(*c*) Blank trials showed no liberation of iodine.

The test was conducted using pure nitrogen as the dispersion medium instead of oxygen. This apparently did not augment the amount of iodine liberated.

CONCLUSION.

1. A simple test, requiring no gas free from carbon monoxide for scrubbing purposes, for the detection of carbon monoxide in medicinal oxygen has been devised.

2. The sensitivity of the test is in the order of 1–100,000.

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LICORICE FERN AND WILD LICORICE AS SUBSTITUTES FOR LICORICE.*

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A preliminary study reported by one of us three years ago (1) indicated the possibility of using the rhizomes of licorice fern, *Polypodium vulgare* L. **var** *occidentale* Hook, in place of the official licorice. We have now completed that study and find increasing evidence for this substitution.

In the meantime attention was called to the common occurrence of wild licorice, *Glycyrrhiza lepidota* (Nutt.) Pursh. Apparently as an outcome of suggestions made at the annual convention of this ASSOCIATION in 1887, McCullough (2) reported an examination of the rhizomes. He found 8.53 per cent of ammoniated glycyrrhizin and 6.39 per cent of the crude acid, which differed considerably in taste from the compound as obtained from licorice.

From the published citations, one would conclude that the rhizomes are similar to those of licorice. Furthermore, we learn that they have been used as a

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tonic by the Pah Ute Indians (3) and that the whites have chewed them in place of tobacco. We are quite puzzled by this situation because our material was bitterish and rather salty, but not sweet and with little resemblance to licorice.

The specimens used by us were identified by Prof. George Neville Jones of the University of Washington. The licorice fern was collected in and near Seattle, the wild licorice near The Dalles, Oregon, in June 1929. The materials were well cleaned, thoroughly dried in air at about 35° C. and ground to fine powders. Proximate analyses gave the following results in percentage, all except the loss of volatile matter being based on the dried sample. The methods used are described in the previous paper (1).

TABLE I.

	Licorice Fern.		Wild Licorice.
	Rhizomes.	Leaves.	Rhizomes.
Loss in air	75.63	74.38	58.97
Loss at 110° C.	79.17	77.10	59.94
Total ash	2.69	6.15	5.18
Acid-insoluble ash	0.27	0.08	0.55
Soluble in ether	7.32	...	1.67
Soluble in chloroform	7.71	...	1.75
Soluble in ethyl acetate	15.59	...	3.66
Soluble in alcohol	35.81	...	14.28
Soluble in water	41.19	...	27.88
Selective extraction			
Petroleum ether total	6.07	3.06	1.41
Non-volatile	6.04	2.88	1.13
Ether total	2.59	3.61	0.94
Non-volatile	2.18	3.48	0.76
Chloroform	1.08	1.69	0.46
Ethyl acetate	2.27	4.16	1.49
Alcohol	21.36	15.57	13.14
Reducing sugars	4.22	17.00	2.29
Sucrose	15.51	0.66	3.64
Starch (Hooker)	6.29	...	3.49
Pentosans (Spoehr)	6.22	...	12.19
Pentosans (A. O. A. C.)	7.75	...	14.63
Nitrogen × 6.25	9.00	...	...
Tannin	2.46	...	None
Alkaloid	None	None	...

The amounts of sugars, starch and tannin are subject to considerable variation with the seasons as would be expected. Sucrose was isolated from all three materials and identified by melting point, specific rotation and ultimate analysis: Found carbon 42.03, hydrogen 6.41; calculated 42.08 and 6.48 per cent.

The leaves of licorice fern were found to contain starch-splitting enzymes using a method previously described for the rhizomes. There are also present in both materials enzymes which will split beta-glucosides.

GLYCYRRHIZIN.

In the present investigation great doubts were early presented on previous presumptions that glycyrrhizin is contained in the rhizomes of either plant. For estimation of its amount there were used the method (I) of Housemann (4) and one (II) which is a modification of several which are found in the literature. The latter is as follows:

Fifty grams of sample were macerated for six hours with diluted ammonia (1:19) and then percolated with additional water until 500 cc. of liquid were obtained. Exactly half of this was treated with excess of dilute sulphuric acid and allowed to stand over night in an ice box. The filtered precipitate was well washed with ice-water, redissolved in the diluted ammonia and again precipitated with acid. After repeating this process once more, the final ammoniacal solution was evaporated and heated to constant weight.

	I.	II.	
Licorice	6.71	11.43	Sweet and light brown
Wild licorice	7.48	9.72	Slightly bitter and light brown
Licorice fern	1.11	9.34	Tasteless and red-brown

The most striking feature was the difference in taste of the residue. Assuming, as seems unavoidable, that all of the glycyrrhizin is present as ammonium salt, the conclusion is that in licorice alone is there more than a mere trace.

The increased amounts in Method II apparently arise in the absence of preliminary extraction by strong alcohol. In order to confirm this, the process was repeated using such a preparatory extraction as outlined by Housemann (4). The results were: Licorice, 7.89; wild licorice, 8.39; licorice fern, 1.87.

It may be concluded, therefore, that neither of the last two contains more than a trace of glycyrrhizin. The former gives as much residue as licorice, but this is without sweet taste, and the much smaller amount from licorice fern is also without sweetness.

As further confirmation, all three were submitted to the process for purification by Tschirch and Cederberg (5). Licorice yielded the characteristic sweet acid which was further purified by crystallization from hot acetic acid. The others, however, gave no crystalline material at all and nothing which corresponded to glycyrrhizic acid. It is interesting to note that the materials obtained from wild licorice were quite bitter throughout.

The absence of glycyrrhizin from these plants again emphasizes the fallacy of drawing conclusions after the usual quantitative methods, and the reports of finding this substance in various plants is usually based upon no other evidence. Because such methods of reasoning proved erroneous here, one could justifiably assume that there must be serious doubts in all other cases, except where the substance was actually isolated and analyzed, as was done by Tschirch for *Periandra dulcis* and for monesia bark.

LEAVES OF LICORICE FERN.

A large quantity of the leaves was exhausted by hot alcohol which, upon cooling, gave 0.86 per cent of a flocculent yellowish precipitate. The solvent was mostly removed from the alcoholic solution by distillation under reduced pressure and the residue was submitted to distillation by steam.

Benzoic Acid.—The distillate, which was acid in reaction, was completely extracted with ether. After drying and removing the solvent by spontaneous evaporation, there was left a crystalline residue which, after purification, melted at 120° C. Solubility and other properties accorded with those of benzoic acid and this was confirmed by converting to ethyl benzoate. The remainder of the distillate still contained acid corresponding to 0.05 per cent of the drug, as determined by titration and calculated for acetic acid. Incidentally benzoic acid was also found in the distillate from wild licorice rhizomes.

Salicylic Acid.—The thick, green residue from distillation by steam was extracted with ether in which only a portion dissolved. From the ether solution 10 per cent solution of ammonium carbonate withdrew more benzoic acid and some salicylic acid, which gave a violet color with ferric chloride and was also converted to methyl salicylate.

Phytosterol.—The residual solution in ether was evaporated to dryness, leaving a black oil. From this hot dilute alcohol extracted a small amount of a crystalline solid, melting point 132–133° C., which gave a white precipitate with digitonin decomposing at 211–212° C. It also responded to the various tests for phytosterol (Schiff, Moleschatt, Salkowski, Burchardt-Liebermann, ferric chloride) and was not saponifiable.

The aqueous solution left upon distillation with steam was found to contain a nitrogenous base and the sugars. Neither this nor the resins were examined further.

Substance Melting at 74° C.—The flocculent material which separated from the original alcoholic solution was purified by crystallization from hot alcohol containing charcoal. It was then perfectly white and melted at 73–74° C., dissolved easily in benzene but only partly so in other solvents and not at all in water or cold alcohol. The substance was not affected by boiling dilute acids or alkalies and did not react with semicarbazide or with benzoyl chloride. Combustion of several samples gave 80.65 per cent of carbon and 12.80 per cent of hydrogen, leaving 6.55 per cent of oxygen. Evidently it cannot be a hydrocarbon, although its inactivity to reagents might so indicate. The small amount of material available prevented any further investigation of this interesting substance.

RHIZOMES OF LICORICE FERN.

During the preliminary extractions it was noted that a substance separated from ethyl acetate on cooling. Later it was found that a preliminary treatment of the rhizomes with chloroform to remove oil and some resin and subsequent extraction with hot ethyl acetate resulted in a white product. The total amount was about 9 per cent of the dry rhizome.

It was sweet and somewhat bitter, soluble in hot water to give a slightly turbid or clear solution on cooling, also in alcohol and fairly so in acetone, but insoluble in other usual solvents. The melting point varied with each batch, somewhere between 90° and 135° C., always with more or less decomposition. Calculated as reducing sugar, the Munson-Walker method showed 15 per cent before hydrolysis and about 45 per cent after. Sucrose could be separated and identified by its melting point, by the specific rotation of +67.2°, and by an ultimate analysis.

An alcoholic solution of the materials was precipitated by ether and the product was dissolved in hot water. Neutral and basic lead acetate were added in excess and the resulting precipitate was suspended in water and treated with hydrogen sulphide. Evaporation of the filtrate gave a small quantity of brownish residue which was free from sugars and possessed a characteristic taste. That it was mixture was concluded from the melting point; there was some darkening at 110° C. but no complete melting below 140° C. Judging from various experiments, it was apparent that the original substance from ethyl acetate contained, in addi-

tion to sugars, a mixture of compounds in small amounts which are, with the sweetness, responsible for the taste and give the resemblance to licorice. The amount present in the rhizomes is too small to be of any importance except to impart this taste.

Polydin.—An alcoholic extract of the rhizomes was concentrated under reduced pressure to a small volume and precipitated by adding eight times as much ether. After standing for several days, the clear supernatant liquor was decanted and evaporated to a small volume. Crystallization set in after about a week to give ultimately 0.75 per cent. The substance obtained was soluble in alcohol, in acetone, in hot water or in spirit of ether, but only partially in hot ethyl acetate and insoluble in chloroform or ether. Purified by repeated recrystallization from a mixture of acetone and water, it was finally obtained in the form of rosettes, melting sharply at 188–189° C. It had no effect on Fehling's solution until after hydrolysis by hydrochloric acid, when it gave an abundant reduction. The addition of ferric chloride to an aqueous solution gave a greenish blue color which could not be obtained in the presence of much acid. Phosphotungstic acid gave to an alkaline solution a blue color. Both of these reactions are characteristic of arbutin which was also indicated by the melting point.

A small portion was suspended in water under an equal volume of ether and hydrochloric acid was added drop by drop over a period of two days, when the mixture was clear. The ethereal solution was separated, dried with anhydrous sodium sulphate and evaporated, leaving a white substance which melted at 167° C. Physical comparison showed that this was not hydroquinone, melting at 169° C., and no quinone could be obtained upon oxidation. From the aqueous solution there was obtained apparently a mixture of osazones, one of which was similar to that of lactose. Hydrolysis of the glucoside, therefore, resulted in a compound melting at 167° C. and one or more sugars.

Ultimate analysis of highly purified samples of the glucoside gave as average carbon 56.32, hydrogen 5.42, per cent. Since it seems to correspond with none so far recorded in the literature, the name polydin is suggested for it. The available material was too small to permit further examination and its minor relation to the problem in question did not appear to warrant additional work.

One experiment was made, however, to see if polydin is potent. A white rat was fed 0.15 Gm. representing 0.8 Gm. per Kg. of body weight. Although this corresponds to approximately two ounces for an average adult person, yet there was no apparent effect on the rat.

Comparative Galenicals.—In the earlier report (1) a series of preparations was made which compared rather unfavorably in taste with those from licorice. In view of our discovery that a previous extraction with chloroform will remove at least a part of the bitter taste, we have repeated these preparations on material which had been so treated. The taste in general was notably improved; the bitterness was not as noticeable and the preparations possessed a smoother and more palatable taste, but they were still somewhat inferior. Addition of certain aromatics, however, gave entirely suitable products and we have no doubt that satisfactory formulas could readily be devised.

Cultivation.—Since the winter of 1929 experiments have been made in growing the fern. Specimens were collected from several places, leaving the plant wholly

intact and with a portion of the moss upon which it grew. These were planted in a frame containing rich, well-fertilized soil and the frame was covered with small panels about 1.5 inches wide and spaced the same distance apart to allow a partially shaded condition. One-half of the frame was then covered with burlap to keep any direct sunshine from the growth underneath.

The plants began to grow immediately but very slowly. Over the period of four years the leaf-growth has been very heavy but the rhizomes are short and compact and do not compare in length with those which have been observed in a natural habitat. The leaves of the plants covered with burlap have been greater in number, more fully developed and of a rich green color, while the leaves not so covered but in similar soil have been shorter, not as thick, and pale green.

In other experiments portions of the rhizome, about 2 to 2.5 inches in length and each containing a growing tip, were planted under various conditions. The first group was planted in soil obtained from their natural surroundings, the second in moss surrounded with rich, loamy soil, the third in the loamy soil originally in the cold frame. After one year the rhizomes were found to have grown from one to two inches, but in none was there a great amount of leaf development.

After the four years of experience in these studies, we are convinced that commercial production would be entirely practicable. It is true that growth is slow under any of the conditions used and that duplication of natural environment would not be feasible. Nevertheless, cultivation could be accomplished under almost any circumstances and determination of the best conditions should be a simple matter. The licorice fern can undoubtedly be grown and harvested at a price far under that of Russian or Spanish licorice. We urge further study of this interesting problem.

SUMMARY.

The rhizomes of wild licorice contain benzoic acid and sucrose but no glycyrrhizin.

The leaves of licorice fern contain sucrose, benzoic and salicylic acids, a phytosterol and an unidentified substance melting at 74° C.

The rhizomes contain no glycyrrhizin in spite of the characteristic, licorice-like taste which was found due to sugars, including sucrose, plus a small amount of an unidentified substance. They contain also a glucoside, which is named polydin, but no alkaloids.

The rhizomes can be used satisfactorily in place of licorice in medicine, especially if previously extracted with chloroform. Experiments in cultivation have indicated that commercial production to economical advantage is very possible.

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