potency of the oil as calculated from the submitted data, are given in the accompanying table. The coefficient of variation in the results obtained by each of the procedures used has been determined. This value was found to be smallest for the determination on the raw oil, greater when the suggested saponification procedure was used and greatest for the optional saponification procedure results. However, the averages of the calculated potencies are in good agreement. The results of this study furnish further basis for the vitamin A value assigned the oil.

PHYSICAL FACTORS OF THE SECOND U. S. P. REFERENCE COD LIVER OIL

Dr. G. S. Jamieson, in charge of Oil, Fat and Wax Investigations, Agricultural Chemical Research Division of the Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture, has examined the new Reference Cod Liver Oil and submits the following report:

Refractive index at 25° C.	1.4771
Saponification value	188.6
Iodine number (Hanus)	172.1
Acid value	0.81
Unsaponifiable matter	0.60
Stearine (cold test)	None
Odor	Slight
Taste	Characteristic mild fishy flavor

A Phytochemical Study of the Root Bark and Fruit of *Cornus Nuttallii**

By Edward Krupski† and Louis Fischer‡

Cornus Nuttallii, commonly called Dogwood and a member of the Cornaceæ family, grows along the Pacific Coast from British Columbia to California, but thrives best in the Douglas Fir forests of the Puget Sound area. This tree was first observed by the botanist Nuttall and later named for him by Audubon (1). Torrey and Gray (2) published the first written description of this plant in their book "Flora of North America."

This tree usually grows from 20 to 30 feet high and from 6 to 8 inches in diameter. It has a dull ashy brown or reddish bark that forms thin scales on old trunks. The twigs are minutely hairy when young, later smooth, and dull red-purple in color. The leaves are deciduous, simple, opposite, three and one-half to five inches long and about one-half as wide. Button-like clusters of very small greenish-yellow flowers which ordinarily bloom in early spring are surrounded by four to six showy white bracts that are commonly taken to be the petals. The thin dry pulp of the drupes, which are bright red, and mature in clusters of 25 to 40 at the ends of the twigs, contains one or two seeds within a stony endocarp.

No record was found of *Cornus Nuttallii* ever having been used medicinally; however, *Cornus florida*, once official in the U. S. P., has been used for many years as a medicinal agent. The bark of the root and trunk of *Cornus florida* was used with some success as a substitute for Cinchona as an antiperiodic and tonic (3). Also, according to Ellis (4), the bark of *Cornus florida* was considered very valuable in the treatment of autumnal fevers; however, the active principle of neither species has been subjected to a pharmacological study in this respect.

EXPERIMENTAL

Collection of Material.—The fruits were gathered during the months of September and October in the immediate vicinity of Seattle. They were separated from the bracts and only the ripe, fully matured fruits were used in this investigation. After drying at a temperature of about 27° C., they were ground to the desired fineness.

The root bark was collected during September, in the vicinity of McCleary, Washington. After uprooting the trees, the roots were scraped clean and the bark peeled. The bark was well dried in the sun and then ground to a fine powder.

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In the proximate analysis of the fruits and root bark, the procedures adopted were from the Methods of Analysis of the Association of Official Agricultural Chemists (A. O. A. C.), fifth edition, and the United States Pharmacopœia (U. S. P.), eleventh revision. All determinations were made in duplicate, the results averaged and reported in percentage of air-dried material.

Moisture.—Two methods were used to determine the moisture; in the first, the ground material was dried at 135° C. for two hours in an electric oven; the other procedure was the official toluene method as described in the U. S. P. The following results, expressed in percentage, were obtained by the two procedures.

	Bark	Fruit
Electric oven method	12.36	6.70
Toluene method	9.88	5.49

Selective Extraction.—Samples of the dried bark and fruit were extracted in a Soxhlet apparatus by the selective extraction method. The percolates were evaporated spontaneously and dried to constant weight in a desiccator. The volatile and non-volatile extracts were also determined and the results expressed, in per cent, as total, non-volatile, and volatile extract.

Selective Extraction of Bark

	Total Extract	Non-Volatile Extract	Volatile Extract
Petroleum ether	1.16	1.02	0.14
Ether	5.84	5.62	0.22
Chloroform	3.78	3.53	0.25
Ethyl acetate	9.02	8.11	0.91
Alcohol	10.14	8.56	1.58
Water	10.93	..	..

Selective Extraction of Fruit

	Total Extract	Non-Volatile Extract	Volatile Extract
Petroleum ether	21.80	21.66	0.14
Ether	2.59	1.80	0.79
Chloroform	0.61	0.48	0.13
Ethyl acetate	8.29	7.47	0.82
Alcohol	3.77	3.33	0.44
Water	8.04	..	..

Ash.—The total ash, water insoluble ash and acid insoluble ash were determined on the ground bark and fruit by methods described in the A. O. A. C. The results obtained, expressed as per cent, were as follows:

	Bark	Fruit
Total ash	9.15	3.23
Water insoluble ash	5.75	2.01
Acid insoluble ash	0.43	0.43

Reducing Sugars.—The quantitative method for determining reducing sugars listed under plant analysis in the A. O. A. C. was used. The weighed samples were neutralized with calcium carbonate and extracted with 80 per cent alcohol. After evaporating the alcohol, the residue was taken up with water and decolorized with lead acetate solution. The excess lead was removed and the reducing sugars were determined in a 50-cc. aliquot using the Munson-Walker method. Calculated in per cent of invert sugar the results were: bark, 6.56; and fruit, 3.75.

Sucrose.—The sucrose of the fruit was determined using the hydrochloric acid inversion method of the A. O. A. C., while the sucrose of the bark was obtained by the official method employing the use of the enzyme invertase.

Results of the sucrose determinations, in per cent, were: bark, 1.50; and fruit, 2.97.

Starch.—The A. O. A. C. quantitative method for the determination of starch was used. The residue remaining after removal of the sugars by hot alcohol was placed in water, warmed to gelatinize the starch and then hydrolyzed with Taka-diastase. After repeated treatment with Taka-diastase, the solution was filtered and further hydrolyzed with hydrochloric acid. The dextrose, present in an aliquot of the hydrolyzed solution, was determined by the Munson-Walker method. The amount of starch present was calculated and found to be 3.97 per cent in the bark and 3.97 per cent in the fruit.

Pentosans.—Using the A. O. A. C. procedure, samples of the bark and fruit were treated, as directed, with 12 per cent hydrochloric acid. Upon complete distillation, the distillate was treated with phloroglucin and from the weight of furfural-phloroglucide the amount of pentosans was calculated. The results, in per cent, were: bark, 10.56; and fruit, 16.55.

Crude Fiber.—This determination was made using the A. O. A. C. procedure which consisted of removing the ether-soluble constituents from weighed portions, then subsequently treating the residue with 1.25 per cent sulfuric acid, followed by digesting with the same strength sodium hydroxide. The loss in weight upon ignition of the acid and alkali treated residue was calculated as crude fiber. The results, in per cent, were as follows: bark, 14.24; and fruit, 26.39.

Protein.—The nitrogenous matter was determined upon the bark and fruit by the Kjeldahl-Gunning-Arnold method. The results were expressed as per cent of nitrogen and per cent of protein.

	Bark	Fruit
Nitrogen	1.66	1.03
Protein	10.36	6.44

Fixed Oil.—A fixed oil was obtained from the fruit by extracting with petroleum ether, using a Soxhlet apparatus. The oil was purified by refluxing an alcoholic solution with purified animal charcoal. After filtering, the solution was allowed to evaporate spontaneously until the odor of alcohol could no longer be detected. The following constants were determined using procedures as described in the A. O. A. C.:

Saponification number	191.45
Acid value	7.09
Ester number	184.36
Iodine number (Hanus method)	90.48
Specific gravity, 20° C.	0.9150
Index of refraction, 20° C.	1.5698

Cornin.—The glucoside, cornin, was extracted using the following procedure. The bark was repeatedly extracted with hot water until the aqueous extract was nearly colorless. The combined extracts were treated with lead acetate, evaporated to a pilular consistency under reduced pressure and extracted with acetone. The acetone was removed by distillation and spontaneous evaporation until crystals of cornin were obtained. These crystals, when recrystallized from acetone, melted at 179° C. The crystals were acetylized using the method of Reichert and Hoffman (5). One part of cornin, 10 parts of cold pyridine and 2 parts of acetic anhydride were mixed and allowed to stand in the cold for 18 hours. Then, while cooling, 75 parts of a 5 per cent solution of sulfuric acid were added. The acetylized product was washed with water, recrystallized from alcohol, and its melting point found to be 134° C.

Further evidence that this product was cornin are the facts that it was found to have an optical rotation of −180.4°, and that it reduced Fehling's solution and ammoniacal silver nitrate solution. Miller (3) reported comparable results for cornin isolated from the root bark of *Cornus florida.* The bark of *Cornus Nuttallii* was found to contain about one per cent of cornin.

Scyllitol.—Scyllitol, a polyhydroxy alcohol, was isolated from the bark by the following procedure. The drug was first macerated with water, then a sufficient quantity of sodium carbonate solution was added to neutralize any free acid present. The bark was then percolated with water until completely exhausted. The percolate was treated with a solution of lead acetate, evaporated under reduced pressure, and extracted with 95 per cent alcohol. The alcoholic solution was partly concentrated and then set aside to allow the scyllitol to crystallize. After several recrystallizations from alcohol, a product, amounting to 0.41 per cent, was obtained that melted at 319° C. Scyllitol was further identified by its acetyl derivative, melting point 291° C. The melting point of scyllitol and the acetyl derivative agree in both cases with those reported by Hann and Sando (6) from *Cornus florida.*

Alkaloids.—In testing for the presence of alkaloids the bark and fruit were extracted separately with one per cent tartaric acid solution. The extracts of each were concentrated, made ammoniacal and extracted with a mixture of one part of chloroform and three parts of ether. After shaking out the organic solvent mixture with one per cent sulfuric acid, the acidic solution was tested with various alkaloidal reagents and in each case negative results were obtained.

SUMMARY

The root bark and fruit of *Cornus Nuttallii* were submitted to proximate analysis. Per cents of moisture, ash, sugars, starch, pentosans, crude fiber and protein are reported.

In the study of the root bark, about one per cent of a glucoside was isolated, purified and identified as cornin by some of its physical properties and the melting point of the acetyl derivative.

The root bark yielded 0.41 per cent of the polyhydroxy alcohol, scyllitol, which was confirmed by the melting point of the alcohol and its acetyl derivative. Neither the fruit nor the bark gave any indication of positive results when examined for the presence of alkaloids.

A fixed oil was separated from the fruit and several of the constants were determined.

REFERENCES

(1) Audubon, J. J., "The Birds of America," The Macmillan Company, New York, N. Y., 1937, p. 367.

(2) Torrey, J., and Gray, A., "Flora of North America," Vol. I, John Wiley & Sons, Inc., New York, N. Y., 1838–40, p. 652.

(3) Miller, E. R., JOUR. A. PH. A., 17 (1928), 744–750.

(4) Ellis, C., *Am. J. Pharm.*, 7 (1835), 265.

(5) Reichert, B., and Hoffman, W., *Arch. Pharm.*, 275 (1937), 474.

(6) Hann, R. M., and Sando, C. E., *J. Biol. Chem.*, 66 (1926), 399–402.