peppermint approaching the *M. viridis* L. parentage and the English peppermint approaching the *M. aquatica* L. parentage.

The American cultivated mint for the distillation of the oil approaches the English type.

The hybrid nature of *M. piperita* L. has been determined by a comparison of morphological characteristics, and not by experimental hybridization. In order to establish the origin of the American peppermint plant it will be necessary to make experimental crosses of the parent plants and produce sufficient oil for phytochemical study.

OENANTHE SARMENTOSA.

BY F. J. GOODRICH AND E. V. LYNN.

The poisonous character of other members of the genus *Oenanthe* has elicited unestablished local statements that the Pacific variety, *sarmentosa,* shows this toxic property. Just how such rumors started and from where they emanated is unknown, but cattle raisers in Washington at least appear sure in their own minds that many fatalities among cattle are due to the plant. Cattle, grazing in the vicinity of the habitat of *Oenanthe sarmentosa,* have been observed eating the tops of the plant growing in and under water, and with no apparent ill effects. This has led to the supposition that a poisonous principle might be present only in the rhizome and roots. Its similarity to wild parsnip may in part account for this view, but the greater share is undoubtedly to be traced to reports regarding the toxic nature of some of the other *Oenanthe.*

Authentic investigations of *Oenanthe sarmentosa* seem to be almost entirely lacking. There are several printed statements to the effect that it is toxic, but no one seems to have taken the trouble to investigate the plant scientifically. As far as could be learned, there has also been no chemical examination of any kind on this particular species. This is rather remarkable because at least one other member of the genus has been quite thoroughly studied. An extensive search of the literature has shown that all of the work on *sarmentosa* has been strictly of a botanical nature, and this has not been of great amount.

It seemed of particular importance, therefore, to subject the plant to minute investigation. Because of its close relationship to other members of the same family and genus, it would appear of great interest to study its distinguishing characteristics. The chemical composition, too, might throw some light upon these and upon the reputed toxicity. With a view to ascertaining whether the plant is poisonous or not, it is also desirable to carry out experiments on animals, using freshly gathered materials and extracts prepared from them.

Another interesting problem in this connection is the question of submergence and its effect upon the botanical structure. It is found growing in, under and out of water. Since some members of this genus apparently are not capable of growing entirely immersed, the possible differences in morphology under such circumstances should undoubtedly be determined. This can only be accomplished by a careful study of the amphibious nature of the plant under different growing conditions.

We have attempted, therefore, (*a*) to study the effects of submergence, (*b*) to determine some of the components and their properties, and (*c*) to settle the question of toxicity.

OTHER SPECIES.

Eight species of *Oenanthe* besides *sarmentosa* are known. Of these eight (*crocata, Phellandrium, fistulosa, filiformus, Lachenallii, tirefolia, Californicum, pimpenoides*), the first three are known to be of a toxic nature. It is apparently not known whether the other five are poisonous or not. Seven are native of either Europe or Asia, or both, and one of them is said to be found native in the United States.

Oenanthe crocata has been mentioned in the literature repeatedly and has been examined chemically a number of times (3, 7, 15, 19). The poisonous character resides chiefly in the rhizomes, which apparently can cause death more quickly than aconite. The principal symptom is similar to the convulsion of picrotoxin, with death finally resulting from general exhaustion and failure of the respiration. The toxic principle, which has been extracted and analyzed, was named oenanthetoxin and given the formula $C_{17}H_{22}O_5$ or $C_{33}H_{44}O_{10}$. Since it is a dark brown, unstable resin like cicutoxin from *Cicuta virosa*, the analysis may be considered as doubtful. The rhizomes also contain 0.1 per cent of volatile oil which has apparently never been examined carefully.

Two other species have been investigated, but only to a small extent. *Oenanthe fistulosa* is known to contain (2) a poisonous substance which is presumably analogous to oenanthetoxin, and which has been named oenanthine. *Oenanthe Phellandrium* is described (21) as bearing both submerged and aerial leaves, the former sometimes having what appears to be aborted stomates.

HABITAT AND DESCRIPTION.

As far as is known to the writers, *Oenanthe sarmentosa* is exclusively a native of the American Pacific coast and not found in any other section of the country. It is very common in Northern California, Western Washington and Oregon, somewhat less in British Columbia and in the Cascade Range, possibly to a limited extent east of the mountains.

The plant is commonly known as water celery or water fennel. Its natural habitat is in low, swampy areas, often flooded in winter and spring, and it is occasionally submerged throughout the growing season. As would be expected, it is usually found growing profusely along the banks of streams and ditches. In spite of the fact that plants are frequently found entirely under water, rapid growth generally extends some of the leaves above the surface.

Oenanthe sarmentosa is perennial, glabrous and mostly aquatic in nature, mature specimens being from two to five feet high. The leaves, the largest of which are at the base and twice branched, are ternate and two-pinnate. The leaflets, which are from 1 to $2^1/_2$ centimeters long, are ovate, accuminate, toothed and often lobed at the base. The stem is smooth, green and hollow, except at the nodes or joints where the leaves are attached. The hollow is formed by the breaking down of the pith, and very young stems may often be solid. The flower clusters are broad and flat and resemble those of many umbellacious plants. The white flowers are in umbels, which are many rayed, have involucres, and prominent calyx lobes. The fruit is globose, glabrous, four millimeters long and slightly flattened laterally. The ribs are obtuse, wide, corky and the lateral ones the largest. The stylopodium is short and conical. There are two oil tubes on the commissure side of the fruit. The lower underground portion of the stem is thickened and fleshy with a series of cross partitions, which in case of older plants divides the interior into hollow chambers. Most of the rhizomes are composed of pith between these partitions, showing that the pith is not here broken down as in the stem. Attached to this thickly tuberous rhizome are numerous roots and root-hairs. Many of the plants have but very little rhizome and the smaller roots constitute the largest part of the underground system. The roots are often several inches in length (22).

GROWTH UNDER WATER.

Many amphibious plants have quite a different anatomical structure, depending upon whether they grow under or out of water. It was, therefore, desirable to study the effects of submergence at different depths and periods and under varying conditions. Investigations were made by growing this species at different levels in water, by excluding varying amounts of light from plants growing under water, by observing the character of new leaves as they developed under the surface, and by comparing the structure of these new leaves with such as had grown out of water. Attention was centered particularly on the structural alterations in the leaves developed under these varying conditions, without any attempt to follow the complete morphological changes.

The number of stomates and size of leaves were found to differ under these two conditions. The rhizomes of healthy, vigorously growing plants, previously freed from leaves, were transferred to an artificial pond whose water-level was raised or lowered, so that at all times it was approximately three inches above the top leaves. In order to obviate influences due to previous removal of old growth, control plants were treated in a similar manner, but not submerged. The leaves growing under water were found to be uniformly wider and to possess a smaller number of stomates, the ratio being about 8:3. In almost every case, however, no matter what the amount of submergence or other conditions of the leaf, the presence of stomates was generally noted.

The comparative growth of seedlings showed a similar condition. During a three-month period from February to May, the plants attained a maximum height of four inches, from June to August one of fifteen inches. In every case those specimens grown nearest to the surface exhibited the most rapid development. Plants retained at a depth of six inches showed distinct progress during March and April and still more pronounced growth during the mid-summer months.

Progress was even evident for plants submerged nine and twelve inches under water, and here again this was greater during the summer period. Below eighteen inches in depth there appeared to be practically no development in either season and disintegration of the plant soon followed.

As expected, the amount of light available to the submerged plant was found to have an important influence upon the growth. The quantity was varied by regulating the size of openings in a metal covering over the pond. Experiments were conducted during the early spring and mid-summer months on plants just under the surface. With the maximum exclusion of light, no apparent development occurred, all parts turned yellow and eventually the leaves dropped. By careful experimentation, it was found that at least 15–20 per cent of the maximum light was necessary for the plant to retain its green color during the growing season. The number and character of the stomates was not appreciably altered by the decrease in the amount of light, provided the green color was retained, but the rate of growth was distinctly diminished.

Exclusion of air from the leaf was also found fatal to the plant. All of the leaves of several individual plants were covered with a thin layer of petrolatum. Within a short time it was noted that development had ceased, the leaves turned yellow and eventually dropped. When the petrolatum was placed on a single leaf, the latter only was affected without influence on other leaves of the plant. It is, therefore, evident that sarmentosa leaves are adapted to carry on metabolic processes in air and under water, and that water itself does not inhibit them, but that closing the stomates by a foreign material is fatal.

HISTOLOGICAL.

As far as could be learned, no histological study has hitherto been made of *Oenanthe sarmentosa.* The microscopical examination of cross-sections from different parts of the plant showed that the structure corresponds to the usual one of a dicotyledon. The sections were made from plants grown above water in soil saturated with moisture, and collected in June 1926.

CHEMICAL EXAMINATION.

The plants were collected during 1925 and 1926 in various localities where conditions were at times quite different. Several hundred pounds of material was obtained at a time when none of the plants showed any indication of flowering, the whole portion above ground being selected. Other collections were made in May, June and July 1925, at which period fully developed flowers and immature fruits were present; the whole plant, exclusive of rhizomes and roots, was again taken. In autumn of the same year mature fruits were gathered separately. At different times throughout the year rhizomes and roots were gathered at various points along the borders of Lake Washington. Each one of these lots was examined separately.

Loss on Drying.—The fresh samples were weighed and allowed to attain constant wieght in the air, the loss being determined. Other portions were dried in an oven at 105° C. The results follow:

	Month of collection.	Loss air-drying. I.	II.	Loss at 105° C. I.	II.
Leaves and Stems	May	...	...	82.67	82.63
	June	79.15	79.36	...	...
Leaves (submerged)	February	...	...	88.08	87.79
	May	...	...	88.36	85.49
Rhizomes and Roots	February	...	...	79.84	77.33
	March	...	...	77.97	...
	August	75.20	...	81.10	...
	November	74.60	...	...	...
Fruit	September	71.55	...	...	...

Extraction.—A convenient quantity of the powdered stems and leaves, and a somewhat larger amount of powdered rhizomes and roots, were extracted separately and successively with various solvents in a Soxhlet apparatus. The solvents were then removed by evaporation at 100° C. The following table gives the results in percentage based upon the dried samples.

	Stems and leaves. I.	II.	Rhizomes and roots. I.	II.
Petroleum ether	1.80	1.56	2.96	2.84
Ether	3.99	3.96	2.62	2.74
Chloroform	0.89	0.84	0.62	0.61
Alcohol	19.81	23.69	29.36	28.97

Alkaloids.—The dried and ground sample (50 Gm.) was exhausted with alcohol containing a small amount of tartaric acid, the alcohol was evaporated, and the residue was partially dissolved in diluted acid. The resulting liquid was subjected to the usual tests for an alkaloid, using Wagner's solution, Mayer's reagent, tannic acid and picric acid. The leaves and stems growing under water, the leaves and stems growing above water, the flowers, the mature fruits and the rhizomes and roots were each tested separately and in all cases found to give no precipitates.

Glucosides.—The dried and ground sample (100 Gm.) was exhausted with boiling water, precipitated with lead acetate solution in the usual way, and the filtrate freed from lead with hydrogen sulphide. The solution was then neutralized and concentrated to a small volume. In other experiments the sample was extracted with boiling alcohol, the solvent evaporated, the residue taken up with water and the above process repeated. None of the portions gave material amounts of separated material, except in the case of the rhizome and roots, which gave a slight crystalline deposit. The latter did not reduce Fehling's solution, but after hydrolysis with dilute acids there was a strong reduction, which would indicate glucosidal nature. The amount was too small to examine further.

Pectins.—The presence of pectins was suspected because, at several times during the examination of the rhizome and roots, gelatinization had occurred. This was confirmed and the amount determined as follows: About 15 Gm. of the dried and ground sample was completely extracted by percolation with hot water, and the resulting liquid evaporated to 25 cc. The crude pectin was precipitated by adding 225 cc. of alcohol and the whole was filtered. The precipitate was dissolved in hot water and the solution evaporated to 40 cc., a few drops of dilute hydrochloric acid being added to dissolve a slight amount of turbidity. To the cooled solution there was added sufficient sodium hydroxide solution to hydrolyze the pectin, and the volume was then made up to 50 cc. After standing for thirty minutes, 40 cc. of water and 10 cc. of dilute hydrochloric acid were added and the solution boiled for five minutes. The precipitated pectic acid was dissolved in alkali and reprecipitated with hydrochloric acid, filtered, washed free from acid, dried and weighed. The yield was 2.36 per cent of the dried rhizomes and roots.

The occurrence of pectins in this plant would seem to offer good commercial possibilities. There is an ever-growing demand in this country for concentrated pectins to be used by the manufacturer and housewife in the making of jellies, jams and other fruit preserves. The apples, used as raw materials, contain about one per cent, which can be obtained from cider residues. Other materials from the vegetable kingdom contain as much or more pectin, but in the majority of cases this is not of the gelatinizing type. Present experiments indicate that the pectins from *Oenanthe sarmentosa* are exceptionally suitable, and the writers are engaged upon further tests to confirm this.

Other Carbohydrates.—The total reducing sugars were extracted from 5 Gm. of the dried rhizomes and roots by digestion with boiling water and the resulting solution was mixed with an equal volume of alcohol and allowed to stand over night. After filtering and washing, the whole was treated with lead acetate solution and made up to volume. The reducing sugars, as determined in the filtrate by the Munson-Walker method, amounted to 3.14 per cent of the dried rhizomes and roots.

Gums or dextrins other than pectins are apparently present in quantity in the rhizomes and roots. This is indicated by the large amount of precipitate by means of alcohol from the aqueous extract. There was also a voluminous precipitate with lead acetate. Sucrose, too, is present in the same material, because during the alcohol extraction crystals, which had deposited on the sides of the cold vessel, were identified as this substance.

The amount of starch in the rhizomes and roots varies considerably according to the time of collection. Fresh material gathered in summer and early fall gave excellent tests with iodine, but other samples at various times gave negative or conflicting results. Quantitative measurements were never satisfactory unless carried out on identical samples, figures being obtained on various samples from 0.5 to 3.0 per cent. The physiological significance of the change in starch content from month to month would be interesting to trace.

Resins.—The resins were extracted with hot alcohol from 4 Kg. of the dried rhizomes and roots. The alcohol was removed by evaporation and the residue was distilled with steam to remove the volatile material. From the aqueous portion a small amount of tar-like material was separated by extraction with ether, this was not further examined. Selective extraction of the main body or resin (105 Gm.) gave the following results:

Petroleum ether	52.5 per cent	Greenish brown
Ether	26.5 per cent	Brown
Chloroform	10.4 per cent	Brown
Ethyl acetate	0.6 per cent	Black
Acetone	0.7 per cent	Black
Carbon tetrachloride	1.8 per cent	Black
Alcohol	2.9 per cent	Black

VOLATILE OILS.

All parts of the plant were found to contain essential oils and those from the leaves and stems, from the rhizomes and roots, and from the fruit were examined. These were prepared by distilling the material with steam, after a preliminary maceration with water for twenty-four hours. The aqueous distillate was extracted with ether, the ether solution was dried, and the ether removed by evaporation in the usual way.

Oil from the Leaves and Stems.—This was of a light yellow color when first distilled, but it darkened perceptibly after standing a few days, the final color being reddish brown. The odor was distinctly aromatic and pleasing, resembling somewhat that of dill oil. From 1045 pounds of the fresh material, corresponding to about 216 pounds dried, there was obtained 41 cc. (37.7 Gm.), a yield of 0.038 per cent, calculated on the dry basis. The physical constants were as follows; specific gravity at 15.5° C., 0.9188; $[\alpha]_D$ at 20° C., + 7.78°; n_D at 20° C., 1.4918;

congealing point —12° C. Distillation of 20 cc. of the oil showed that it is principally composed of terpenes:

161–165°	1.0 cc.	170–175°	5.0 cc.	190–210°	1.0 cc.
165–170°	6.5 cc.	179–190	4.0 cc.	Residue	2.5 cc.

The third and fourth fractions were tested for phellandrene, with negative result.

Upon standing, the oil deposited small yellow crystals which were identified as almost pure sulphur, melting point 113° C. The deposition of sulphur would indicate the presence of some of its compounds in the oil. The total amount of sulphur in the oil was determined by heating with carbonate and nitrate and by subsequent conversion to barium sulphate. The result showed 1.28 per cent., corresponding to approximately 3 per cent of sulphur compounds calculated as diallyl sulphide.

Reference to the literature shows that very few volatile oils contain sulphur compounds. Most of these are obtained from the Liliaceae, the Cruciferae, or related families. Oil of peppermint was found by Power to contain dimethyl sulphide, and oil of asafetida is known to be principally composed of several compounds whose structure has not been determined. Volatile oils from other families may also contain the element sulphur, but the occurrence in the labiates (peppermint) and in the umbellates (asafetida) is distinctly exceptional.

Since the oil gave an aldehyde reaction, it was examined for furfural (12) which very often is associated with sulphides. The oil was dissolved in the proportion of 0.5 cc. to 50 cc. of aldehyde-free alcohol ad transferred to a colorimeter tube, kept at a temperature of 15° C. Colorless aniline (2 cc.) and 0.5 cc. of hydrochloric acid (specific gravity 1.125) were added and the whole mixed thoroughly. After thirty minutes, a distinct red color indicated the presence of furfural in quantity.

Oil from the Rhizomes and Roots.—This was of a light color when first distilled, but after standing a few days became brownish yellow. Its odor was much less pleasing than that from the stems and leaves. From 135 pounds of the fresh material, corresponding to 33.4 pounds dried, there was obtained 13.8 cc. (12.8 Gm.), a yield of 0.1 per cent on the dry basis. The physical constants were as follows: specific gravity at 15.5° C., 0.9304; $[\alpha]_D$ at 20° C., + 7.52°; n_D at 20° C., 1.4898. Distillation of 12.5 cc. of the oil indicated that it contains a fairly large percentage of oxygenated compounds:

171–175°	1.0 cc.	185–195	3.0 cc.	Residue	2.0 cc.
175–185	2.5 cc.	195–220	4.0 cc.		

The fractions boiling below 185° C. were tested for phellandrene by a method given below. A trace of crystalline solid was formed in the test, but the amount was so small that it was impossible to identify it as phellandrene nitrite. As with the oil from the leaves and stems, there was deposited upon standing a small amount of sulphur, and the quantity in the oil was found to be less than 0.1 per cent. This oil was also found to contain furfural.

Oil from the Fruit.—This was of a light yellow color, darkening upon a few days standing to a brownish yellow. The odor was distinctly aromatic, resembling eucalyptus oil somewhat, and quite as pleasing as that from the leaves and stems. From 43.8 pounds of the fresh material, corresponding to 12.5 pounds dried, was obtained 21.7 cc. (19.7 Gm.), a yield of 0.35 per cent on the dried basis. The physical constants were as follows: specific gravity at 15.5° C., 0.9032; $[\alpha]_D$ + 7.52 at 20° C.; n_D at 20° C., 1.4942; congealing point —10.5° C. Distillation of 15 cc. of the oil indicated mostly terpenes:

161–170°	0.5 cc.	174–185	4.0 cc.	Residue	1.5 cc.
170–174°	8.0 cc.	185–200	1.0 cc.		

Upon standing the oil deposited sulphur, just as that from the other oils. Analysis of the whole oil showed that it contained 0.91 per cent, which corresponds to 2.1 per cent of sulphur compounds calculated as diallyl sulphide. Tests for furfural were entirely negative, which is very surprising.

The fraction boiling between 170° and 174° was found to contain a considerable quantity of the hydrocarbon, phellandrene, identified by conversion to the nitrite. A solution containing 5 Gm. of sodium nitrite in 8 cc. of water was layered beneath a petroleum ether solution of the fraction. Glacial acetic acid (5 cc.) was added with shaking to bring the evolved nitrous ether into intimate contact with the ethereal solution. At the same time the temperature was kept down by means of an ice-bath. The precipitate was filtered off, washed with water and then methyl alcohol, and recrystallized several times from chloroform. The melting point was found to be 103° C. Since α-phellandrene forms dextro and levo nitrites melting at 112–3° C. and 105° C., respectively, since the corresponding nitrites from β-phellandrene melt at 102° C. and 97–98° C., respectively, it is reasonable to conclude that the fruit oil of *Oenanthe sarmentosa* contains α-phellandrene.

TOXICOLOGICAL.

As has already been mentioned, there have been various unconfirmed rumors that *Oenanthe sarmentosa* is poisonous to animals. Regardless of the fact that no proven fatalities are on record, there is a distinct avoidance of it by cattle growers in the northwest. One of the chief reasons for this is the great similarity to wild parsnip, genus *Cicuta*. Because this question has never been investigated scientifically, experiments were here made to determine what grounds there are for this belief in toxicity.

Guinea-pigs and white rats were fed with the whole plant and with portions and extracts. They did not eat the leaves and stems nor the rhizomes with any great relish, but ingested enough to show absolutely that the material is not toxic. Various parts of the plant were then extracted separately with various solvents—petroleum ether, ether, chloroform, ethyl, acetate, acetone, carbon disulphide, alcohol and water—and, after removal of the solvent by evaporation, the extracts were forcibly fed to guinea-pigs and white rats. The doses employed varied from 0.2 Gm. to 0.5 Gm. In no case was there any sign of a convulsive effect or, indeed, of poisoning in any degree. On one or two occasions the animals lost some appetite and were not quite as active, but this was only temporary.

SUMMARY.

1. The leaves of *Oenanthe sarmentosa*, growing submerged, possess stomates and develop in inverse proportion to the distance from the surface; below 18 inches

there is no growth. At 2 to 3 inches the plant requires at least 15 per cent of maximum light. The plant does not survive when the leaves are covered with petrolatum.

2. Considerable quantities of pectin are found in the rhizome and this may be of commercial importance. Starch is present to a variable extent, reducing sugars to the amount of 3.14 per cent. Gums or dextrins and resins were found in large quantities, and sucrose also occurs to some extent.

3. Alkaloids were not found, but there may be small amounts of glucosides.

4. All parts of the plant contain volatile oils, the chief characteristic of which is the content of sulphur, that from the stem and leaf containing the most. α-Phelandrene is evidently a component of the fruit oil, while furfural is contained in that of the leaf and that of the rhizomes. The physical constants of each oil are given.

5. The loss on drying was determined for the fruit, the leaves and stems, and for the rhizomes and roots. Figures were also obtained for the amount of extracts by selective extraction.

6. Evidence is presented to show that there is no basis for the present widespread assumption that the plant is poisonous to animals.

BIBLIOGRAPHY.

(1) Comerais and Dufeillay, *Jour. Chim. Med.* (1830), 459.
(2) Gerding, *Am. J. Pharm.*, 21, 68; *J. prakt. Chem.* (1848), 44, 175.
(3) Christison, *Ibid.*, 34 (1862), 273.
(4) Vincent, *Jour. de Pharm.*, 46 (1864), 140.
(5) Jones, Brit. "Yearbook of Pharm." (1885), 436.
(6) Poehl, *Chemist and Druggist* (Feb. 9, 1895).
(7) Poehl, *Arch. exptl. Path. Pharmacol.*, 34 (1896), 258.
(8) Haensel, *Pharm. Ztg.*, 43 (1898), 760.
(9) Holmes, *Pharm. J.*, 4 (1902), 14, 431.
(10) Holmes, *Ibid.*, 4 (1904), 20, 585, 690 and 788.
(11) Kondakkow and Schindelmeister, *J. prakt. Chem.* (2), (1905), 72, 193.
(12) Tolman, *Jour. Amer. Chem. Soc.*, 29 (1906), 1629.
(13) Kondakkow, *J. prakt. Chem.* (2), (1908), 78, 42.
(14) Holmes, *Pharm. J.*, 4 (1911) 32, 430.
(15) Tutin, *Ibid.*, 4 (1912), 33, 296.
(16) Thompson, *Chemist and Druggist*, 93 (1920), 966.
(17) Checkley, *Pharm. J.*, 104 (1920), 44.
(18) Styger, *Schweiz. Apoth. Ztg.* (1920), 57, 183 and 188.
(19) Moffet, *Irish Naturalist*, 29, 13; *Bot. Abs.*, 5 (1920), 2132.
(20) Nicholson, *Jour. of Botany*, 58 (1920), 201.
(21) Arber, "Water Plants" (1920), 166, 229, 312.
(22) Frye and Rigg, "Flora of the Northwest" (1914), 280.

COLLEGE OF PHARMACY,
UNIVERSITY OF WASHINGTON,
SEATTLE, WASH.

RULING ON DELETED OFFICIAL PREPARATIONS.

Prohibition Commissioner Doran has suspended for one year the requirement of the regulations that manufacturers of preparations deleted from the latest editions of the U. S. P. and N. F. must secure special permits to manufacture such preparations and must apply for same on Form 1404 supplemental.