PHYTOCHEMICAL STUDIES OF SEVERAL OREGON PLANTS I.*

BY H. M. BURLAGE.

ARTEMISIA HETEROPHYLLA, NUTT., COMPOSITÆ (*A. suksdorfii*, PIPER).

A mild climate and an abundance of rainfall for the greater portion of the year present ideal conditions for plant growth in northern California and the Pacific Northwest. The vegetation, therefore, includes a wide variety of plants, many of which, no doubt, have a medicinal value and have never been studied. With this idea in mind a systematic study of some of these plants, particularly those which showed evidences of a volatile oil as one of the important and conspicuous constituents has been undertaken. This report is a preliminary one on investigations which were started in July 1927.

An examination of literature reveals that approximately three hundred and eighty species of Artemisia have been identified (1). Forty-six species have been subjected to a chemical study. Only a few of these, however, have been examined with any degree of completeness and such studies have been confined chiefly to an examination of the volatile oil if present in sufficient quantity and to detect the presence of santonin, an expensive and valuable medicinal agent, which has been found in a few species. Twenty species are listed in a text on Northwest Flora (2), six of which have been examined to various degrees and reported in literature. A complete bibliography of these studies will be included in a later and more complete report.

A. heterophylla, Nutt. is a perennial plant, 90–150 cm. high, having, when crushed, a penetrating camphoraceous odor and a bitter taste. The leaves are alternate, numerous, oblong-lanceolate, acute, entire to cleft or lobed, 6–20 cm. long, 3–8 cm. wide, of bright green color above, and white-tomentose beneath. The heads are numerous, arranged in pyramidal panicles, which are dense and 15–45 cm. long, small (3–4 mm. high), and many-flowered. The flowers are pale yellow or yellowish and tubular; the disc flowers are perfect and fertile; the marginal flowers are pistillate and fertile. The involucre is pale green, glabrous to pubescent and cylindric; the tegules are obtuse and hyaline-margined. The flowers when observed hastily remind one of the Golden-rod, hence the plant has been given the common name "Golden-rod Sage."

The herb grows profusely throughout the western portion of the Pacific Northwest along roadways, the seashore, fence rows and more abundantly in regions that are apt to be overflooded during the rainy months. An abundant supply of moisture seems necessary for vigorous growth since it has been found that plants growing on hillsides and banks where sub-irrigation is not adequate were found to be low and decidedly woody.

As stated above the plant is often taken for a Golden-rod but when examined more closely the foliage is found to be quite different and to possess a characteristic odor. Examination of literature indicates that this species has evidently not been examined. Since the plant grows so abundantly in this region a chemical study to ascertain the possibilities of the herb for commercial use was thought to be advisable.

Since, in general, there is a great analogy between the species of the same

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genus, both morphologically and chemically, the results which have been found in studies on other species have been carefully examined.

Without doubt, the volatile oil is an important ingredient and this report will include a preliminary investigation of the plant and its volatile oil.

The material used in the present investigation was collected chiefly in Linn county of the Willamette valley near Corvallis, Oregon, during 1927–1928. In order to determine, if possible, the proper time of collection for maximum oil content or for other valuable properties, the plant was harvested during several months, each lot being treated separately.

Samples of the fresh material were dried to constant weight at room temperature and then at 105° C. The total ash and acid-insoluble ash were determined. Small portions of the air-dried drug were subjected to successive extractions with petroleum ether, ether, alcohol, and water and the residues carried to constant weight at room temperature and then at 105° C. The results obtained were calculated on a completely dry basis. The results follow:

TABLE I.

	Leaves.			Tops.	
Total ash	7.73%			8.12%	
Acid-insoluble ash	1.84			2.26	
Loss on air-drying	61.24			65.12	
Loss at 105° C.	4.98			5.15	
Total volatile	66.22			70.27	
Petroleum ether extract total	3.72	3.40	.....	2.06	2.01
Non-vol. at 105° C.	3.20	2.88	...	1.52	1.35
Ether extract total	4.41	4.06	...	2.34	2.39
Non-vol. at 105° C.	3.01	2.86	...	1.87	1.63
Alcohol extract total	3.20	5.34	...	2.72	2.35
Non-vol. at 105° C.	2.60	3.66	...	2.24	1.90
Water extract at 105° C.	14.33	17.19	...	12.34	11.14

THE VOLATILE OIL.

The leaves and tops were harvested by stripping from the stalk of the plant. Each lot was subjected to steam distillation until no more oil was obtained. The latter was separated by means of an automatic separator, the watery liquid was cohobated, and the final traces of oil were removed by ether extraction. The collected oil from each lot was dried over anhydrous sodium sulphate and measured in a graduated cylinder. The physical constants were determined. The results are shown in the following table:

TABLE II.

Collection.	Yield.	Color.	d_{20}.	$(\alpha)_{D20}$.	n_{D20}.
Sept. 1927	0.86%	Olive to golden	0.9478	+23.48°	1.4196
October*	0.88	Golden to straw	0.9492	+32.10°	1.4944
November†	0.77	Straw	0.9187	+28.74°	1.4896
December	0.69	Straw	0.9546	+26.78°	1.4880
July 1928	1.10	Blue	0.9639	+39.95°	1.4824
August*	1.72	Green-yellow	0.9530	+13.08°	1.4852
September†	1.02	Blue-green	0.9601	− 0.91°	1.4902

(Solubility 1 part in 1.1 parts of 80% alcohol (by vol.).)

* Full flowering. † First killing frost.

Seasonal observations do not show sufficient regularities to draw any definite conclusions as to the proper time to gather the herb in order to obtain maximal yields of oil. It would seem, however, that the proper time is at full flowering. The summer of 1927 according to the laity was not a normal growing season for Oregon; the late spring was followed by a correspondingly late fall and late flowering season. The summer of 1928 was quite normal and it will be noted that the time of flowering was much earlier and the yield of oil much higher. A sharp decline in the yield of oil is noted after the first killing frosts, indicating consumption of the oil in catabolic processes or to frost effects on cell structure with a subsequent evaporation of the volatile oil.

In most cases the oil showed the presence of a blue constituent which was not so evident in samples procured from materials affected by frost. Later work showed the blue substance to be affected by acid; this might indicate the presence of a plant indicator, which was highly soluble in the oil. Fractionations showed the blue constituent to be present in the higher boiling fractions, indicating a possible presence of azulene, a blue-colored constituent often present in volatile oils. The blue color of azulene, however, is quite permanent; the blue fractions obtained soon lost this color, indicating some substance that is readily oxidized. It is hoped that subsequent experiments will indicate what this substance might be. In general, it is noted that there is a maximum value for all physical properties with a decline (?) after the first killing frost, indicating a sharp change in chemical composition of the oil.

In order to gain some idea of the components of the oil, each lot was examined for total esters, free acids, aldehydes and ketones, alcohols and phenols. The acid value and the saponification value were determined by the Pharmacopœial processes (3) and the ester number found by subtraction. The acetylization was made according to Semmler (4) and the value calculated by subtracting the saponification value before acetylization from that after acetylization. The percentages of free acids, phenols, and aldehydes and ketones were estimated by shaking known volumes of the oil successively and respectively with 5 per cent sodium carbonate, 5 per cent sodium hydroxide, and 35 per cent sodium bisulphite solutions in a flask with a graduated neck and observing the dimunition in volume. The results follow:

TABLE III.

Leaves and tops.	Acid value.	Sapon. value.	Acetyl. value.	Ester value.	Free acids.	Phenols.	Aldehydes, ketones.
September 1927	2.75	41.37	46.30	38.62	9.0%	6.0%	3.0%
October	8.26	35.40	40.44	27.14	19.0	6.0	4.0
November	10.73	42.22	191.94	31.49	21.0	6.0	4.0
December	12.63	51.97	201.43	39.34	..	...	...
July 1928	3.20	24.66	49.70	21.46	4.0	6.0	0.0
August	3.02	23.36	48.20	20.34	6.0	8.0	2.0
September	2.83	36.82	81.40	33.89	10.0	4.0	2.0

A study of the data above shows the effect of normal growth and natural destructive agencies as frost on the respective chemical constants indicating energy changes necessary for the maintenance of the life processes within the plant.

HERACLEUM LANATUM, MICHX. (UMBELLACEÆ.)

H. lanatum, commonly known as Cow Parsnip, is a tall stout perennial, 1–2.5 m. high, and wooly. It is named after Hercules because of its size and stoutness. The stem is grooved, 3–8 cm. thick. The leaves are large, ternately compound with leaflets, 10–25 cm. wide, and round cordate. The flowers are white, involucre deciduous; umbels large with many rays, 5–15 cm. long. The fruit is broadly obovate, 8–12 mm. long, and dorsally much flattened. A microscopic study of the herb will be included in a later report.

The herb is widely spread throughout the central, Rocky mountain and western portions of the United States according to various books on flora (5). In the Willamette valley of western Oregon the plant thrives in great abundance along the banks of streams, drainage ditches, and fence rows.

The writer's attention was attracted to the plant by reports from various sources of fatal poisoning of cattle when the young shoots of the plant are eaten in the spring. Pammel (6), however, states that the fresh leaves have been eaten by the Indians and that a bitter principle, heraclin, $C_{32}H_{29}O_{10}$ is present. Hyams (6), on the contrary, states that the herb is poisonous and Halsted (6) reports that blisters are produced by the plant. Tests show that methyl alcohol is present and it has been asserted that the poisonous properties might be due to this substance; it is doubtful, however, that this alcohol is present in the quantity necessary to produce fatal effects unless consumed in extremely large quantities.

A study of the literature reveals that five species have been examined more or less completely. The oil of *H. giganteum*, Hort. (*H. villosum*, Fisch.), and *H. sphondylium*, L. have been examined quite thoroughly but no mention has been made of a poisonous constituent in the plant. It was decided to examine the oil of the species *lanatum* and carry on an exhaustive study of the constituents of the herb in order to ascertain the presence of poisonous ingredients other than methyl alcohol. The present report includes preliminary studies on the various extracts and the constants of the volatile oil. A complete bibliography will be included in a later report.

The air-dried ground fruits were subjected to successive extractions with petroleum ether, ether, alcohol, and water and the residues were brought to constant weight at room temperature and finally at 105° C. The results follow:

TABLE IV.

	Stems.	Fruits.	
Total ash	6.20%	6.50%	
Acid-insoluble ash	1.27	5.30	
Petroleum ether extract total		4.37%	3.04%
Non-vol. at 105° C.		4.25	2.71
Ether extract total		2.44	2.36
Non-vol. at 105° C.		2.25	2.32
Alcohol extract total		3.98	3.41
Non-vol. at 105° C.		...	3.38
Water extract total		18.29	13.78
Non-vol. at 105° C.		14.77	10.72

The ash was found to contain considerable amounts of iron.

THE VOLATILE OIL.

Preliminary experiments showed the volatile oil to be chiefly in the fruits; the stems although possessing the characteristic odor of the oil yielded but a very

small amount. It was also found necessary to dry and grind the fruit in order to bring about a complete removal of the oil by steam distillation without a great deal of trouble and expenditure of time. The fruits were collected during various months of the year and each lot was steam distilled in order to determine if possible the proper time of gathering.

The oil from each lot was dried over anhydrous sodium sulphate and was then measured. The physical constants were determined and the yield of oil calculated on the dried herb.

TABLE V.

Collection.	Yield.	Color.	d_{20}.	$(\alpha)_{D20}$.	n_{D20}.	Sol. in 80% alcohol.
June 1928	0.26%	Straw	0.8698	+0.93°	1.4288	.....
July	0.57	Pale straw	0.8680	−0.28°	1.4290	.....
July 1929	..	Very pale	0.8640	−0.07°	1.4268	1 in 1.2 or more
Oil of H. sphondylium (7)						
(1) Umbel oil	0.08	Brown-yellow	0.9273*	−0.80°	....	1 in 1.1, turbid in more
(2) Fruit	0.90	....	0.8744	+0.63°	....	1 in 0.8 and more
(3) Fruit	1.21	....	0.8798	+1.10°	....	1 in 1 and more
Oil of H. giganteum (7, 8, 9)						
(1)	3.6	....	0.8722	+1.23	....	1 in 1.8
(2)	2.9	....	0.8738	+1.00°	1.4240	1 in 1
(3)	..	....	0.8695	+0.35°	....	.....

* 15° C.

It has been found that the time of collection for maximal yield of oil is the stage just before the fruits assume the "dead" ripe appearance. Much loss of fruit is encountered by shattering if the time of gathering is postponed until a later time than that mentioned above. The fruits of the June gathering above were green to slightly brown; the July collection consisted of fruits entirely brown. A decided increase in oil yield is noted.

TABLE VI.

Oil.	Acid value.	Sapon. value.	Acetyl value.	Ester value.	Free acid.	Phenols.	Aldehydes, ketones
June 1928	5.30	249.70		244.40	2.0%	1.0%	1.0%
July	3.40	266.90	48.00	263.50	5.0	2.0	2.0
July 1929	2.40	256.30	33.20	253.90	1.0	1.0	1.0
Oil of H. sphondylium (7)							
(1) Umbel	16.2	164.8	31.1	148.6	...	...	...
(2) Fruit	15.9	231.3	54.0	215.4	...	...	...
(3) Fruit	7.3	249.7	26.6	242.4	...	...	...
Oil of H. giganteum (7, 8)							
(1)	1.6	286.7	25.9	288.3	...	...	...
(2)	3.7	284.7	26.3	281.0	...	...	...

The table shows a reasonable comparison with other samples of oil of the same genus. A more complete examination of the oil is being conducted to determine, if possible, all of its constituents.

Each lot was examined for total esters, acids, aldehydes, and ketones, and phenols. The acid and saponification values were determined by the Pharmacopœial methods (3) and the ester number found by subtraction. The acetyl

value was determined by the method described by Semmler (4) and calculated by subtracting the saponification value before acetylization from that obtained after acetylization. The percentages of free acids, phenols, aldehydes and ketones were estimated by shaking definite quantities of the oil successively and respectfully in a flask with a graduated neck with 5 per cent sodium carbonate, 5 per cent sodium hydroxide, and 35 per cent sodium bisulfite and observing the dimunition in volume of the oil. The results are shown in Table VI.

MELISSA OFFICINALIS, L., MENTHACEÆ.

Lemon mint, Balm, Common Balm, or Garden Balm—common names for the herb—attracted the writer's attention because of several inquiries received concerning the commercial possibilities of the plant. It is to be found in dense patches in the Williamette Valley of Oregon and examination of the source of supply showed these beds to be escaped from gardens and farm yards where the mint had been grown for flavoring purposes and because of its pleasant, characteristic lemon-like odor. The patches were sturdy growths of unusual and profuse size, indicating that the herb might easily be grown for commercial use if the demand were sufficient for its cultivation. Literature revealed very little concerning the constants and chemical constituents of the oil derived from the herb. It was decided to confine our efforts to a study of these values.

The plant has been described in "Gray's Manual" (10) and more completely in a treatise on northwest flora by Frye and Rigg (10).

The herb shows the presence of 74.5% total volatile material; total ash content was 6.2%; and insoluble ash 5.0%; iron and aluminum were indicated in the ash. When subjected to steam distillation and subsequent cohobation and extraction of the aqueous distillate the herb yielded approximately 0.13% oil with a golden-yellow color and a pleasant lemon-like odor. A table of comparison of the constants follows:

TABLE VII.

	Oregon oil.	Spanish oil (11).	Oil of *Melissa calamintha* (11). 1.	2.
d_{20}	0.9632	0.8910*	0.8759	0.8771‡
$(\alpha)_{D20}$	−10° 6′	+2° 8′**	−28° 12′†	−16° 57′
Solubility in alcohol	Insol. in 20 parts of 80% alcohol	Sol. 1 part in 2 parts of 80% alc.	Insol. in 10 parts of 90% alcohol	Insol. in 10 parts of 90% alcohol
N_{d20}	1.4999	1.4704**	1.4951†	1.4911
Acid value	9.28	.....	.....	.....
Saponification value	34.41	.....	4.5	8.3
Acetyl value	43.30	236.0	.....	38.95
Ester value	25.13	27.4	4.5	8.3
Free acids	2.0%	.....	.....	.....
Phenols	4.0%	.....	.....	.....
Aldehydes and ketones	17.0%	42.0%	.....	.....

* 25° C. ** 22° C. † 16° C. ‡ 15° C.

Flatau and Labbe (12) on examination of a Spanish oil reported 6 per cent citronellal, 20 per cent geraniol and 12 per cent linalol (after saponification).

Since there is no particular demand for the oil no further work was attempted.

MICROMERIA DOUGLASII, BENTH., MENTHACEÆ (*M. chamissonis*, GREENE).

M. douglasii, commonly known as Yerba Buena or Tea Vine, is a prostrate perennial vine, 30 to 100 cm. in length with orbicular or ovate, and dentate leaves, 6 mm. to 50 mm. long. The

flowers are small, few, in the leaf axils, white or pale lavender. The herb is found growing in shaded places and more profusely along shaded banks of streams. It is easily identified by its pleasant minty and balsamic odor which distinguishes it from the very similar Twin-flower (*Linnaea americana*, Forbes, *Caprifoliaceæ*). It differs further from this plant by having the characteristic square stem of the mint family. A survey of literature shows that all of the physical and chemical constants have not been reported and it was deemed advisable to report such determinations on the Oregon product.

Lynn and Cheng (13) have found that drying the herb before steam distillation tends to dissipate the volatile components almost entirely. Our experience substantiates this statement and accordingly the fresh herb was subjected to steam distillation. Apparently the proper time to collect for maximum yield of oil was just before or at the time of flowering. Experiments, however, were not carried out to prove this statement. Cohobation and extraction yielded 0.25 per cent of a pale green oil with a delightful balsamic, minty odor. Upon standing the color of the oil gradually changed to a golden tint.

A solid substance deposited in the condenser upon cohobation. This substance with respect to some of its physical properties resembled menthol. The amount, however, was too small to permit further study. Lynn and Cheng (13) have noted this solid substance while Power and Salway (14) have reported a semi-solid substance which they identified as palmitic acid. It is quite likely, however, that menthone and menthol are present, since Murayama (15) has identified these substances in the oil of *M. japonica*, Miq. (yield of oil -0.7%) and since analogy in composition is generally shown between species. Owing to the small amount of oil present in the herb, for which apparently there is no great commercial demand and to the difficulties involved in gathering no further work was done other than determinations of physical and chemical constants reported in the following table of comparison:

TABLE VIII.

	Oregon oil.	Western oil (14). A.†	B.*
Yield	0.25%	0.16%	0.5%
d_{20}	0.9217	0.9244	0.9450
$(\alpha)_{D20}$	$-21°\ 3'$	$-22°\ 48'$	$-26°\ 44'$
Solubility in alcohol	Sol. 1:10 of 80% alc.	Not sol. in 10 vol. 70% alc.	Easily sol. in 70% alc.
N_{d20}	1.4572	.....	.....
Acid value	6.06	.....	.....
Saponification value	27.01	.....	.....
Acetyl value	55.54	.....	.....
Ester value	20.95	.....	.....

† Pale yellow-brown oil.

* Yellow-brown oil obtained by alcoholic extraction.

SUMMARY.

1. A preliminary report on *Artemisia heterophylla*, Nutt. and its oil is made. The various extracts of the herb and constants of the oil are found. Work is being continued and an extended bibliography will be offered later.

2. A preliminary report of *Heracleum lanatum*, Michx. and its oil is given. The various extracts and constants of the oil are given with comparative values for other oils of the same genus. Examination of the oil and the herb is being continued to ascertain any poisonous ingredients. The results of the examination and an extended bibliography will be offered in a later report.

3. The physical and chemical constants of the oil of *Melissa officinalis*, L. and *Micromeria douglasii*, Benth. are reported.

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PEPPERMINT MONOPOLY IN JAPAN REFUTED.

At a meeting called by the Japanese Minister of Home Affairs, at which the governors of all the prefectures in Japan were present, it was stated that, if a peppermint monopoly were established, dementholized peppermint oil of uniform grade could be offered to exporters and the monopoly would be able to curtail sales of peppermint oil and thus stabilize the price. No particular details as to the method of procedure were discussed, and it is believed that at present no investigation of this matter is being made either by the Monopoly Bureau, or by the Hokkaido Prefectural Government.

One of the largest dealers and exporters of peppermint oil states that dealers were not in sympathy with the idea and that resolutions to that effect were drawn up and presented to the Department of Commerce of Japan. (Assistant Trade Commissioner H. B. Titus, Tokyo.)

EFFECT OF METHANOL ANTI-FREEZE ON HEALTH.

Surgeon General H. S. Cumming of the United States Public Health Service, when asked regarding the statement issued December 6th by the Bureau of Mines on the effect of methanol anti-freeze on health, stated that the Public Health Service had kept in touch with the observations being made by the Bureau of Mines on the subject, and that he felt there was need for the immediate release of information which would protect the general public. It appeared from the study so far that there was much more danger from exhaust gas (carbon monoxide) than could possibly come from methanol when used strictly as an anti-freeze liquid in automobiles.