CONCLUSIONS.

In the study of the physiological qualities of *Ephedra nevadensis*, the following points seem especially pertinent:

1. The drug seems to be possessed of some slight diuretic effects. This characteristic is best shown in the infusion form. From this, it may follow that the water-soluble constituents are of a blenorrhetic nature. The fact that the alcoholic and chloroformic extracts do not possess this property further proves the fact that these constituents are of the water-soluble group.

2. The drug is not strongly active, for doses representing from 5 to 15 Gm. of the drug produce only slight stimulation. This proves that the drug does not belong to the exceedingly active class of medicinal plants.

3. The slight constipating after-effects following the prolonged administration of the fluidextract mitigates its ability to increase excretion through the kidneys. This may be said to be due to the tannin present.

4. The absence of the alkaloid, *Ephedrine*, precludes the use of *Ephedra nevadensis* as a circulatory stimulant, and the other effects ascribed to Ephedrine.

From the recapitulation of the evidence, there seems to be no distinct advantage to be gained by the introduction of *Ephedra nevadensis* into medicine. The properties usually ascribed to it are such that the need is well supplied by some well established therapeutic agent. Outside of its domestic use, very little may be said in its favor.

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EXAMINATION OF ASARUM CAUDATUM.

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A year ago a preliminary report (1) on a few Northwestern plants contained reference to one of the wild gingers. We have now submitted this herb to a more extended examination, with special regard to the interesting volatile oil. Since other members of the genus are now used to some extent in medicine and in perfumery, it was deemed important to learn whether the Washington variety might be employed in the same way.

Asarum caudatum (Lindl.), *Aristolochiaceæ*, is a perennial, possessing stem-like rhizomes, 2 to 8 mm. thick, with branches 10 to 30 cm. long, having a penetrating, ginger-like odor, and a spicy, acrid taste. The roots are thick, fibrous and fleshy. The leaves are evergreen, reniform, cordate, entire, sparsely hairy throughout, and with long petioles. The flowers are solitary, peduncled, brownish purple or mottled, and borne near the ground. The calyx is campanulate or hemispheric, with oblong-lanceolate, attenuated lobes, 2 to 5 cm. long. The stamens are twelve in number, the placentae are parietal and intruded. The fruit is coriaceous, sub-globose or bursting irregularly, the seeds are flat.

The herb grows profusely in moderately damp places, shady woods, and on steep hillsides, all of which are usually enriched with an abundance of leaf mold. The plant has been found on rocky slopes with some exposure but, under such conditions, the rhizomes become shortened, woody, much branched, and jointed, the leaves are much smaller, and the patches are considerably matted and less extensive. Preliminary tests to determine the limiting circumstances in attempts to cultivate caudatum having given very favorable results, we are at present engaged in extensive experiments to ascertain the best growing conditions, the yield, and the changes in oil content under varying methods of growth.

Six species of asarum have previously been examined, three of them quite completely. Miller (2) found that A. arifolium gives an oil which contains *l*-pinene, eugenol, safrol, methyl eugenol, methyl isoeugenol, asarone, a sesquiterpene, and an unidentified phenol. A. Bicknelli (reflexum) was studied by Kraemer (3) who compared the anatomy with that of other species. The oil from *A. blumei* was found by Schimmel & Co. (4) to contain eugenol, safrol, and a terpene. According to Asahina, (5) the Chinese drug *ToKo* consists of the entire plant. Asahina has also reported that the Chinese drugs, *Sai-Sin* and *Si-Sin* are derived from *A. Sieboldii* (Mig.). Holmes(6), in 1879, described the rhizome and root from the latter and stated that the rhizome is used as a drug in Japan under the title of *To-Sai-shin*.

As early as 1807, *A. Europæum* was studied by Lassaigne and Feneulle (7), and later by a number of others (8). The oil has been found to consist, among other substances, of asarone (3,4,6-trimethoxy-allyl-benzene), *l*-pinene, methyl eugenol, and dimethyl protocatechuic acid. There are also statements in the literature that the plant contains starch, tannin, resin, gluten, citric acid, albumin, malates, and a yellow coloring matter.

Several investigators have examined *A. canadense*, Canada snake root (14). The most valuable contributions to the knowledge of this plant, however, have been made by Power (9). The rhizome was found to yield 1.15 per cent of a volatile oil, a large amount of starch and gum, some resin and fat, a yellow coloring matter, sugar, and an alkaloid. The ash was also examined in detail. The volatile oil contained an unidentified phenol, pinene, *d*-linalool, asarol ($C_{10}H_{18}O$), *l*-borneol, *l*-terpineol, geraniol, methyl eugenol, palmitic acid, acetic acid, and a lactone.

The material used in our present experiments was collected in the White River Valley, Washington, during 1925–26. In order to determine, if possible, the proper time of collection for maximum oil content or for other valuable properties, the plant was harvested at several seasons, each lot being treated individually. The leaves, including petioles, were separated from the rhizomes and roots.

Samples of the fresh material were dried in the air to constant weight and then at 105° C. Small portions of the air-dried samples were subjected to successive extractions with petroleum ether, ether, alcohol, and water, the residues being carried to constant weight at below 70° C. and then at 105° C., and calculated on the completely dry basis. The results follow:

	Rhizomes and Roots.		Leaves and Petioles.	
Loss on Air-drying	78.39		81.53	
Loss at 105° C.	9.33		1.59	
Total Volatile	87.72		83.12	
Petroleum Ether Extract Total	2.73	2.74	2.94	2.21
Non-volatile at 105° C.	1.28	1.39	1.23	1.04
Ether Extract Total	0.71	0.51	1.67	1.70
Non-volatile at 105° C.	0.67	0.44	1.15	1.55
Alcohol Extract	17.32	20.41	18.77	15.38
Water Extract	....	19.72	22.16	20.11

THE VOLATILE OIL.

Each lot of the herb was carefully washed and air-dried and then ground to a coarse powder in a disc mill. After maceration over night with water, the separate samples were subjected to steam distillation until no more oil was obtained. The latter was separated as far as possible and the watery liquid cohobated, the final traces of oil being removed by ether extraction. The collected oil was subsequently dried over calcium chloride and measured in a graduated cylinder. The physical constants of each lot were determined and the yield of oil calculated on the dry basis. The amount obtained from the leaves was so small that the rotation could not be determined on the separate samples, so all of them were combined for this constant. The results are shown in the following table:

	Rhizomes and roots.				Leaves and petioles.			
Collection.	Yield.	d_{20}.	$[\alpha]_{D20}$.	n_{D20}.	Yield.	$[\alpha]_{D20}$.	D_{20}.	n_{D20}.
Summer	2.08%	0.9831	−0.32	1.5118	0.18%	0.9993	+0.55*	1.5146
October	4.06	0.9377	−3.26	1.5000	0.91	0.9299	.....	1.5060
November	3.09	0.9463	+1.21	1.5075	0.67	0.9290	.....	1.5004
December	2.94	0.9627	−2.45	1.5098	0.75	.....	.....	1.5006
April	2.47	1.0017	−0.80	1.4992	....	.....	.....	.....

*Combined samples.

It is thus seen that the leaves and petioles contain but a very small amount of oil, which is undoubtedly furnished chiefly by the latter. In subsequent distillations attention is being almost entirely devoted to the rhizome and roots. It will also be noted that early fall collections give higher yields than at any other time of the year. The physical constants of the oil and, therefore, the chemical composition vary somewhat with time of harvest but this is probably only qualitative.

In order to gain some idea of the components, each lot was examined for total esters, acids, aldehydes and ketones, alcohols, and phenols by the usual methods. The acid value and saponification value were determined by the pharmacopœial processes and the ester number by subtraction. The acetylization was made according to Semmler (10) and calculated by subtracting the saponification value before from that after acetylization. The free acids, phenols, and aldehydes and ketones were estimated by shaking successively and respectively with 5 per cent sodium carbonate, 5 per cent sodium hydroxide, and 35 per cent sodium bisulphite solutions, and observing the diminution in volume. The results are shown in the following table:

Rhizomes.	Acid value.	Sapon. value.	Acetyl value.	Ester value.	Free acid.	Phenols.	Aldehydes.
Summer	5.60	38.45	26.73	34.35	9.1%	2.2%	8.0%
October	1.61	25.86	58.51	24.25	15.9	4.8	3.2
November	1.89	36.32	47.41	34.43	16.2	4.1	5.8
December	5.05	26.98	30.50	21.93	11.3	8.3	0.0
April	3.13	33.03	41.69	29.90	4.0	10.0	4.1
Leaves	7.24	41.43	48.81	34.19	30.0	9.5	2.4

Tests were made by the usual methods for sulphur and nitrogen compounds, with negative results. The mixed rhizome oils were submitted to a methoxy determination by Gregor's modification (11) of Zeisel's method, resulting in a number of 181.3 or 18.13 per cent of CH_3O.

The total oil from all collections of rhizomes and roots was combined and fractionated several times, the final result being shown in the table below, all temperatures being corrected.

Up to 90° C.	5.1%	203–214	2.3%	246–251	4.1%
90–151	2.4	214–224	2.6	251–261	2.4
151–182	7.7	224–235	5.7	Residue	5.3
182–203	2.1	235–246	60.3		

The first four fractions were again distilled several times, using smaller ranges of temperature, with the following results:

Up to 70° C.	15.2%	121–151	7.8%	172–182	18.8%
70–90	7.5	151–162	7.7	182–203	12.6
90–121	7.8	162–172	22.7		

Identification of Pinene.—Fraction 151–162° C. was cooled to —10° C. by means of a freezing mixture and a stream of nitrosyl chloride slowly passed into it. A bluish green color was immediately formed and the addition of absolute alcohol caused the precipitation of white crystals, which were identified as pinene bisnitrosochloride by a melting point of 103° C. This was confirmed by conversion (12) to the nitrolpiperidide, melting point 118° C.

The fraction boiling between 235° C. and 246° C., which constituted the bulk of the oil, was found to contain no free acids nor aldehydes and but a small amount of a phenol (eugenol ?) which was not identified. The saponification value was found to be 24.9, corresponding to about 8.71 per cent of ester calculated as geranyl acetate. The acetyl value was 7.2, corresponding to 1.98 per cent of alcohol calculated as geraniol. The methoxy number was 241.6, or 24.16 per cent of CH_3O, corresponding to 69.5 per cent of methyl eugenol. The figures found, therefore, indicated that most of the fraction consisted of methoxy derivatives of hydrocarbons.

Identification of Methyl Eugenol.—About one Gm. of the fraction was dissolved in 20 cc. of ether and the solution cooled by a freezing mixture. Bromine was added drop by drop to the cooled solution with shaking until a permanent red color was obtained. The red solution was decolorized by sulphur dioxide and evaporated to remove excess of the reagents and ether. The brown syrupy residue was dissolved in alcohol and the solution allowed to evaporate spontaneously, producing very beautiful needles of tribrom methyl eugenol with a melting point of 78° C. The presence of methyl eugenol was further confirmed by oxidation according to Wallach (13) to veratric acid, melting at 180–181° C.

Azulene.—The residue from the fractional distillations, when heated under a vacuum, gave at 230° (20–40 mm.) a blue oil having all of the properties of azulene, often found by others and already reported as existing in other asarum oils.

Asarone.—During the distillation of the residue, crystals were deposited in the condenser, in the receiver, and in the distilling flask. These were collected in ether and allowed to crystallize, later being recrystallized from alcohol. The melting point was found to be 61° C. which, with the boiling point and the fact that asarone is found in other species, is considered adequate identification. Crystalline asarone could also be separated from the original oil by cooling in a freezing mixture. Of the other varieties of Asarum, *arifolium* and *Europæum* oils contain asarone, but there seems to be none in that of *Canadense.*

From our work thus far we would estimate that the oil from rhizomes and roots of *Asarum caudatum* contains about ten per cent of azulene, as much or more asarone, sixty to seventy-five per cent of methyl eugenol or closely related methoxy compounds, a small amount of pinene, and probably other terpenes. We are at present engaged in separating and identifying the substances which exist in smaller amounts.

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