AN EXAMINATION OF CEANOTHUS VELUTINUS.*

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This member of the buckthorn family is an evergreen shrub found from British Columbia to California and from the Rocky Mountains to the Pacific Coast. It is commonly known as sticky laurel or as mountain balm, but also by other names. It grows best in sandy or rocky soil in the hills and is found profusely on logged-off land, of which the acreage on the coast is large and constantly increasing. Wahlenberg (5) has recommended it as an aid in reforestation.

The leaves are covered with a sticky substance of cinnamon-like odor, which the flowers also possess in a magnified degree. Because of this waxy matter the plant is considered a serious fire-menace in the Californian forests.

The plant has previously been examined three times, but not exhaustively. In 1916, Scalione and Blakemore (2) made an investigation, particularly of the wax, 7.3 per cent, and of the tannin, 17.3 per cent. In 1922, Howard (3) classified the leaf-wax as a balsam because it yielded a volatile oil and cinnamic acid. A year later Lynn, Lee and Clausen (4) distilled an oil from the leaves and suggested that it is largely aldehyde.

The eastern species, *Ceanothus americanus*, has been examined extensively and repeatedly since the middle of the last century. An alkaloidal mixture, ceanothyn, is well known in certain medical circles; it is obtained from the roots and root-bark.

The material used in this work was gathered mostly near Seattle, but also partly near Missoula, Montana. It was freed from foreign matter, cleaned well and dried in the air. Some of the roots and leaves were powdered before use.

Histological.—In order to throw some light on the origin of the sticky coating, which is found in great abundance on the leaves, permanent and temporary sections of the latter were made. These revealed at frequent points pear-shaped structures descending from the upper epidermal cells, the point near the surface consisting of two parallel rows of large, pentagonal cells. The two rows separated half way down and formed the circular end. Within this cavity was found a network of smaller, thick-walled cells which undoubtedly is conductive tissue and forms a canal through the leaf. Of course, the stomates are on the under side of the leaf, not on the upper surface. While the interior portion of this peculiar structure was easily stained by various reagents, the large outer cells were unaffected by any of them. Although the content would suggest a secretory layer, there is no reason to relate the structure to secretion. Dr. Eames, of Cornell, expressed the opinion that the cylinders are bundle-sheaths and probably not linked with the balsam, and in this Dr. Rigg of Seattle concurs. It is interesting to note, however, that the structure is not possessed by any of six other species of *Ceanothus*, which also do not have the balsamic coating. These are *prostratus*, *americanus*, *ovatus*, *pubescens*, *fendleri* and *thyrsiflorus*. The structure was found to be missing in the leaf-bud and in very immature leaves. It evidently appears at the time when the tissues are first differentiated according to function.

Proximate Analysis.—This was made in the usual way on the leaves and on the root-bark.

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	Leaves.	Root-Bark.
Loss in air	50.6	48.3
Ash	4.5	6.5
Petroleum-ether extract	12.8	0.5
Ether extract	12.8	2.2
Crude fibre	12.3	17.6
Total nitrogen × 6.25	10.6	..

Selective Extraction.—The bark, wood and flowers were each submitted to selective extraction by the solvents given. The results follow:

	Bark.	Wood.	Flowers.
Petroleum ether	1.18	0.75	2.84
Ether	1.89	0.27	2.48
Alcohol	12.40	4.51	13.85
Acetone	0.42	0.59	3.47
Carbon disulphide	0.04	0.14	None
Ethyl acetate	0.07	0.04	None
Water	8.36	3.72	16.70

Volatile Oil.—The flowers furnished 0.1 per cent of an oil which was apparently unlike that from other parts of the plant. The total amount of flowers available was not sufficient to warrant any examination of the oil.

From the leaves by distillation with steam, there was obtained 0.14 to 0.21 per cent of oil, the amount being largest during the winter months and gradually decreasing up to the time of flowering in May and June. Judging by the index of refraction, however, the oil obtained at different times of the year was constant in composition. It was reddish brown in color and possessed a strong, aromatic and pungent odor. The constants were: specific gravity at 20° C. 0.9565; index of refraction at 20° C. 1.509 to 1.542; specific rotation $-12.5°$; saponification number 148.4, 142.0 and 155.0; 22 per cent soluble in sodium hydroxide; 20 per cent soluble in sodium bisulphite solution.

The whole oil was fractionated and refractionated several times at 5-mm. pressure with final results as given in the table:

Fraction, Degree.	Per Cent.	Approximately at 760 Mm., Degree.	n at 20° C.
65– 80	7.5	165	....
80– 90	8.0	190	1.5360
90– 94	14.0	211	1.5600
94– 98	7.5	214	1.5691
98–120	4.5	230	1.5031
120–125	4.0	239	1.4859
125–128	4.5	252	1.5015
128–135	3.0	259	1.5110
135–145	5.0	266	1.5150
145–157	9.5	273	1.5180
157–165	7.0	280	1.5200
Residue	25.5	...	....

The chief constituents were found to be ethyl and cinnamyl cinnamates, the former in fractions 8–10 and the latter in these and the residue. Saponification of the oil boiling at 132–135° gave cinnamic acid, melting at 132° C. and oxidizing with potassium permanganate to benzaldehyde. The separated aqueous solution contained ethyl alcohol which was isolated and identified by conversion to the benzoate and acetate. Cinnamyl cinnamate, melting point 43–44° C., was separated by treatment with warm alcohol and was further characterized by saponification to cinnamic acid and cinnamyl alcohol, whose phenyl urethane melted at 90° C.

Salicylaldehyde, semicarbazone melting at 225° C., was apparently the chief component of fractions 2 and 3, which gave with ferric chloride a deep violet color.

The fraction boiling at 126–128° probably contained esters of an alcohol with a strong odor like geraniol, although a diphenylurethane melting at 56° C. and a phthalate melting at 204–205° C. could not be referred to a known alcohol. The acids from saponification were cinnamic acid and probably valeric acid, the latter partly identified by odor and boiling point.

The lowest fraction contained terpenes which were not identified. From Fraction 2 a bromide was separated with a melting point of 105° C. (limonene?) and from Fraction 4 a bromide melting at 124° C. (dipentene?). Fraction 10 also furnished a bromide melting at 92° C. No further study was made of these. The residue contained, with the cinnamyl cinnamate, a paraffin melting at 62° C.

It is interesting to note that cinnamic aldehyde could not be identified in any of the fractions, notwithstanding that the odor is suggestive and that others have predicted its presence.

Alkaloid.—Preliminary experiments proved that the leaves do not yield alkaloids, but that some could be extracted from the bark and more from the root-bark. For further study a large quantity of the latter was collected, dried and ground to a coarse powder. After considerable experimentation, it was found best to extract first with chloroform, from which the alkaloidal material was removed by means of very dilute sulphuric acid. This solution gave with ammonia a copious, curdy, white precipitate which could again be collected by chloroform. After repetition of the process several times, the precipitate was collected and dried, the yield being approximately 0.1 per cent.

It was very soluble in chloroform and fairly so in hot alcohol or hot methyl alcohol, but was only slightly soluble in the cold alcohols, in ether, acetone, petroleum ether, benzene or carbon tetrachloride. It was practically insoluble in water. By means of hot methyl alcohol, there were obtained minute crystals in the form of raphides or needles, which were perfectly colorless. This crystalline material decomposed when heated slowly and melted at indefinite points; when the temperature was raised quickly, the melting point was fairly constant at about 270° C. with decomposition.

Analysis by combustion gave figures which agreed closely with the simple formula, $C_{23}H_{26}N_2O_4$. Those for six consecutive determinations of carbon and hydrogen and three for nitrogen were as follows:

	1.	2.	3.	4.	5.	6.
Carbon	69.7	70.0	69.9	69.8	70.2	70.0
Hydrogen	7.0	7.0	7.1	6.8	7.0	7.2
Nitrogen	7.2	7.3	7.3	....	....	....

Calculated for $C_{23}H_{26}N_2O_4$: Carbon 70.0, hydrogen 6.5, nitrogen 7.1.

To determine the molecular weight by the freezing-point method was impracticable because of insolubility in the proper liquids. The boiling-point method also proved unsatisfactory, because the small amount of material precluded using a suitable apparatus like those of McCoy or Cotrell. The figure obtained in one experiment was 125, calculated 394.

Precipitating agents, except tannic acid, gave very satisfactory reactions with acidified aqueous solutions.

Reagent.	Precipitate.
Mayer	Very small white granules
Sonnenschein	Amorphous, light blue
Scheibler	Amorphous, white
Wagner	Amorphous, brown
Wormley	Amorphous, yellow
Picric acid	Granular crystals
Platinum chloride	Small, light yellow, granular crystals
Cold chloride	Amorphous, yellow
Hydrobromic acid	Amorphous, white
Hydriodic acid	Amorphous, brown

A few color reactions were noted, although no attempt was made to study these completely. Froehde s reagent gave a green color changing to brown and then back to green. Marquis' reagent yielded a green turning slowly to a brown. Mandelin's reagent produced a yellow color which later became brown. No reactions were obtained in the Vitali test, with concentrated ammonia water or in the fading purple test as for strychnine. Nitric acid gave a blue-green color which soon changed to purple and finally faded. Sulphuric acid produced a yellowish brown and finally a purple. Hydrochloric acid gave a beautiful blue which later became green.

The hydrochloride was prepared, by passing dry hydrogen chloride into a chloroformic solution, as a white, agglutinated mass which was very unstable and gave no definite melting point. It was completely decomposed at 240° C. Analysis by the usual methods gave 5.4 and 5.8 per cent of chlorine, compared to 8.2 per cent is calculated for a monohydrochloride.

The small amount of material available prevented any further study, but plans are now being laid to collect larger quantities of root-bark and to examine the alkaloid more carefully, chemically and pharmacologically.

Coloring Matter.—Extracts of leaves, bark and root-bark contained coloring agents which did not respond to the Bornträger test, in spite of the fact that many of the Rhamnaceæ contain anthraquinone derivatives. Aqueous or alcoholic extracts of the root-bark were of a bright red color, from which ammonia precipitated a gelatinous, purple substance. This was soluble in acid to give a red solution, but was not soluble in any of the organic liquids. Qualitatively it gave several reactions which are characteristic of anthraquinone derivatives and we plan to study it further.

Dermatitis.—In 1905, Rooney (1) described somewhat a dermatitis which was attributed to *Ceanothus velutinus*, and a number of persons in Washington have orally reported similar experiences. In view of the wide-spread occurrence of this plant, it is very possible that many cases of poisoning laid to species of *Rhus* are in reality caused by *Ceanothus*. With the aid of Drs. Wm. Clausen and Henry Odland, both dermatologists, it was found that such a dermatitis could be caused on a small percentage of subjects by either the leaves or an ethereal extract of them, but that aqueous solutions are inactive. One of the writers was continually affected during collection of material.

The eruption appeared on the skin one or two days after contact. The first symptoms were itching and burning, with subsequent redness, and finally vesicles appeared on the surface. On the face the skin became bright red and the eyelids and surrounding tissues were markedly swollen. These symptoms continued for two or three weeks after exposure and then gradually subsided. Simultaneously, desquamation occurred in the form of small scales and large flakes of dried epidermis. Treatment by the antigens of poison oak or ivy was of no prophylactic or curative value, but the conditions were relieved by the usual moist compresses and soothing lotions.

SUMMARY.

The leaves of *Ceanothus velutinus* present microscopically a peculiar structure which is not found in other species and may be related to the characteristic balsamic coating. They furnish 0.14 to 0.21 per cent of an oil which is chiefly ethyl and cinnamyl cinnamates, with smaller amounts of salicylaldehyde, terpenes and esters of an unidentified alcohol with cinnamic and probably valeric acids. The root-bark furnishes about 0.1 per cent of alkaloid, part of which was obtained in the crystalline state. Analysis indicated the formula $C_{23}H_{26}N_2O_4$. Some study was made of its properties and reactions. Reports of a dermatitis from the leaves were confirmed and the symptoms are described.

REFERENCES.

(1) Rooney, *California State Journal of Medicine* (1905).
(2) Scalione and Blakemore, *Ind. & Eng. Chem.*, 8 (1916), 411.
(3) Howard, JOUR. A. PH. A., 11 (1922), 19.
(4) Lynn, Lee and Clausen, *Ibid.*, 12 (1923), 420.
(5) Wahlenberg, *J. Agr. Research*, 41 (1930), 601.

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THE INTERNATIONAL STANDARD FOR THE ŒSTRUS-PRODUCING HORMONE.

At the request of the officers of the Permanent Commission on Biological Standards of the Health Organizations of the League of Nations, the Board of Trustees of the U. S. Pharmacopœial Convention has agreed to assume the responsibility for distributing the International Standard for the Œstrus-Producing Hormone in the United States. Supplies of this material have just been received from the National Institute for Medical Research, London, where the International Standard has been prepared. This Standard is now available for the use of manufacturers of preparations of this Hormone, for the purpose of establishing for their products a uniform potency in the terms of the International Unit; one International unit consists of 0.0001 mg. of the Hormone issued by the League of Nations. This material is also available for those carrying out important therapeutic researches in this field.

A memorandum suggesting the course to be followed in using the International Standard, has also been supplied by Dr. H. H. Dale, Director of the National Institute for Medical Research. Those who are interested in securing this memorandum or the International Standard, should communicate directly with E. Fullerton Cook, Chairman of the U. S. P. Committee of Revision, 43rd Street and Woodland Avenue, Philadelphia.