The total amount of crystalline, sterol-precipitating material, *i. e.*, digitonin, thus obtained was about 155 Gm. corresponding to 1.16 per cent of the seed. It should be remembered, however, that a small amount of precipitated digitonide was not resolved into its components, also that some of the digitonin had been separated from the alcoholic extract as insoluble digitonide, having been formed during the process of extraction. As yet it has not been ascertained whether the digitonide, removed as previously described, constitutes all of the digitonide formed during the extraction. As previously pointed out it seems reasonable to assume that digitonide may be precipitated in the tissue during the process of extraction, hence may be lost in the marc.

That the 155 Gm. of material, separated as described, consisted of digitonin was demonstrated not only by its method of preparation which is sufficiently specific to exclude other compounds, but by its m. p. 227° to 240° (10) (not very conclusive it is true), its ability to precipitate sterols (quite conclusive), also by a positive Keller color reaction (11).

The defatted alcoholic concentrate from which the digitonin had been precipitated with cholesterol was set aside to be investigated at a later date, if deemed desirable.

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LABORATORY OF EDWARD KREMERS,
MADISON, WIS.

TWO SPECIES OF THE GENUS LEDUM.*

BY RUSSELL A. CAIN AND E. V. LYNN.

Several years ago we reported (1) on the volatile oil of *Ledum grœnlandicum* Oeder., which grows abundantly in the numerous bogs of Washington. We have now collected specimens of this plant from a dry bog near Seattle and examined them more carefully. We have also gathered samples of *L. columbianum* Piper, which is found along the coast of Oregon and Washington. It is differentiated from *L. glandulosum* by some and by others is considered identical. It is distinguished from *L. grœnlandicum* by being glabrous and by its larger, not revolute-margined leaves, which are not tomentose. Like the other species it is reputed to be poisonous to stock, but no one has hitherto investigated it scientifically.

* Scientific Section, A. PH. A., Madison meeting, 1933.

A preliminary study having demonstrated that the composition is little influenced by the wetness of the soil in which the plants grow, the samples were collected at random. They were thoroughly cleaned, allowed to dry in the air and then submitted to a proximate analysis, using the A. O. A. C. methods.

	L. grœnlandicum.		*L. columbianum.*	
	Leaves.	Flowers.	Leaves.	Flowers.
Loss on air-drying	42.22	70.12	62.50	79.55
Loss at 100° C.	6.16	7.53	6.55	8.11
Loss at 110° C.	6.93	8.13	7.44	9.00
Ash	2.76	4.35	4.22	3.97
Ash, acid-soluble	2.69	3.58	3.90	3.71
Selective Extraction.				
Petroleum ether	5.48	3.36	6.61	2.98
Volatile	0.45	0.18	0.54	0.39
Ether	6.33	2.78	4.04	3.06
Volatile	0.24	0.28	0.53	0.41
Chloroform	1.78	1.12	1.40	1.24
Alcohol	34.12	38.39	28.16	31.19
Water	7.56	8.43	14.43	12.32

Alkaloid.—Since the plants are considered poisonous to stock and because the leaves have been employed considerably in medicine and for killing insects, there was a possibility that an alkaloid is the responsible agent. Accordingly 100 Gm. each of the leaves and of the roots of both plants were separately exhausted with alcohol containing 1 per cent of tartaric acid. The resulting solutions were concentrated under reduced pressure to a syrupy consistency, poured into water and filtered. The filtrates were evaporated under reduced pressure to a small volume, made slightly alkaline with ammonia and extracted with 1 per cent sulphuric acid. The acid solutions were treated separately with Wagner's, Marme's, Mayer's and Scheibler's reagents, with gold chloride, picric acid and tannic acid. Since none of these materials gave any precipitate, we conclude that alkaloids are absent from the roots and leaves of these two species.

VOLATILE OIL FROM L. GRŒNLANDICUM.

Immediately after being picked and separated from the stems, some of the leaves were transferred to a large still and subjected to distillation with steam. From 362 pounds there was obtained a total of 226 cc., or 204 Gm. of oil, representing a yield of approximately 0.12 per cent, 0.21 per cent on the dry basis. By a previous maceration with water before distillation, this yield could be increased by about 50 per cent.

The oil was light amber in color, possessed a characteristic odor and was neutral to litmus. On standing it darkened somewhat and the odor became more pronounced. The constants were: d_{25} 0.9031; $[\alpha]_D^{20}$ +1.36°; n_D^{20} 1.4900; acid number 2.46; saponification number 28.81 (ester as bornyl acetate 9.32 per cent); after acetylization 97.20 (combined alcohol as borneol 7.24 per cent, free alcohol as borneol 11.57 per cent); methoxyl value 13.19 (Zeisel method) equivalent to 3.45 per cent of methyl eugenol.

Free Acids.—From 200 cc. of the oil the free acids were extracted by means of five per cent sodium carbonate solution which was washed with ether to remove adhering oil and then evapo-

rated to a small volume. After acidification with sulphuric acid the residue was distilled and acetic acid identified in the distillate by characteristic reactions. It is possible, judging by odor, that there is also a small amount of butyric or valeric acid. The total free acid amounted to about 2.5 per cent.

Phenols.—From the residual oil there was extracted by 5 per cent potassium hydroxide solution 0.49 Gm. of phenols, or 0.27 per cent. The index of refraction at 25° C. was 1.5045. Carvacrol was identified by color reaction with ferric chloride, by the Flückiger test and by the phenyl urethane melting at 135° C. The total amount of phenols in the oil, as determined in a cassia flask, was found to be 11 per cent. Since carvacrol can be extracted from its alkaline solution, the total percentage in the oil may be much more than 0.27.

Levo-α-phellandrene.—The remaining oil was distilled repeatedly up to 105° C. at 35 mm., giving a distillate of 36.5 cc. which was found to be almost entirely α-phellandrene. It boiled at 170–175° C. at atmospheric pressure, had a specific gravity of 0.8628 at 20° C. and a specific rotation in alcoholic solution of −10.45° at 20° C. The nitrite when purified had a melting point of 113° C.

Combined Acids.—The rest of the oil was saponified with alcoholic potash and the alkaline solution was separated carefully and evaporated to a small volume. This was then acidified and distilled and acetic acid was identified in the distillate as before. Apparently there was also at least one more acid in the distillation residue, but it was in very small amount.

The bulk of the oil after saponification was submitted to fractional distillation at 30–35-mm. pressure and repeatedly refractionated. The first 2 cc. were found to be entirely α-phellandrene.

Levo-borneol.—At 140–142° C. there was obtained a crystalline solid and a liquid, both of which gave identity tests for borneol. The melting point of the crude solid was 203° C. and a phenyl urethane could be obtained from either portion melting at 138° C. The specific rotation of an alcoholic solution of the solid was found to be −37.64°, which indicated that the borneol is chiefly levo. It is probably present in the oil, partly free and partly as acetate.

Levo-α-caryophyllene.—The third fraction, boiling at 145–170° C., was found to contain caryophyllene. The blue nitrosochloride melted at 175° C., by rapid heating at 177° C., with decomposition. The nitrosate melted at 166° C. with decomposition, which is somewhat higher than that usually given. The specific rotation in alcoholic solution of this fraction was found to be −5.77°.

Ledum Camphor.—Although all attempts to freeze out this compound from the upper fractions failed, its presence was strongly indicated by the preparation of a phenyl urethane melting at 145° C. Hjelt (2) gives this as 144–145° C. There was also found a small amount of caryophyllene in this same portion. The presence of a ketone, such as was reported by Lomidse (3) in *Ledum palustre*, could not be established.

The highest fraction was dark blue and probably contained azulene. It gave a red solution in syrupy phosphoric acid and with hydrochloric acid in acetic acid solution a red color changing to violet. The crystalline picrate was not obtained.

OIL FROM THE FLOWERS.

From the fresh flowers of *Ledum grœnlandicum* there was obtained by distillation with steam 0.058 per cent of oil, or 0.195 per cent on the dry basis. This was mildly aromatic, of a red color and slightly acid to litmus. The constants were: d_{25} 1.0332; n_D^{20} 1.51025; acid number 28.03; ester number 77.97; after acetylization 161.31. These would indicate a much higher content of alcohols and esters than in the leaf oil.

VOLATILE OIL FROM L. COLUMBIANUM.

From 397 pounds of fresh leaves, collected in June near North Bend, Oregon, there was obtained a total of 704 cc. or 641.4 Gm., which represented a yield of 0.35 per cent, or 0.95 per cent on the dry basis. Previous maceration with water before distillation increased this yield by 60 per cent.

The oil was neutral to litmus, amber in color and possessed a mild odor similar to that from the leaves of *L. grœnlandicum*. The constants were: d_{25} 0.9111; $[\alpha]_D^{25}$ +9.13°; n_D^{20} 1.4954; acid number 0.716; ester number 17.83 (6.24 per cent as bornyl acetate); after acetylization 79.56 (combined alcohol as borneol 4.90, free alcohol 12.07 per cent as borneol); methoxy value 10.73 (2.80 per cent as methyl eugenol).

Free Acids.—From 200 cc. of the oil the free acids were extracted by means of 5 per cent sodium carbonate solution as before. Acetic acid was identified in the same way, its amount being less than 0.1 per cent. There was also possibly a small amount of butyric or valeric acids.

Phenols.—Estimated volumetrically by a cassia flask, the amount of phenols was about 5 per cent, but by extraction with alkali and subsequent washing of the latter with ether only 0.15 per cent could be obtained. This had an index of refraction of 1.5134 at 20° C. Carvacrol was identified as before by color tests and by a phenyl urethane melting at 135° C.

From the remaining oil the terpenes were separated by repeated distillation up to 100° C. at 35-mm. pressure. The distillate, amounting to about 60 cc., was then carefully fractionated at ordinary pressure.

Levo-α-pinene.—In the first fraction, boiling point 155–162° C., d_{20} 0.8501, n_D^{20} 1.4700, $[\alpha]_D^{20}$ in alcoholic solution—27.04°, a nitrosochloride was obtained which melted at 103° C. Pinene was further identified by oxidation to pinonic acid whose semicarbazone melted at 204° C.

Levo-α-pinene.—The second fraction, constituting over half of the terpenes, distilled at 162–170° C., d_{20} 0.8553, n_D^{20} 1.4750, $[\alpha]_D^{20}$ −16.85° in alcohol. Oxidation by the method for β-pinene gave an acid melting at 95° C. instead of 125° C. Although none of the other terpenes could be identified, the presence of β-pinene must remain in doubt.

Dextro-α-phellandrene.—The third fraction, boiling at 170–175° C., d_{20} 0.8401, n_D^{20} 1.4772, $[\alpha]_D^{20}$ +26.68°, comprised about 10 per cent of the oil. It consisted almost entirely of phellandrene, nitrite melting at 105° C.

The remainder of the terpene fraction boiled at 175–185° C. and was dextro. Neither dipentene nor limonene could be identified.

Combined Acids.—The oil was saponified with alcoholic potash and the alkaline solution was separated and evaporated to a small volume. After acidifying and distilling, acetic acid was identified in the distillate. There was probably a small amount of other acids, judging by the odor and by the fact that ether extracted a few drops from the residue.

Levo-borneol.—The rest of the oil was submitted to fractional distillation at 30–35-mm. pressure and repeatedly refractionated. The first portion was practically solid and consisted of borneol, melting point 203° C., d_{20} 0.8972, n_D^{20} 1.4891, $[\alpha]_D^{20}$ in alcohol −33.59°, phenyl urethane melting at 138° C. It amounted to about 15 per cent of the total oil.

Levo-α-caryophyllene.—The second fraction, boiling point 170–185° C., d_{20} 0.9082, n_D^{20} 1.5054, $[\alpha]_D^{20}$ in alcohol −1.42°, was found to be caryophyllene. The blue nitrosochloride melted at 177° C. and the nitrosate at 166° C., both with decomposition. The amount was about 10 per cent.

Ledum Camphor.—The third fraction, boiling point 195–200° C., d_{20} 0.9414, $[\alpha]_D^{20}$ +15.27°, n_D^{20} 1.5120, which made up 15 per cent of the oil, gave copious quantities of a phenyl urethane melting at 145° C., which served to indicate ledum camphor. All attempts to freeze out the material failed and at no time could solid ledol be obtained.

As with the oil from *L. grœnlandicum*, no semicarbazone could be obtained from any of these fractions, which would seem to indicate that the ketone of Lomidse (3) is not present.

The highest fraction was dark blue probably because of the presence of azulene. Although the characteristic color reactions were obtained as before, no crystalline derivative could be made.

Columbenol.—By subjecting the original oil to a temperature of −15° C. for several hours, there was obtained from it about 10 per cent of crystals which we were unable to identify with any known compound and have tentatively named columbenol. The compound, crystallized from alcohol and then from ligroin, was in the form of beautiful prismatic plates, varying somewhat in general appearance depending upon the solvent. The constants found were: melting

point 55.7° C.; boiling point with some decomposition 277° C.; $n_D^{58.5}$ 1.5169, n_D^{62} 1.5155; $[\alpha]_D$ in 1 per cent alcoholic solution +5.2°; molecular refraction at 20° C. about 73. Analysis gave: Carbon 82.45, 82.70, 82.55, 82.46; hydrogen 10.55, 10.04, 10.17, 10.13 per cent. Calculated for $C_{15}H_{22}O$ requires 82.53 and 10.32 per cent.

Columbenol is colorless, odorless and bland in taste, is neutral to litmus, freely soluble in all of the usual solvents except water or dilute alcohol. It dissolves in sulphuric acid to a red-brown color and in nitric acid to a light brown, both turning gradually darker. A mixture of the two acids gives a violent reaction, finally resulting in an almost black solution. Addition of sulphuric acid to a chloroformic solution gives a deep red color which slowly changes to purple. No chemical derivatives could be prepared, such as phenyl urethane, semicarbazone, oxime, benzoate, etc. Bromine was not decolorized in ether solution notwithstanding that the molecular, refraction indicated an unsaturated compound. It is, therefore, impossible to classify columbenol but since dehydration seemed to form a hydrocarbon, we have assumed that it is a tertiary alcohol of the sesquiterpene series.

OIL FROM THE FLOWERS.

From the fresh flowers of *L. columbianum* was obtained 0.59 per cent of oil or 2.87 per cent on the dry basis. It was neutral, light amber and strongly aromatic, d_{20} 1.0182, $[\alpha]_D^{20}$ in alcohol −8.41°, n_D^{20} 1.5119, acid number 7.27, ester number 23.61 (as bornyl acetate 8.26 per cent), after acetylization 128.65 (combined alcohol 6.49, free alcohol 28.89 per cent as borneol). By freezing, columbenol could be separated from the oil in about the same proportion as in that from the leaf.

ARBUTIN.

This glucoside is reported to be present in some species of *Ledum*, including *L. grœnlandicum* (4), but we have been unable to find any record of its isolation. Uloth obtained hydroquinone by dry distillation, but most of the evidence seems to be based upon color reactions using an aqueous solution.

We have extracted the leaves of both our own species with water in the usual way in order to determine if arbutin is present. After precipitation with neutral and basic lead acetate and removal of the excess lead, the solution was evaporated under reduced pressure to a small volume. Since nothing separated even after some time, the syrupy residue was extracted with spirit of ether and the solvent was evaporated. The addition of ferric chloride to an aqueous solution of the residue gave a dark blue color, while phosphomolybdic acid produced an intense blue in an ammoniacal solution (Jungmann's test). Since these are typical of arbutin, we admit the possibility of its presence, but could not prepare hydroquinone, quinhydrone or quinone from the aqueous residue. In any event the amount must be small because it would otherwise have separated from the evaporated solution. Extraction of uva-ursi in a similar manner gave crystals of arbutin which responded to the above color reactions and yielded readily hydroquinone and its oxidation products.

ERICOLIN.

Throughout the literature are numerous references to a glucoside, ericolin, and to its occurrence in several plants, chiefly ericaceous. It was first separated by Rochleder and Schwarz (5) from the leaves of *Ledum palustre* L. as a brownish yellow resin with an intensely bitter taste. Upon hydrolysis they obtained a volatile oil which Willigk (6) assumed to have the same composition as the oil obtained

directly by distillation with steam. Although others have claimed that about thirty other plants contain ericolin, including *Ledum grœnlandicum*, we can find no reference to actual separation and purification of a glucoside. The only evidence is based upon the oily hydrolytic product, which was given (7) the formula $C_{20}H_{26}O$.

In order to learn more about this substance, we have worked with the aqueous extract as obtained under arbutin, using the leaves of each of our own species. The syrupy residue was extracted by a mixture of alcohol and ether and the resulting solution was dried and evaporated. The residue was made acid with sulphuric acid and distilled with steam, giving a product which had a strong odor typical of the higher fractions previously described. Extraction with ether yielded an amber-colored oil in small amount, n_D^{20} 1.5213 for *L. grœnlandicum*, 1.5130 for *L. columbianum*. In each case there could be prepared from this oil a phenyl urethane melting at 145° C. We conclude from these results that the leaves of both species contain a glucoside which hydrolyzes to give ledum camphor or a similar substance. If this glucoside be the unknown ericolin, which could not be isolated in the pure state, the formula given above for the hydrolytic product may not be right.

TOXICITY.

Emulsions of the two oils were separately fed to adult white rats in increasing doses up to 1.44 Gm. per Kg. weight. Except for some slight irritation during administration, the oils gave no outward physiological effects.

The powdered leaves of each species were rolled with glucose into pills and fed to a rabbit in doses up to 10 Gm. of the leaves per Kg. weight. Again no observable effects were noted.

Finally adult white rats were fed equivalent doses of columbenol, with similar negative results.

We can safely conclude that the leaves and oils of our two species are certainly not poisonous under the conditions here, because the doses used were extraordinarily high.

SUMMARY.

After a partial analysis of the leaves of *Ledum grœnlandicum* and of *L. columbianum*, during which alkaloids were found absent, the volatile oils were more carefully examined.

Fresh leaves of the former yielded 0.12 to 0.18 per cent of oil containing: 20 per cent of l-borneol, partly as acetate; 15 per cent each of 1-α-phellandrene, 1-α-caryophyllene and ledum camphor; a smaller quantity of phenols, chiefly carvacrol; some free acetic and probably other acids; probably some azulene. The fresh flowers gave 0.058 per cent of oil with much different constants.

Fresh leaves of the other species yielded 0.35 to 0.56 per cent of oil containing: 3 per cent of 1-α-pinene; 17 per cent of l-borneol, partly as acetate; 15 per cent each of ledum camphor and an unidentified terpene, probably 1-β-pinene; 10 per cent each of d-α-phellandrene, 1-α-caryophyllene and columbenol, a stearoptene, probably $C_{15}H_{22}O$; a small amount of phenols, chiefly carvacrol; some free acetic and other acids; probably some azulene. The fresh flowers gave 0.59 per cent of oil with quite different constants and containing about 10 per cent of columbenol.

Contrary to previous reputation, they were found not poisonous to rats or rabbits, even when given in enormous doses.

No evidence could be found for the presence of arbutin which had been claimed as a constituent of *L. grœnlandicum*. The glucoside ericolin may be in the leaves of both species, as attested by hydrolysis to an oil probably containing ledum camphor. However, since no one has ever isolated this glucoside in the pure state from any vegetable source, we have doubts as to the uniformity of its composition.

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SEATTLE, WASH.,
June 20, 1933.

DETERMINATION OF CERTAIN MEDICAMENTS UNDER THE INFLUENCE OF LIGHT.*

BY H. V. ARNY, A. TAUB AND R. H. BLYTHE.

I.—INTRODUCTION.

This report covers the third and fourth years of research on the deterioration of chemicals and pharmaceuticals when stored in colored glass containers. A complete report of the work of the years 1929–1931 conducted by Dr. Abraham Steinberg under the personal direction of Professor Abraham Taub and the senior author was presented by Dr. Steinberg as an "Arbeit" submitted in partial fulfilment of the requirements set for the degree of Doctor of Pharmacy of Columbia University, while the material in condensed form was published as a paper by Arny, Taub and Steinberg in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, 20 (1931), 1014 and 1153.

During 1931–1932, a second fund of $2000 was raised to continue the work where it was discontinued by Dr. Steinberg. The second fund of $2000 was obtained through the generosity of the following firms and organizations:

Subscriptions of $100 each from Burroughs Wellcome & Co., London and New York; Dow Chemical Co., Midland, Michigan; Hynson, Westcott and Dunning, Baltimore, Md.; Lehn & Fink, Bloomfield, N. J., and New York City; Merck & Co., Rahway, N. J., and New York City; Charles Pfizer & Co., Brooklyn and New York City; Smith, Kline & French Laboratories, Philadelphia, Pa.; E. R. Squibb & Sons, Brooklyn and New York City; the Upjohn Co., Kalamazoo, Mich.; and the Proprietary Association.

A subscription of $200 from William R. Warner & Co., Inc., New York.

A grant of $300 from the Breitenbach Fund of the College of Pharmacy of the City of New York. A grant of $500 from the Board of Trustees of the U. S. Pharmacopœial Convention.

As research fellow for 1932–1934, the senior author selected Rudolph H. Blythe, B.S., who gave the subject his devoted attention during two years as a stu-

* Joint Session, Scientific Section and Section on Practical Pharmacy and Dispensing, Washington meeting, 1934.