

THE LEAF OIL OF DOUGLAS FIR.*

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Pseudotsuga taxifolia (Poir.) Britt., commonly known as Douglas fir, Douglas spruce, red fir, yellow fir or Douglas pine, is a tree which grows to a height of from 180–190 feet and is from 3.5 to 6 feet in diameter. It has been introduced in various countries throughout the world but is native to the western part of North America from British Columbia to central California at altitudes of from sea level to 6000 feet. The soil and climatic conditions under which it grows vary greatly, however, a well-drained soil and a mild climate together with a moderate amount of rainfall seem to best favor its growth. It usually grows in pure dense stands although frequently it is mixed with Western hemlock.

The oil from the leaves of Douglas fir has previously been obtained and in some instances examined although the data in many cases is quite incomplete. Sweet in 1908 (1) distilled the oil from the leaves of trees growing in Washington. Besides recording a few physical constants he stated that the oil contained camphene and probably limonene. Benson in 1912 (2) subjected the oil, obtained from the leaves of Washington trees, to fractional distillation but reported no constituents. In a later paper (3) Benson together with Thompson and Wilson reported the presence of camphene and borneol in the leaf oil examined.

Schorger in 1913 (4) examined several samples of leaf oil obtained from trees growing in California. In addition to the physical constants he reported the presence of furfural, alpha pinene, beta pinene and borneol, as well as the possible presence of limonene, dipentene and acetic acid.

In 1920 Bennett (5) distilled the oil from the leaves of trees growing in England. Geraniol, bornyl acetate, either dipentene or limonene, and a trace of citral were reported as being present.

The oil from the leaves of Douglas fir, growing in Russia in the Ssuchum district, was examined in 1925 by Rutovskii, Vinogradova and Koslov (6). The physical constants were determined, but no constituents were reported as having been identified.

The following year E. Alinari (7) in Italy, found that the leaves from trees growing in that country yielded an oil which contained chiefly beta pinene together with small amounts of geraniol and nerol. Traces of unidentified higher fatty acids were also present.

EXPERIMENTAL.

The leaves and twigs, from which the oil was distilled, were gathered during the months of August and September 1934, from trees growing in the vicinity of Seattle, Washington. From 2025 pounds of the fresh material there were obtained, by steam distillation, 7374 cc. of oil which represented a yield of 0.8 per cent. The oil was yellow in color and possessed a pleasant aromatic odor.

The physical constants were determined in the usual manner and were as follows: d_{20} 0.8696; N_D^{20} 1.4732; $[\alpha]_D^{22}$ -23.5 ; saponification number 25.4; acid number 1.65; ester number (calculated) 23.75; per cent of ester (calculated as geranyl acetate) 8.3; saponification number

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after acetylation 63.3; per cent of total alcohol (calculated as geraniol) 17.2; per cent of free alcohol (calculated as geraniol) 10.7.

Free Acids: Capric Acid.—Extraction of the oil with a 5 per cent aqueous sodium carbonate solution and subsequent extraction of the acidified aqueous solution with ether, yielded 2.899 Gm. of a brown viscous liquid, which represented 0.33 per cent of free acids. It possessed an extremely pungent odor, and was strongly acid to litmus. Steam distillation of an ethereal solution of the residue yielded 1.01 Gm. of an insoluble volatile acid. Silver salts prepared from the insoluble volatile acid contained 37.2 and 38.0 per cent, respectively, of free silver. Silver caprate contains 38.5 per cent silver; therefore it was concluded that the precipitate consisted of the silver salt of capric acid and hence the insoluble portion of the distillate consisted chiefly if not entirely of capric acid. Although traces of soluble volatile acids may have been present in the aqueous portion of the distillate no satisfactory identifications could be made.

Phenols: Salicylic Acid.—Extraction of the oil with a 5 per cent aqueous solution of sodium hydroxide, and subsequent extraction of the acidified aqueous solution with ether yielded 0.6168 Gm. of crystalline residue which was equivalent to 0.07 per cent of phenols. The crystals, after recrystallization from ether, melted at 157° C., the melting point of salicylic acid. The addition of a few drops of ferric chloride solution to an alcoholic solution of the crystals produced a purple color. The odor of methyl salicylate was produced when the crystals were heated with a mixture of methyl alcohol and concentrated sulfuric acid. As a final test, 5-nitrosalicylic acid was prepared by heating the crystals with nitric acid. The melting point of the purified product was 224° C. Salicylic acid, therefore, was identified as the phenol present.

Fractionation.—The oil from which the free acids and phenols had been removed was washed with water and dried. The total of 948 cc. of remaining oil was separated into two parts by fractional distillation at 25-mm. pressure. That portion which boiled below 100° C. and represented nearly 75 per cent of the original oil was repeatedly refractionated and the following fractions were obtained:

Fraction No.	Boiling Point.		Amount.	Sp. Gr. 20° C.	N _D ²⁰ .	[α] _D ²⁰ .	Color.
	At 25 Mm.	At 769 Mm.					
I	0-75°	156-161°	119 cc.	0.8799	1.4753	-32.95°	Colorless
II	75-76	163	70	0.8633	1.4744	-34.75	Colorless
III	76-82	163-165	324	0.8625	1.4771	-29.37	Colorless
IV	82-100	165-177	176	0.8720	1.4824	-15.28	Colorless

Levo Alpha Pinene.—A portion of the first fraction was oxidized to pinonic acid by means of an 8 per cent aqueous solution of potassium permanganate. The semicarbazone of the acid was prepared and, after washing with dilute alcohol, melted at 201° C. There was no depression of the melting point when the crystals were mixed with the semicarbazone of pinonic acid prepared from oil of turpentine. The inability of Sweet (1) to detect the presence of alpha pinene in the leaf oil may have been due to the fact that reliance was placed entirely upon the nitrosochloride test alone, which has since been found to be unsatisfactory when applied to oils possessing such a high rotatory power. The writer was likewise unable to prepare the nitrosochloride.

Levo Camphene.—Fraction II was hydrated according to the method of Bertram and Walbaum (8). Not all of the oil responded to this treatment and hence it was found advisable to discard that portion which was not hydrated and which formed a separate layer. The hydrated oil was saponified with alcoholic potassium hydroxide and as a result isoborneol was obtained which when purified by recrystallization from petroleum ether melted at 204° C. The phenylurethane after purification melted at 136° C. The melting point of the crystals therefore together with that of the phenylurethane served to identify isoborneol, the hydration product of camphene.

Levo Beta Pinene.—The third fraction yielded large amounts of nopinic acid when oxidized with an alkaline solution of potassium permanganate using Wallach's method (9). The crystals melted at 126-127° C. after purification by recrystallization from ether. The preparation and identification of a relatively large amount of nopinic acid indicated that the fraction consisted chiefly of beta pinene.

Dipentene.—A portion of Fraction IV was dissolved in glacial acetic acid and brominated according to Wallach's method (10). The solid derivative when crystallized from ethyl acetate melted at 114° C. By repeated recrystallization from the same solvent the melting point was raised to 119° C. which is somewhat lower than that given for dipentene tetrabromide. In order

to confirm the presence of dipentene the dihydrochloride derivative was prepared which when purified melted at 50° C. Because of the large yield of derivatives it was assumed that the fraction consisted mainly if not entirely of dipentene.

Saponification of the Higher Boiling Portion.—That portion of the oil which boiled above 100° C. at 25-mm. pressure and totaled 190 cc., and represented approximately 19 per cent of the original oil, was saponified with alcoholic potassium hydroxide. After saponification the oil was separated, washed, dried and submitted to repeated fractionation at 16-mm. pressure. The following fractions were obtained:

Fraction No.	Boiling Point, 16 Mm.	Amount.	Sp. Gr., 20° C.	N_D^{20} .	$[\alpha]_D^{25}$.	Color.
I	100–140° C.	114 cc.	0.909	1.4841	–13.55°	Yellowish
II	140–165	4	0.891	1.5095	0.0	Brownish
III	165–190	5	0.858	1.5133	0.0	Bluish green
IV	190–above	4	Tarry residue

Geraniol.—The first and largest fraction which distilled between 100° and 130° C., was separated into several portions the greatest amount of which boiled between 125° and 130° C. It was this latter portion that gave the most satisfactory derivative of geraniol. The diphenylurethane was prepared by treating the fraction with diphenyl carbamine chloride in pyridine according to the method of Erdmann and Huth (11). The purified crystals melted sharply at 82° C. The preparation of the derivative, geraniol diphenylurethane, established the presence of the alcohol geraniol.

Azulenogenic Sesquiterpene.—Although the third fraction because of its bluish green color was suspected of containing azulene, no positive results could be obtained, probably due to the fact that if azulene were present it was only in a very small amount. When a chloroformic solution of bromine was added to a small amount of the fraction, as outlined by S. and H. Sabetay (12), a bluish green color was obtained which was indicative of an azulenogenic sesquiterpene.

No constituents could be identified in Fraction II, while Fraction IV which possessed a slightly burnt odor indicating partial decomposition was not examined.

Combined Acids: Capric Acid.—The alkaline liquid from the saponification was concentrated, acidified with dilute sulfuric acid and distilled with steam. The distillate consisted of a soluble and an insoluble portion. The insoluble layer which floated on the surface of the distillate measured 2 cc. and from it was prepared the silver salt in the usual manner. Analysis of the samples of silver salt showed them to contain 38.2 and 38.8 per cent, respectively, of silver. The silver salt of capric acid contains 38.66 per cent of silver, hence the results here obtained indicated that the insoluble combined acid was capric acid.

Acetic Acid.—Acetic acid was identified as being present in the aqueous portion of the distillate by the usual general qualitative tests.

SUMMARY.

The fresh leaves and twigs yielded 0.8 per cent of oil which consisted of 75 per cent of terpenes and 19 per cent of higher boiling constituents including an alcohol and sesquiterpenes. The composition of the oil was found to be about: 12 per cent levo alpha pinene; 7 per cent levo camphene; 33 per cent levo beta pinene; 18 per cent dipentene; 12 per cent geraniol partly as the caprate or acetate; capric acid was present in both the free and combined state, while acetic acid was found in the combined state only. The phenols, which amounted to 0.07 per cent consisted chiefly if not entirely of salicylic acid.

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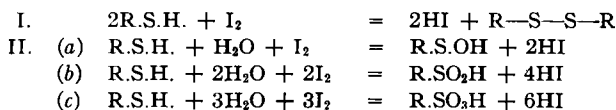
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THE ASSAY OF CYSTEINE HYDROCHLORIDE.*

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The assay of cysteine has been discussed by Okuda (1) and by Lucas and King (2). Their methods involve the use of aqueous solutions and require exact control of acidity, iodine concentration and temperature. Moreover, the factors used to calculate cysteine are more or less empirical; such factors are not as desirable to use in quantitative analysis as stoichiometrical ones derived directly from chemical equations. We have succeeded in avoiding their use.

In an aqueous acid solution, cysteine can react in more than one way. If we indicate the cysteine molecule as R.S.H., the reactions can be expressed by the equations which follow:



The oxidation of cysteine with iodine in aqueous acid solution proceeds in accordance with II as well as I. The equations under II require water as a reactant, while the equation under I does not.

We have found that the oxidation of cysteine proceeds substantially according to Equation I when carried out in such a manner that at the end of the titration the solvent medium consists of ethyl alcohol containing not more than 20% water by volume. The use of alcohol as a titrating medium and of *N*/10 aqueous iodine as oxidant permits a rapid and practically quantitative estimation of cysteine in cysteine hydrochloride monohydrate. It avoids the undesirable use of empirical factors and at the same time simplifies the determination. Another advantage in the use of alcohol as solvent is that the cysteine formed, or its salt (hydrochloride or hydroiodide) separates from the reaction mixture as a white crystalline precipitate, thus favoring the reaction in the sense given, *i. e.*, a reactant is removed from the reaction phase.

Table I gives the results obtained by the Okuda (1) method using the stoichiometrical factor derived from Equation I and an empirical factor derived from Okuda's discussion.

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