

there is no significant difference; that is, the protein in the younger plants was more profoundly affected by this condition than that from the seed stage, which was very little changed.

2—The differences between total and pure protein are greater in the alfalfa cured in the shade than in that cured in the sun or open field; that is, the proportion of pure protein decreases during the process of drying. This is most noticeable in the earlier stages. The hay cured in the sun contains a larger per cent of pure protein than that cured in the shade.

In comparing the figures obtained on the samples for 1914 with those of 1915, important differences are noticed. The per cent of pure protein in the total protein is less in every instance in the samples of 1915, and differences between the alfalfa cured in the shade and that cured in the sun are less. The more unfavorable drying conditions of 1915, with the necessity of curing under cock covers, allowed a prolongation of the vital activity of the protoplasm, resulting in protein cleavage. The per cent of pure protein in the total protein is from 6 to 9 per cent less in the alfalfa dried in the shade in 1915 than in the same lot in 1914, while the 1915 samples dried in the open field show about 9 per cent less than the 1914 samples. The larger, heavier plants of 1915 took longer to dry. The stems, which dry the slowest, show the greatest differences in comparing the samples of the two years, and the leaves, which dry the fastest, show the least difference.

These facts point to a very profitable line of investigation in regard to the chemical changes which take place under different conditions of haymaking. These changes no doubt include not only the proteins, but also the carbohydrates, fats, and phosphorus compounds. The feeding value of hay is affected not only by mechanical losses due to handling, and the changes due to bacterial action, but also by chemical changes which are little known or noticed.

#### SUMMARY

I—The alfalfa cut in the bud stage had the largest ash and crude protein and the smallest crude fiber and nitrogen-free extract.

II—In each successive stage the crude fiber and nitrogen-free extract increases, and the crude protein and ash decrease. In pounds per ton the alfalfa cut in the earlier stages has more of crude protein and less of crude fiber.

III—The total amount of any or all nutrients produced per acre depends to a large extent on the yield, as shown by the fact that in 1914 the greatest amount of nutrients was obtained in the bud stage, while in 1915 the full bloom gave the greatest amount of total nutrients.

IV—The leaves and stems differ in content of ash, ether extract, and nitrogen-free extract, but the greatest difference is in the per cent of crude protein and crude fiber. The leaves contain over  $2\frac{1}{2}$  times as much protein as the stems, while the stems contain over  $2\frac{1}{2}$  times as much crude fiber as the leaves.

V—In harvesting and handling there is a large loss of leaves, which loss affects the composition of the hay in an increase of crude fiber and a decrease of crude protein.

VI—The alfalfa cured in the sun has a larger pure protein content as determined by Stutzer's method, than that cured in the shade. This difference is so great as to more than offset the influence of the loss of leaves. The differences in respect to pure protein content were most pronounced in the alfalfa cut in the earlier stages.

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#### THE VOLATILE OIL OF *CALYCANTHUS OCCIDENTALIS*

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*Calycanthus occidentalis*, or *Butneria occidentalis*, also popularly known as spice-bush, belongs to the family *Calycanthaceae*, 4 or 5 species of which are found in the United States. The species named is limited to northern California and southern Oregon and is rarely found in large patches, but usually scattered along stream banks or river bottoms or on the lower slopes of hillsides. It is an unarmed shrub, which sometimes reaches a height of 10 ft. It bears an abundance of ovate to oblong lanceolate leaves, somewhat scabrous and of a bright green color, and dark red flowers from 1 to  $1\frac{1}{2}$  in. long. The leaves, bark and wood are all strongly aromatic; the flowers less so.

Considerable interest has been attached to some of the other species of this genus on account of their physiological action.

An alkaloid was discovered in the seeds of *C. glaucus* *C. fertilis*, by R. C. Eccles,<sup>1</sup> in 1888, and named by him Calycanthine. This was confirmed by H. W. Wiley<sup>2</sup> in 1889. H. M. Gordin,<sup>3</sup> in a number of papers, has shown the presence of two alkaloids, calycanthine and isocalycanthine.

The volatile oil of *Floridus* has been studied by Miller<sup>4</sup> and his co-workers and found to consist of pinene, cineol, borneol, bornyl acetate, salicylic acid, possibly linalool and some other esters besides the bornyl acetate. The cineol predominated.

#### EXPERIMENTAL

Four hundred pounds of leaves and twigs from Oriental, California, that had been kindly supplied by officials of the United States Forest Service, at San Francisco, were submitted to steam distillation under a pressure of 4 lbs. The yield of oil obtained from the united distillates amounted to about 0.27 per cent of the weight of the original material.

In transit considerable decomposition of the leaves had taken place and the water distilling over with

<sup>1</sup> Eccles, *Proc. Am. Pharm. Assoc.*, **36**, 84 and 382.

<sup>2</sup> Wiley, *Am. Chem. J.*, **11**, 557.

<sup>3</sup> Gordin, *Proc. Am. Pharm. Assoc.*, **52**, 345; **53**, 224.

<sup>4</sup> Miller, *J. Am. Chem. Soc.*, **26**, 2182.

the oil was strongly alkaline. After removing the oil the alkaline distillates were treated with an excess of hydrochloric acid and evaporated to dryness on the water bath. The residual salts were found to have an odor very suggestive of aliphatic amines; they were crystallized from absolute alcohol and the chlorine determined in both the alcohol-soluble salt and the alcohol-insoluble salt. The former determination gave 37.48 per cent chlorine, which corresponds with the theoretical percentage of chlorine in trimethylamine hydrochloride (*i. e.*, 37.17 per cent). The alcohol-insoluble salt gave 66.18 per cent chlorine which corresponds with the percentage of chlorine in ammonium chloride (*i. e.*, 66.37 per cent). A distillation on some fresh leaves failed to show either of these constituents and whether the trimethylamine and ammonium hydroxide were due to the decomposition of the protein matter alone or whether they were derived from the protein material plus some easily decomposable alkaloid has not been definitely settled. Qualitative determinations with phosphomolybdic acid demonstrated the presence of basic bodies in the leaves. These bodies failed to respond to tests for tannins: they were probably alkaloidal in character. The total nitrogen content was found to be 3.75 per cent by weight of the dry leaf. This nitrogen content is abnormally high if due to protein material alone.

A second sample of the plant, which was grown in a garden at Berkeley and weighed some 30 lbs., was separated into twigs and leaves and the two portions distilled separately while still fresh. The yield of oil from the leaves was about 0.15 per cent and from the twigs 0.37 per cent.

#### PHYSICAL CONSTANTS OF THE OIL

The oil had a greenish yellow appearance, a bitter taste, and a camphoraceous odor. The constants were as follows:

Density at 25° C.....	0.9295	Free acid.....	0.05
Rotation.....	+7° 28'	Saponification value....	54.3
Index of refraction $N_D^{20}$	1.4713	Acetyl value.....	33.5

The oil is soluble in all proportions in 90 per cent alcohol, and in 15 to 16 volumes of 70 per cent alcohol by volume.

#### CHEMICAL EXAMINATION OF THE OIL

**ALDEHYDES AND KETONES**—The oil did not respond to decolorized magenta or sodium bisulfite solution. When tested with phenylhydrazine a distinct cloudiness was produced. This would indicate the presence of a ketone that does not respond to the magenta or bisulfite test, such as camphor.

**FREE ACID**—The free acid value of the oil was so low that it was impossible to identify the acid.

**COMBINED ACIDS**—One hundred grams of oil were saponified with alcoholic potassium hydroxide by heating on the water bath with a reflux condenser for half an hour. Water was added to the mixture and the oil layer separated out. After distilling off

the excess of alcohol on the water bath the alkaline solution was extracted with ether to remove any adhering oil. The remaining solution was evaporated to a small volume, acidified with sulfuric acid and distilled with steam. The distillate was extracted with ether. The ether was evaporated spontaneously, leaving a reddish brown crystalline residue. When tested with ferric chloride a purple color was formed, indicating salicylic acid.

The aqueous portion remaining after the ether extraction was neutralized with sodium carbonate. An aliquot of one-tenth of the original solution was concentrated to a small bulk and precipitated in three fractions with silver nitrate. Fraction 1 yielded 1.0560 g. of Ag salt; Fraction 2, 0.5431 g. of Ag salt; Fraction 3, 0.2114 g. of Ag salt. A perceptible reduction of the silver nitrate showed the presence of formic acid. On ignition the following percentages of silver were obtained from the silver salt: Fraction 1, 65.85 per cent Ag, Fraction 2, 53.1 per cent Ag, Fraction 3, 39.9 per cent Ag. Acetic acid yields 64.6 per cent Ag, butyric acid 55.3 per cent Ag, valeric acid 51.6 per cent Ag, and capric 38.67 per cent Ag.

The combined acids consisted mainly of acetic and salicylic with traces of formic, capric and possibly butyric, or valeric, or a mixture of the two.

#### FRACTIONATION OF THE OIL

The oil was fractionated with a 3-bulb Le Bel fractionating column, the results being given in the table.

TABLE I—FRACTIONATION OF THE OIL OF *CALYCANTHUS OCCIDENTALIS* SHOWING PHYSICAL PROPERTIES OF EACH FRACTION

Fraction No.	Fractionation Temperatures °C.	Percent Distilled over	Specific Gravity 15° C.	Specific Rotation at 15° C.	Index of Refraction 15° C.
1	154–170	8.51	0.8810	+16.42°	1.4590
2	170–180	54.83	0.9119	+5.85	1.4585
3	180–190	9.47	0.9215	+6.56	1.4650
4	190–200	2.48	0.9290	+9.52	1.4665
5	200–220	8.75	0.9635	+36.42	1.4817
6	220–over	10.22	0.9365	+18.85	1.4793
7		5.01	0.9171	...	1.4884

Fractions 5, 6 and 7 were made under reduced pressure at 150–180°. The boiling points of these fractions were redetermined at atmospheric pressure.

From the fractions in Table I it will be seen that this oil contains 5 groups of constituents, the first distilling between 154–170°, the second between 170–190°, the third between 190–220°, the fourth between 220–230°, and the fifth over 230° C.

#### CHEMICAL EXAMINATION OF THE FRACTIONS

**PINENE**—Fraction 1 consisted mainly of pinene. The nitroso chloride was prepared according to the method of Wallach<sup>1</sup> and was found to melt at 102.3°; the pure nitroso chloride melts at 102–103°. The medium value of the specific rotatory power indicates that although the pinene is mainly of the dextro-rotatory form some of the laevo-rotatory form is also present. A quantitative estimation of pinene in the original oil was made by distilling off a fraction between 155 and 170° C. and placing a portion of this fraction in a cassia flask and adding a 50 per cent

<sup>1</sup> Wallach, Liebig's Ann., 245, 251; 253, 251.

resorcinol solution, which absorbed the cineol. From the residual volume it was estimated that about 8.3 per cent of the original oil consisted of pinene.

**CINEOL**—Fractions 2, 3 and 4 combined and refractionated between 175 and 180°. After twice refractionating, the portion boiling at 176° was collected. The physical constants of this fraction and of the melting point of the iodol show that this fraction is mainly cineol.

	Fraction	Pure Cineol
Boiling point.....	176-177°	176°
Density at 20° C.....	0.930	0.929
Index of refraction at 20° C.....	1.4561	1.4559
Melting point of iodol.....	111.2°	112°

A quantitative determination by the resorcinol method gave 60.32 per cent cineol by weight of the original oil.

**BORNEOL AND CAMPHOR**—Fractions 4 and 5 were combined and redistilled under vacuum. They had a strong camphoraceous odor and on freezing crystals separated out which were very difficult to remove from the adhering oil. An attempt to prepare the semicarbazone by the method of Tiemann<sup>1</sup> gave no results.

Phenylhydrazine produced a distinct cloudiness, showing the presence of a ketone. When the fraction was heated to vaporization, and the vapor permitted to cool on a microscopic slide, a white sublimate was obtained which was found to consist of two distinct substances, one crystallizing in plates, the other in smaller irregular aggregates. This would point toward a mixture of borneol and camphor. A portion of this fraction was oxidized with Beckmann's chromic acid mixture, the acid neutralized and the mixture distilled with steam. The semicarbazone was prepared from the product and was found to have the melting point 235.8°: camphor semicarbazone melts at 236-238°. This fact, as well as the evidence of a ketone in the original oil, points to a mixture of borneol and camphor. If the acetyl value be considered due to borneol then there would be 9.21 per cent borneol present. This value is a little high, as some of the sesquiterpene alcohols present combined to form the ester with acetic anhydride, thus raising this constant.

**METHYL SALICYLATE**—Fraction 6 had a strong pleasant odor of esters. Since salicylic acid was found in the original oil, methyl salicylate was suspected. This fraction was treated with cold *N*/5 potassium hydroxide solution in order to form the soluble potassium methyl salicylate salt. After extracting the remaining oil the alkali solution was neutralized with dilute acid and again extracted with ether. On spontaneous evaporation of the ether a small quantity of oil remained, having the characteristic odor of methyl salicylate. Such a small quantity was obtainable that the index of refraction alone was determined and found to be 1.5298. The index of refraction of pure methyl salicylate is 1.536 to 1.538 at 20° C. A further amount of the oil was saponified with concentrated hot alkali solution. The solution was acidified and extracted with ether. The ether extract yielded

a few crystals which gave the characteristic color reactions for salicylic acid. From these data and from the boiling point of the fraction in which this substance appears it can be safely concluded that a small quantity of methyl salicylate was present in the oil.

**LINALYL ACETATE**—The remainder of Fraction 6 had a very pleasant bergamot odor. When saponified with alcoholic potash the saponification number was found to be 277.9. The alcoholic solution was diluted and the excess alcohol distilled off on the water bath. The solution was acidified and extracted with ether. Silver nitrate was added to the solution; the per cent of silver in the silver salt obtained was found to be 64.12 per cent Ag: acetic acid combines with 64.46 per cent Ag. The empirical formula for the ester would then be  $C_{10}H_{17}OOC.CH_3$ . The ether extract from this solution was evaporated spontaneously and the residual oil refractionated. The greater proportion distilled between 197 to 198°. The specific gravity was found to be 0.8793; the index of refraction at 20° C. was 1.4691. If from this data the molecular index of refraction is calculated it is found to be 48.77. The theoretical molecular index of refraction for a compound of formula  $C_{10}H_{17}OH$  with two double bonds is 48.86. This would indicate linalool. The only other alcohol having this structure is geraniol which has a higher boiling point and a greater specific gravity than linalool. The presence of linalool was confirmed by preparing the phenyl urethane derivative which had a melting point at 65.4°: linalool phenyl urethane melts at 65 to 66°.

**SESQUITERPENE ALCOHOLS**—These remained in the fractions boiling above 230° C. Owing to the small quantity of material and due to the fact that considerable polymerization had taken place, it was impossible to determine what they were. They constituted less than 5 per cent of the original oil.

#### SUMMARY

The volatile oil of *Calycanthus occidentalis* is made up of the following constituents in the proportion given:

	Per cent
Pinene ( <i>d</i> and <i>l</i> ).....	8.30
Cineol.....	60.32
Borneol.....	9.21
Camphor.....	...
Methyl salicylate.....	...
Linalyl acetate.....	18.99
Sesquiterpene alcohols.....	...

In the above estimation of the percentages of constituents present, the pinene and cineol were determined directly. The borneol was calculated from the acetyl value: this is perhaps slightly in error, due to the presence of sesquiterpene alcohols. The linalyl acetate was estimated from the saponification value, and is slightly in error, due to a small amount of methyl salicylate.

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<sup>1</sup> Tiemann's *Ber.*, 27, 1, 815.