

an acid solution above that spontaneously produced in the extract retards or inhibits this action.

No digestion of egg albumin could be detected by the proteases in any part of the plant, but this substance invariably retards the action of the enzyme on casein. It was also found that the inhibiting influence of egg albumin on the casein digestion was proportional to the time of action and not to the temperature.

It is hoped that a more detailed investigation of individual members of the alfalfa enzymes can be undertaken in the near future.

RENO, NEVADA.

THE VOLATILE OIL OF CALYCANTHUS FLORIDUS.

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The plant family Calycanthaceae comprises two genera and six or seven species, natives of North America and eastern Asia. The family is represented in the United States by four or five species of the genus *Calycanthus* L. (*Butneria Duhamel*), all but one of which are found in the south eastern states. The remaining one is native to California. All of these are aromatic shrubs from two to ten feet high, growing on hill-sides and along streams. The flowers of the eastern species appear early in the spring, are brownish to brownish purple and exhale a delightful fragrance, compared by some to that of strawberry. Owing to their aromatic properties these plants are known commonly as Sweet Scented Shrub, Strawberry Shrub, Carolina Allspice, Florida Allspice, etc. The California species is known also by the names Spice Bush, Spice Wood, Wine Flower, etc. Plants of this latter species are a little larger than the others and have somewhat larger flowers of a livid red color.

Though this genus may not be rightly considered as being of much economic importance, two of its species, namely, *Calycanthus floridus* and *C. fertilis* (*glaucus*) are cultivated as ornamental shrubs both in this country and in Europe. Of further interest may be mentioned its reputed medicinal and poisonous properties. Thus, according to the National Standard Dispensatory, the root, leaves and bark of *C. fertilis* are much used as an antiperiodic. This undoubtedly means as a so-called domestic remedy. Kings' American Dispensatory states that the same species has been suggested as a stimulant, antiperiodic and an aromatic, while the root is said to be emetic. There are also reports of its having been poisonous to cattle. Other writers on medicinal plants include also the *C. floridus*. With the exception of the single phytochemical group of alkaloids practically nothing is known of the chemistry of this genus.

In 1888 the alkaloid calycanthine was discovered in the seeds of *Caly-*

canthus glaucus by R. G. Eccles¹ and a year later his work was confirmed by H. W. Wiley² who separated also about 47% of a fixed oil. Since then several papers have been published by H. M. Gordin³ who has shown that two alkaloids, calycanthine and isocalycanthine occur in the seeds.

According to the experiments of A. R. Cushney⁴ on the pharmacological action of calycanthine, its effect on the spinal cord is about the same as that produced by strychnine poisoning, though the spasms are shorter. "In mammals, calycanthine seems to act as a stimulant to the spinal cord and as a depressant to the heart. In frogs it has, in addition, a weak, curare-like action on the terminations of the motor nerves. The symptoms are so similar to those described in cattle from poisoning from calycanthus that there can be no doubt that the alkaloid is the chief poisonous constituent."

In 1912 Mirande⁵ reported finding in 100 parts of fresh leaves of *Calycanthus floridus* 0.004 of HCN; the same amount in 100 parts of the leaves of *C. laevigatus*, and 0.016 in 100 parts of leaves of *C. occidentalis*.

In 1912 H. W. Brooks⁶ examined the roots, leaves and flowers of *C. floridus* for alkaloids and obtained results which make the presence of alkaloids very probable, especially in the leaves.

Experimental.

The volatile oil used in this investigation was separated by steam distillation from material collected mainly in the vicinity of Auburn, Ala. According to the "Flora of the Metamorphic Region of Alabama," by F. S. Earle, our material consisted mainly, if not entirely, of *Calycanthus floridus*. Most of the material was collected after the leaves had fallen, but any leaves still remaining were removed. The shrubs were cut off just above the ground, allowed to become air dry, coarsely comminuted and then distilled with steam.

Three samples of oil were obtained, one in 1908-09, one in 1909-10 and another in 1911-12. The yield was 0.53%, 0.25% and 0.39%, respectively. The smaller, younger plants gave the best yield. This might be expected, since most of the oil is found in the bark, especially in that of younger stems.

Physical and Chemical Properties of the Oils.—In general appearance these oils were very similar, of a light yellowish color, a warm aromatic taste and a pleasant, refreshing camphoraceous odor suggestive of cineol.

¹ *Proc. Am. Pharm. Assoc.*, 36, 84 and 382 (1888).

² *Am. Chem. J.*, 11, 557 (1889); *Am. J. Pharm.*, 62, 96 (1890).

³ *Proc. Am. Pharm. Assoc.*, 52, 345 (1904); *Ibid.*, 53, 224 (1905); **THIS JOURNAL**, 27, 144 (1905); *Proc. Am. Pharm. Assoc.*, 56, 805 (1908); *Ibid.*, 57, 889 (1909); *J. Am. Pharm. Assoc.*, 1, 849 (1912).

⁴ **THIS JOURNAL**, 27, 155 (1905).

⁵ *Compt. rend.*, 155, 783, 925 (1912).

⁶ Unpublished manuscript.

All the samples were soluble in all proportions in 90% alcohol. Samples I and III were soluble in 16 and 25 parts, respectively of 70% alcohol.

The specific gravity at 25°/25° was 0.9209, 0.9161, 0.9136.

Optical rotation in 100 mm. tube was +2.85, +2.84, +6.6.

Index of refraction at 26°, 1.4675, 1.4713, 1.4753.

Saponification number 12.5, 14.40, 16.6.

Saponification number of acetylated oils 75.1, —, 65.7.

Calculated as borneol and bornyl acetate these oils contained 4.37%, 5.04%, 5.81%, respectively, of bornyl acetate, while Sample I contained 18.44% free borneol, and Sample III 14.46% of free borneol.

Tests for Aldehydes and Ketones.—With Schiff's reagent and sodium bisulfite, negative results were obtained; but phenylhydrazine, when applied according to the directions of Mulliken¹ produced cloudiness indicating the presence of a ketone. If a ketone is present it occurred in very small amount and is probably one which does not react with sodium bisulfite.

Test for Methoxy Compounds.—When tested by the Zeisel method all samples gave negative results, showing the absence of compounds containing the methoxyl group.

Identification and Quantitative Estimation of Cineol.

All fractions formed an addition product when treated with iodol. This, after recrystallization from benzene, melted at 111° with decomposition. Assayed by the official Phosphoric Acid Method, Sample I gave 36% of cineol and Sample III 69%. By the Resorcin Method Sample I gave 35% and Sample III 71%. The great difference in cineol content of the two oils may be explained in part by the fact that oil No. I was obtained from older plants and apparently contains a higher percentage of pinene.²

Examination for Phenol.—The oil was first shaken with a weak solution of sodium carbonate in order to remove free acid, then shaken several times with a 2.5% solution of potassium hydroxide; the caustic alkali solutions were extracted with ether to remove unchanged oil, then acidified with dilute sulfuric acid and again extracted with ether. After evaporation of the ether there remained a small amount of a reddish brown substance in which, on standing, a very small amount of a crystalline solid appeared. This was pressed between filter paper and tested with an alcoholic solution of ferric chloride, which gave a dirty greenish brown color. When water was added to this a purple color was formed. This is an indication of salicylic acid.

Saponification and Fractionation of the Oils.—Sample I was boiled an

¹ "Identification of Pure Organic Compounds," Vol. I, 134.

² It seems to be generally considered, however, that the younger the plants the larger the percentage of terpenes. "The Volatile Oils," Gildemeister-Kremers, Vol. I, p. 285.

hour on a water bath with an excess of 0.5 *N* alcoholic solution of potassium hydroxide. The alcohol was then distilled off on a water bath, the residue mixed with water, the oil separated, washed, dried and fractionated three times under a pressure of 3 mm. Table I shows the fractions obtained, with the rotation, index of refraction and density of each.

TABLE I.

No. of fraction.	Boiling temp.	$d_{25}^{25^{\circ}}$.	α_D .	$n_{D27^{\circ}}$.
1.....	50-55°	0.9064	+ 3.3°	1.4580
2.....	55-60°	0.9090	+ 4.1°	1.4585
3.....	60-80°	0.9203	+ 2.1°	1.4647
4.....	80-90°	0.9227	+ 9.2°	1.4796
5.....	90-100°	0.9380	+14.3°	1.4854
6.....	100-110°	0.9431	+11.7°	1.4895
7.....	110-120°	0.9401	+ 8.3°	1.4948
8.....	120-130°	0.9340	+ 4.9°	1.4982
9.....	130-140°	0.9571	- 1.1°	1.4987

These fractions were then distilled under atmospheric pressure, yielding fractions with the following boiling temperatures:

1, 155-160°; 2, 160-170°; 3, 170-176°; 4, 176-180°; 5, 180-190°; 6, 190-200°; 7, 200-210°; 8, 210-220°; 9, 220-230°; 10, 230-240°.

Sample of oil No. III, after removal of phenol, was saponified, then fractionated four times under a pressure of 15 mm. and once under atmospheric pressure. Table II shows the fractions obtained with the rotation, index of refraction and density of each.

TABLE II.

No. of fraction.	Boiling temp.	$d_{25}^{25^{\circ}}$.	α_D .	$n_{D26^{\circ}}$.
1.....	160-170°	0.898	+ 5.5°	1.45959
2.....	170-175°	0.904	+ 4.3°	1.45859
3.....	175-185°	0.921	...	1.46429
4.....	185-200°	1.46806
5.....	200-210°	0.939	+15.8°	1.47681
6.....	210-220°	0.943	+17.8°	1.48179
7.....	220-240°	0.926	...	1.48711
8.....	240-260°	0.926	+ 9.95°	1.49668
9.....	260-273°	0.895	- 4.7°	1.48841

In addition to these fractions, there was obtained from the alcohol which was distilled off from the saponification mixture a small amount of an oil which boiled between 155° and 160°.

Identification of Pinene.—The fraction boiling at 155-160° was treated by the method of P. Ehestädt¹ for the preparation of pinene nitrosyl chloride. The product obtained was purified by dissolving it in chloroform and precipitating by the addition of methyl alcohol. The substance thus obtained melted at 101-102°. A nitrobenzylamine also was prepared which melted at 122-123°. The preparation of these two com-

¹ Report of Schimmel & Co., April, 1910, 164.

pounds may be considered as proof of the presence of pinene. As shown by the optical rotation it is a mixture of dextro- and levo- α -pinene with the dextro- form slightly predominating. The low dextro-rotation may also be accounted for in part by the presence of cineol, which is optically inactive. The index of refraction of the lowest boiling fractions is lower than that of pinene while the density is higher. Both of these points of difference are likewise explained by the presence of cineol.

Identification of Cineol.—The fractions boiling between 170° and 180° in the case of oil No. I, and those boiling between 170° and 185° in the case of oil No. II, were composed chiefly of cineol, as shown by the odor, boiling temperature, density, the iodole test and by the fact that the greater part was dissolved by a 50% solution of resorcin.

Test for Linalool.—Fractions boiling between 180° and 200° were oxidized with chromic acid mixture, the mixture neutralized and distilled with steam. The liquid recovered had a light yellow color and an odor suggesting that of citral. This was treated with semicarbazide hydrochloride according to Zelinsky¹ for the preparation of semicarbazones. The substance obtained was recrystallized from methyl alcohol and the melting point determined. It began to soften at 193° and melted at about 200° . Citral forms two semicarbazones, one melting at 164° and the other at $171-172^{\circ}$. Camphor semicarbazone melts $236-238^{\circ}$. The product obtained in this experiment was probably a mixture of citral semicarbazone and camphor semicarbazone, since borneol was proved to be a constituent of the next higher boiling fraction.

Test for Camphor. Identification of Borneol.—The fractions boiling between 200° and 240° were fractionated again twice, collecting in one fraction all that portion which distilled between 200° and 220° . Some of this was treated with hydroxylamine hydrochloride in the usual manner for the preparation of camphor oxime. The result was negative. The remainder of this fraction was oxidized with Beckmann's chromic acid mixture, the acid neutralized and the mixture distilled with steam. The oil obtained was treated with hydroxylamine hydrochloride. An oxime was obtained which, after recrystallization from diluted alcohol, had the melting point, $118-119^{\circ}$. The melting point of camphor oxime is generally given as $117-119^{\circ}$.

The high boiling portion of the oil was not identified. Judging from the boiling temperature, the index of refraction and the density, it probably consists of one or more sesquiterpenes. As will be seen from Table III

TABLE III.

	Boiling point.	Density.	Rotation.	Index of refraction.
Caryophyllene	$258-260^{\circ}$	0.908 at 15°	-8.7°	1.4997 at 20°
Fraction No. 9	$260-273^{\circ}$	0.895 at 25°	-4.7°	1.4884 at 26°

¹ Ber., 30, 1541 (1897).

there is a fair degree of agreement between the physical constants of caryophyllene and those of one of the high boiling fractions of oil No. III.

But an attempt to prepare caryophyllene hydrate resulted negatively. The amount at hand was not sufficient for further tests.

Identification of Acids.

The alkaline liquid resulting from the saponification of oil No. I was evaporated to a small volume, acidified and distilled with steam. The acid distillate was neutralized with sodium hydroxide and precipitated with silver nitrate. Very decided reduction of the silver occurred, indicating the presence of formic acid.

The distillate from oil No. I was neutralized with sodium carbonate and evaporated to a small volume. Oil No. I was shaken with a 2-3% solution of sodium carbonate, the aqueous liquid evaporated to a small volume and mixed with the concentrated distillate, the mixture acidified and distilled with steam. The distillate was neutralized with sodium hydroxide and precipitated with silver nitrate. The dried salt gave upon ignition 50.2% of metallic silver.

The alkaline liquid resulting from the saponification of oil No. III was concentrated, acidified and distilled with steam. The distillate was collected in two portions, neutralized with ammonia and precipitated with silver nitrate. The amount of the first precipitate was very small. The precipitate from the second fraction yielded, upon ignition, 63.66% of metallic silver.

From the portion remaining in the flask a silver compound was prepared which, upon ignition, left a residue of 38.16% of metallic silver.

The silver salts of some of the fatty acids which occur in volatile oils contain the following percentages of silver:

Of acetic acid, 64.64%; butyric acid, 55.34%; valerianic acid, 51.64%; caproic acid, 48.39%; caprylic acid, 42.59%; capric acid, 38.67%.

The results obtained indicate therefore that the oils contain formic acid, acetic acid and at least one other (higher) fatty acid.

Summary.

From the foregoing investigation it follows that oil of *Calycanthus floridus* contains the following compounds, the cineol predominating:

1.....	<i>d</i> - α -Pinene	5.....	Linalool?
2.....	<i>l</i> - α -Pinene	6.....	Bornyl acetate
3.....	Cineol	7.....	Salicylic acid
4.....	Borneol	8.....	One or more esters in addition to bornyl acetate