

cc. of concentrated hydrochloric acid at 0° and then diluting to 100 cc. with distilled water. The solution was then filtered to clarify it. Several rotations were taken at 17° and remarkably constant results were obtained. In fact, the measurements ranged between the values recorded above.

While nitro-fibroin was found to be optically active, on the other hand, the alanine and 3-nitrotyrosine obtained by hydrolysis were both optically inactive. In this connection it is interesting to note that 3-nitrotyrosine, prepared from natural tyrosine (from silk) by action of nitric acid,¹ is likewise optically inactive.

Summary.

Nitro-fibroin² has been hydrolyzed by digestion with hydrochloric acid and 41.10 per cent of its molecule accounted for in the form of glyco-coll, alanine, and 3-nitrotyrosine. The protein is optically active, but on hydrolysis leads to the formation of optically inactive alanine and 3-nitrotyrosine. The relative proportion of tyrosine to alanine and glyco-coll in silk fibroin is 1 : 2.1 : 3.6, while in our nitro-fibroin the ratio of nitrotyrosine to alanine and glyco-coll is 1 : 1.7 : 4.4.

Tyrosine linked in fibroin is not attacked by nitric acid of specific gravity 1.12 at ordinary temperature with substitution of two nitro groups *ortho* to the phenolic hydroxyl group of this amino acid.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE BUREAU OF PLANT INDUSTRY.]

VOLATILE OIL OF *EUTHAMIA CAROLINIANA* (L). GREENE.

By G. A. RUSSELL.

Received May 2, 1916.

Euthamia Caroliniana (L). Greene (Family Compositae), is found growing in moist, sandy old fields throughout the United States from Massachusetts to Florida and west as far as Texas. The plant is indigenous to these states and occurs in greatest abundance near the coast, especially in Florida. It is rarely ever found on new land or in woods, but is very frequently found in abundance in old, moist lake bottoms or abandoned fields. It flowers from September to November and each plant produces an abundance of small lemon-yellow blossoms.

Distillation of the Volatile Oil.—On September 8, 1914, Mr. S. C. Hood, scientific assistant in charge of the Florida station of the Office of Drug and Poisonous Plant Investigations of the Bureau of Plant Industry, distilled 83 kilograms of the fresh herb gathered just previous to the flowering stage. The material was gathered in old fields adjacent to the city of Orlando in Orange County, Fla. While yet in the fresh unwilted

¹ Johnson and Kohmann, *Loc. cit.*

² Johnson, Hill and O'Hara, *Ibid.*

state the plants were distilled and yielded 576 g. of oil. The yield calculated with reference to the fresh herb was 0.693%. The oil was pale yellow in color and possessed a pleasant aromatic odor.

On January 11, 1916, a chemical examination of the oil was undertaken. The color had not changed on storage. The odor was characteristic of the plant when crushed between the fingers, somewhat sweetly aromatic and not unpleasant. The taste was acrid, slightly bitter and left a strong terebinthinate after-taste manifested at the base of the tongue.

Physical and Chemical Constants.—The specific gravity of the oil at 23° was found to be 0.8587. The low figure pointed to a predominance of terpenes in the composition of the oil. The index of refraction was 1.4805 and the specific angle of rotation at 23° was $-10^{\circ} 48'$.

No free acids were present in the sample of oil examined. The saponification value was 6.35, which is equivalent to 2.10% of esters calculated as acetate of $C_{10}H_{18}O(CH_3COOC_{10}H_{17})$. The acetylation value was 25.3, which on calculation reveals 7.01% of alcohols in the original oil. The ester number of 6.35 is equivalent to only 1.66% of alcohols. There are, therefore, 5.35% of free alcohols (as $C_{10}H_{18}O$) in the original oil.

Solubility of the Oil.—The oil was readily soluble in 6 volumes of 90% alcohol and clear in excess. It was also soluble, with slight cloudiness, in 9 volumes of 70% alcohol, and did not become clear on adding an excess of 50 volumes. The solubility results all point to a considerable quantity of terpenes present in the oil.

Tests for Phenols and Aldehydes.—Five cubic centimeters of oil were shaken with a 5% solution of sodium hydroxide and the mixture allowed to stand for twenty-four hours. No diminution was noted in the volume of oil. The alkaline solution was then removed, acidified with sulfuric acid and distilled with steam. The distillate tested with ferric chloride gave negative results. There were no phenols present in the sample of oil examined.

A quantitative test with Schiff's reagent revealed the presence of aldehydes. The characteristic purple color was produced after the mixture of oil and reagent had stood for about thirty minutes. A small amount of aldehydes was present in the oil.

Combined Acids in the Oil.—A portion of the oil was saponified with alcoholic potassium hydroxide; water was added and the whole heated on a water bath until the alcohol was expelled. The oil which separated out was removed and the aqueous alkaline solution shaken out with ether to remove any adhering oil. The alkaline solution was then reduced to a small volume on a water bath, treated with an excess of sulfuric acid and distilled with steam. The distillate was found acid to litmus and possessed a sharp though not a marked disagreeable odor. The distillate

was neutralized with 0.02 *N* potassium hydroxide and precipitated with 0.1 *N* silver nitrate. Reduction of the silver salt took place almost immediately. From the odor of the distillate, the nonseparation of oily globules on the same, and the quick reduction of the silver salt, the conclusion is drawn that the combined acid or acids present are low in carbon content and consist probably of esters of acids lower in carbon content than propionic acid.

Fractionation of the Oil.—A portion of the oil was first saponified and then fractionated under atmospheric pressure. Fractionation was carried on in a 200 cc. round bottom flask fitted with a Hempel distilling column having a neck 10 centimeters in length from constriction to delivery tube. The neck was filled three-fourths full with glass beads. Heat was applied by means of an air bath. An Anschütz thermometer was used and no corrections of temperatures were made.

TABLE I.—FRACTIONATION OF THE SAPONIFIED OIL.

Frac- tion.	Tempera- ture.	Per cent. distilled.	Color.	Odor.	Density at 23°.	$[\alpha]_D$ at 23°.	N_D at 23°.
1	170°	1.52	Water white	Slight odor of pinene	0.7992	...	1.4663
2	170-175°	51.44	Water white	Sweetly aromatic	0.8414	-3° 3'	1.4756
3	175-177°	18.04	Water white	Sweetly aromatic	0.8431	-4° 6'	1.4770
4	177-179°	9.42	Water white	Sweetly aromatic	0.8461	+13° 0'	1.4770
5	179-185°	0.50	Light yellow	Somewhat decomposed
6	185°+	19.58	Dark brown	Decomposed

Since the results obtained by fractionating the saponified oil pointed to the presence of an abundance of one constituent in this oil, a small portion of the original oil was fractionated under atmospheric pressure. The same distilling apparatus and the same source of heat was employed as in the fractionation of the saponified oil.

TABLE II.—FRACTIONATION OF THE ORIGINAL OIL.

Frac- tion.	Tempera- ture.	Per cent. distilled.	Color.	Odor.	Density at 23° C.	$[\alpha]_D$ at 23°.	N_D at 23°.
1	176°	50	Water white	Sweetly aromatic	0.8435	-2° 6'	1.4742
2	176-180°	25	Water white	Sweetly aromatic	0.8660	+9° 36'	1.4814
3	180°+	25	Yellowish brown	Slightly decomposed

A third portion of the original oil was then fractionated under 45 mm. of pressure and eight fractions secured. Heat was applied by means of an air bath and the same distilling apparatus used as in the previous fractionations.

Examination of the Fractions.—Fraction 1, which possessed a slight odor of pinene was tested by means of the nitroschloride reaction.¹ A deep blue coloration was obtained on adding 22% of the prescribed amount of hydrochloric acid. On further addition of this reagent the color disappeared. The pinene in the oil is present only in a minute amount.

¹ O. Wallach, *Annalen der Chemie*, Vol. 245, p. 251.

TABLE III.—FRACTIONATION AT THE ORIGINAL OIL UNDER 45 MM. PRESSURE.

Fraction.	Tem- perature.	Per cent. distilled.	Color.	Odor.	Density at 23°.	$[\alpha]_D$ at 23°.	n_D at 23°.
1	79-91°	5	Water white	Slight odor of pinene	0.8438
	91-92°	12					
	92-93°	13					
	93-94°	17.1					
	94-95°	5					
2	91-95°	47.1	Water white	Aromatic	0.8439	- 3° 36'	1.4765
	95-96°	8					
	96-97°	6					
	97-98°	2.9					
	98-100°	1.0					
3	95-100°	17.9	Water white	Aromatic	0.8444	- 3° 36'	1.4795
4	100-105°	5	Water white	Aromatic	0.8470	+ 5° 18'	1.4844
5	105-115°	5.4	Light yellow	Aromatic	0.8669	+ 19° 48'	1.4878
6	115-150°	4.6	Light yellow	Sweet aromatic	0.9056	- 34° 18'	1.4937
7	150-180° ¹	5	Yellow	Slightly decom- posed	0.9286	- 63° 18'	1.5042
8	Residue	10	Dark reddish brown	Slightly decom- posed

A purple color developed on allowing a portion of the fraction to stand in contact with Schiff's reagent for thirty minutes, indicating a trace of aldehydes.

Fractions 2, 3 and 4, which contain the major portion of the fractional oil, were examined for dipentene. Wallach² gives the following constants for dipentene: B. p., 177-178°; Sp. gr., 0.845 at 20°.

The boiling point of the major portion of the oil was found to be 170° to 179°, 80.42% passing over at these temperatures. The specific gravity of the fractions obtained at atmospheric pressure was from 0.8414 to 0.8461 at 23°. A more careful fractionation at 45 mm. pressure gave fractions whose specific gravity at 23° was 0.8439 to 0.8444, which corresponds very closely with those stated in the literature.

On shaking a portion of the fractions with an equal volume of concentrated sulfuric acid, a strong odor of hydrogen sulfide was developed, indicating the presence of dipentene.³

On treating these fractions with alcoholic sulfuric acid at a temperature of 50° a compound was obtained that had no optical activity.

A weighed amount of each of the fractions was treated with bromine. After standing for several days crystals separated out. These crystals of dipentene tetrabromide recrystallized from acetic ether melted at 123-124°. The fraction consist mainly of dipentene.

¹ At 180° decomposition becomes marked as indicated by a sudden fall in the temperature.

² *Ann.*, 245, 197.

³ Heusler and Pond, "Chemistry of the Terpenes," 89 (edition).

On shaking Fraction 5 with an equal volume of concentrated sulfuric acid a slight odor of hydrogen sulfide was noted. On treating with bromine a slight amount was absorbed, but no crystals separated on standing. Testing for limonene by means of the nitrosochloride gave a blue coloration at first, but this color rapidly disappeared. Evidently there is little, if any, limonene in the oil.

Fractions 6 and 7, which consist of 9.6% of the original oil, are levorotatory and of relatively high specific gravity. Their composition was not established.

Summary.

The sample of volatile oil of *Euthamia caroliniana* (L). *Greene* examined, consists mainly of dipentene with a trace of pinene and possibly a small amount of limonene. No free acids were present, although the oil had been distilled and stored for about 15 months. A small percentage of combined acids, probably formic and acetic were present. Esters were present to the extent of 2.10% calculated as $\text{CH}_3\text{COOC}_{10}\text{H}_{17}$. The presence of aldehydes was established by means of Schiff's reagent. The total amount of alcohols present was 7.01%, of which 5.35% are free, and 1.66% combined. In addition to the compounds identified, a compound or compounds having levorotatory properties and a comparatively high density, are present to the extent of approximately 10%. That portion of the sample of oil that boiled above 180° at 45 mm. pressure with decomposition constitutes approximately 10% of the volume of the original oil.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

THE CHEMICAL COMPOSITION OF OSCILLARIA PROLIFICA.

By B. B. TURNER.¹

Received April 4, 1916.

This plant, one of the *Cyanophyceae*, was first brought to the attention of the previous authors in connection with the systematic examination of the public water supplies of Massachusetts under the direction of the State Board of Health. This study was undertaken and carried on for a number of years prior to Mrs. Richards' death, because the plant is a type of blue-grass alga that has caused much trouble at water works and to consumers. It was felt that by a study of the plant some plan could be devised for successfully dealing with the class.

The life history and chemical composition of the alga have already been the subject of three papers.² It is there described how the alga

¹ This work was made possible through a fund established as a memorial to the late Ellen H. Richards.

² Isabel F. Hyams and Ellen H. Richards, *Technology Quarterly*, 14, 302 (1901); 15, 308 (1902); 17, 270 (1904).