

established that with the Brignall method there is less tendency toward the formation of enol acetates when pulegone is present than there is when the U. S. P. procedure is employed. The elimination of saponification after acetylation is also a factor in favor of the Brignall method when oils containing pulegone are being assayed.

The following constituents from among the twenty-two listed (3) for American peppermint oil have been identified in these oils: inactive α -pinene, cineol, *l*-menthone, *l*-menthol (both free and combined), pulegone (and its hydrolysis product 1,3-methylcyclohexanone).

The presence of an appreciable amount of 3,6-dimethylcoumarone tetrahydride has been detected in the Oregon oil. This constituent, first reported in 1930, is characterized by a high degree of dextro-rotatory power, a relatively high specific gravity and a rather unpleasant odor.

Small amounts of *d*-isomenthone have been found associated with pulegone in the Washington, D. C., oils. Pulegone and *d*-isomenthone are also reported to have been found together in the oil from the quite unrelated *Hedeoma pulegoides* (American Pennyroyal).

d-Neomenthol is thought to have been present in small amounts. The odor of the saponification residues in particular sug-

gested this isomeric menthol, but identification was not conclusive.

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Florida Volatile Oils.* III.—*Pycnanthemum Muticum* (Michx.) Pers. Assay Methods and Minor Constituents

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The volatile oil of *Pycnanthemum muticum* (Michx.) Pers. also known as *Koellia mutica* (Michx.) Britton or Mountain Mint was

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reported on by Werner (1) in 1931. He identified pulegone 62-72%, *d*-menthol 15%, *d*-menthone 8%. He also found an unidentified ketone, traces of acetic and formic acids, a small amount of a non-volatile acid and an indication of terpenes and sesquiterpenes. Since then plots have been continually cultivated in the Florida Medicinal Plant Garden and recently in different sections of the state. The oil has aroused much

interest because of its high pulegone content which may be utilized for the production of menthol by hydrogenation. It can also be used as a satisfactory substitute for oil of Pennyroyal. Therefore it seemed worth while (a) to improve the method of assay for pulegone which has been long and tedious and has not given uniform results in the hands of many workers; (b) to determine the physical and chemical properties of this oil from different plantings; (c) to redetermine its non-pulegone constituents.

Gildemeister (2) states that although attempts have been made repeatedly to work out a general method for the determination of aldehydes and ketones, this problem has not yet been solved in a satisfactory manner. Failure has been due to the fact that not all aldehydes and ketones react equally well with a given reagent, but that in each case only a few yield quantitative results.

The bisulfite method for the assay of ketones, introduced first in 1890 by Schimmel and Co. (3), was selected for this investigation. On this particular oil it requires as much as 15 to 20 hours. Several modifications of this method were made in an effort to make it more rapid and efficient. As reported by Werner (1) menthone occurs in this oil, but it is well known that this ketone does not combine with acid sulfite.

Gildemeister (2) advises that too much free sulfurous acid retards the reaction and recommends neutralizing it with sodium carbonate. This we have found necessary. Baeyer (4) recommends the addition of 25% of ethyl alcohol to an oil to assist the combination of a ketone with the acid sulfite. This did not prove satisfactory. Neither did such solvents as dioxane, propylene glycol, diethylene glycol, ethylene glycol, propylene glycol laurate and pyridine. Different percentages of ethyl alcohol were then tried. Best results were obtained by using 10 cc. of ethyl alcohol with 10 cc. of oil and neutralizing the excess sulfur dioxide with sodium hydroxide. When the bisulfite method was so modified it was found to be superior to the hydroxylamine method and lowered the time of pulegone assay to five hours.

Florida climatic conditions permit two crops annually. Three different lots of oil were used, each from the first seasonal crop. Two were produced in the Florida Medicinal Plant Garden in 1939 and 1940. One was from the 1940 crop produced by the United States Sugar Corporation at Clewiston, Florida. Second crops were not distilled but used for propagation.

The physical and chemical properties and pulegone content of the three lots of oil did not differ materially. In addition to the constituents identified by Werner (1) limonene was found.

EXPERIMENTAL

I. ASSAY METHODS

1. *General Bisulfite Method.*—See Gildemeister (2). A 10-cc. sample of the 1939 crop when treated by this method and heated for eight hours gave only 8% (pulegone) absorption by volume.

2. *Neutralization of Free Sulfurous Acid.*—The residual oil was again treated as above except that the free sulfurous acid was neutralized with NaOH T.S. after the oil and the bisulfite solution were mixed in the flask. Phenolphthalein was used as an indicator. While heating the solution turned red due to NaOH set free which was neutralized from time to time with 30% NaHSO₃ solution. After six hours of heating 65.9% additional (pulegone) absorption took place.

3. *Effect of Ethyl Alcohol.*—To 10 cc. of the 1939 oil 2 cc. of ethyl alcohol was added and the general bisulfite method repeated without neutralizing the free sulfurous acid. After eight hours of heating 16% (pulegone) was absorbed.

4. *Effect of Alcohol and Neutralization.*—(a) The residual oil from the last procedure was again extracted with 30% bisulfite solution neutralizing from time to time the free sulfurous acid with NaOH T.S. After six hours of heating 53% more ketone (pulegone) was absorbed.

(b) Four 10-cc. samples of the 1939 oil were each diluted with 10 cc. of ethyl alcohol and treated in like manner. After five hours of heating the absorption was 91.5, 86.0, 86.0, 85.0%—av. 87.1%. Further heating did not increase the absorption. Neither did standing at room temperature for two months. When three samples of pure pulegone were so treated 100% absorption was found.

5. *Substitution of Alcohol by Dioxane.*—Experiment (3) was repeated using dioxane instead of alcohol. Result, 5% absorption.

6. *Other Solvents.*—Equal volumes of the oil were found completely soluble in propylene glycol, diethylene glycol, propylene glycol laurate and pyridine. It was insoluble in ethylene glycol. Addition of 30% NaHSO₃ solution to the glycol mixtures of the

oil caused the oil to separate completely. With propylene glycol laurate a slight emulsion formed. One volume of pyridine is nearly soluble in four volumes of 30% NaHSO₃ solution and completely soluble in five volumes. Propylene glycol, its laurate and pyridine were selected for study.

7. *Effect of Propylene Glycol and Neutralization.*—Three 10-cc. samples of the 1939 oil were treated as in (4) using 10 cc. of propylene glycol. Average of results, 84% absorption.

8. *Effect of Propylene Glycol Laurate and Neutralization.*—Three 10-cc. samples of the 1939 oil were treated as in (4) using 1 cc. of propylene glycol laurate. Average of results, 30% absorption.

9. *Effect of Pyridine.*—Three 10-cc. samples of the 1939 oil were each diluted with 10 cc. of pyridine and heated for five hours with 30% NaHSO₃ in a cassia flask. Since pyridine is a base, no alkali was needed. Average of results, 86% absorption.

10. *Identification of Pulegone.*—In each of the above extractions the ketone absorbed was regenerated by separating the aqueous bisulfite portion from the residual oil, making alkaline with Na₂CO₃ and steam distilling. In each case pulegone was the only compound absorbed. It was identified by its odor, taste, boiling point, specific gravity, refractive index, optical rotation, semicarbazone, 2,4-dinitrophenylhydrazone and bisnitroso compound.

It thus appears that assay method (4b) is the best. The use of pyridine gives good results but its odor and toxicity are undesirable.

II. COMPARISON OF OILS

The oils from the 1939 and 1940 crops at the University of Florida and 1940 crop from Clewiston are compared in Table I.

Table I.—Comparison of Oils

Properties	1939 Crop, U. of F.	1940 Crop, U. of F.	1940 Crop, Clewiston
Color	Reddish brown	Golden yellow	Golden yellow
Odor	Mint-like	Mint-like	Mint-like
Taste	Mint-like	Mint-like	Mint-like
Sp. gr. ^{20°} / _{20°}	0.9321	0.9326	0.9320
<i>n</i> _D ^{20°}	1.4837	1.4842	1.4845
[α] _D ^{20°}	+23.32	+22.37	+22.21
Pulegone	87.3%	89.3%	90.2%

III. EXAMINATION OF CONSTITUENTS

The oil from the 1940 crop at the University of Florida was examined for non-pulegone constituents. Its physical properties and pulegone content are shown in Table I. By the U. S. P. XI procedure the following were found: A. V., 0.73; S. V., 12.47; E. V., 11.74. The latter corresponds to 4.15% of an alcohol C₁₀H₂₀O, calculated as acetate. Acetyl value, 60.6 corresponding to 16.88% of an alcohol C₁₀H₂₀O. According to Schmidt (5) pulegone probably forms enol acylates giving an *apparent* acetyl value. Tests for phenols and aldehydes were negative.

Preliminary Treatment.—From 1205 cc. of oil the acids were extracted with Na₂CO₃ solution and set aside for investigation at a future date. Pulegone was next extracted by NaHSO₃ according to assay procedure (4b). Residual oil was washed with water, dried over anhydrous Na₂SO₄ and filtered. Amount, 125 cc. *n*_D^{25°} 1.4663. One cc. of it yielded a small amount of an unidentified semicarbazone, m. p. 256–7° C., after repeated crystallization from dilute alcohol.

Fractionating Equipment.—The spiral screen packed column and still head described by Lecky and Ewell (6) were built in the Drake Laboratory, University of Florida, and used for this investigation. The washers in the column had an outside diameter of 7/16 inch and a center diameter of 3/32 inch, through which a nickel rod was inserted to provide support. Length of packing, 4 feet, 7 turns per inch in Pyrex glass 27/64 inch diameter. A common type heating jacket was used with two other insulating jackets. Flask and column were electrically heated using transformer controls. Automatic pressure control was used as described by Hershberg and Huntress (7). Efficiency of the column was determined by the method of Morton (8) using a mixture of carbon tetrachloride and benzene. The data of Rosanoff and Easley (9) for composition of gaseous and liquid phases were used. Twenty theoretical plates were found. In fractionating the oil it was found that this column was very sensitive to minute temperature changes, particularly at 5-mm. pressure. This may be due to two factors: (1) fluctuations in the line voltage; (2) the small diameter of column as compared to length. In another investigation the column operated much better at 20-mm. pressure. Voltage regulators or thermostat controls would be an added advantage.

The still head was designed for operation at total reflux with intermittent take-off, so that the distillate was collected in 2-cc. portions. After 18 portions were collected the heat could not be regulated for the reasons explained above and the flooding could not be stopped. The rest of the oil was collected in portions larger than 2 cc. without being able to record the vapor temperature. In view of these facts, it was decided to identify some of the minor constituents present in the first portions obtained. Table II gives the data of the fractionation of 120 cc. of residual oil.

The first vapors were condensed in the cold trap when the pressure was 7.6 mm. and without applying any heat to the distilling flask. This portion was too small for identification. The exact temperature of the last four portions could not be determined due to the flooding in the column. The boiling points were determined by a micro apparatus (10). The specific gravities were determined by using an ampul which held 1.3290 Gm. of distilled water at 25° C.

Limonene.—The properties of fractions 5 and 6 indicated limonene which was established by a tetrabromide derivative, m. p. 101° to 102° C., by Fisher-

Johns melting point apparatus. Fraction 6 did not yield a bromide.

Sylvestrene.—Physical properties of fractions 6 and 8 suggested sylvestrene but it failed to give the sulfuric acid color reaction or a tetrabromide.

Cineol.—Results were negative when an attempt was made to make the resorcinol derivative using fractions 3, 10 and 21.

Table II.—Fractionation of Non-Pulegone Constituents

Fraction	B. P., ° C., 5 Mm.	B. P., ° C., 760 Mm.	Sp. Gr. $\frac{25}{20}$	n_D^{20}
1	34.0–34.5	168.0	0.8401	1.4698
2	37.5–38.0	...	0.8391	1.4690
3	38.5–39.0	171.5	0.8374	1.4693
4	39.5–40.0	174.5	0.8440	1.4694
5	39.5–40.0	175.0	0.8442	1.4700
6	42.0–42.5	178.0	0.8519	1.4741
7	42.5–44.0	178.5	...	1.4750
8	47.0–47.5	179.5	0.8601	1.4664
9	51.5–52.0	...	0.8924	1.4590
10	63.5–64.0	204.0	0.8963	1.4570
11	64.0–64.5	203.5	0.8963	1.4575
12	63.0–63.5	205.5	0.8972	1.4550
13	67.0–67.5	...	0.8971	1.4589
14	67.0–67.5	211.0	0.8977	1.4591
15	67.5–68.0	211.0	0.9005	1.4587
16	71.0–71.5	211.5	0.9005	1.4610
17	72.5–73.0	...	0.9040	1.4646
18	73.5–74.0	...	0.9052	1.4669
19	63.0–65.0
20	65.0–72.0
21	67.0–75.0
22	72.0–75.0

Menthone.—This was identified in fraction 12 by means of b. p., sp. gr., n_D^{20} and its semicarbazone which melted at 183° C. after repeated crystallization from methyl alcohol.

Menthol.—The properties of Fractions 15 and 16 indicated menthol which was substantiated by preparing a phenylurethane, m. p. 107° to 108° C.,

recorded for *d*- and *l*-menthol 108° to 109° C. The stereoisomerism of the menthol was not determined.

SUMMARY

1. Assay method (4b) is recommended for determination of pulegone in the volatile oil of *Pycnanthemum muticum* (Michx.) Pers. which reduced time of assay from approximately 20 or more hours to five hours.

2. Pulegone content and physical properties of three different lots of oil have been determined and found to be very close.

3. Limonene has been identified, not previously reported.

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Derivatives from Hydrogenated Castor Oil

I. 12-Hydroxystearic Acid and Its Alkyl Esters*

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Although 12-hydroxystearic acid was first synthesized at the beginning of the century

(1) and has been obtainable from hydrogenated castor oil since 1921 (2), little has been done until recently (3) to investigate its pharmaceutical potentialities. Nor has any extensive work been done upon the synthesis and industrial applications of its derivatives. This hydroxy acid, of relatively high melting point and molecular weight, and bearing two functional groups, should provide interesting and useful synthetic compounds. With an annual pro-

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