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## Florida Volatile Oils. II.—*Mentha Piperita* L.\*

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In 1930, Dr. B. V. Christensen, Director of the School of Pharmacy, University of Florida, reported to the 4th Annual Symposium of the Genus *Mentha* (1) that a supposedly true type of *Mentha piperita* L. grown in the University's Medicinal Plant Garden the previous season had yielded an oil which assayed only 7.23% menthol and contained about 80% pulegone. He pointed out that the plant may have undergone dehybridization or a delayed reduction in the process of metabolism and expressed the hope that further study might explain the low menthol content of peppermints grown in the South.

This report was apparently based on the work of Hiner (2) who investigated oils produced in 1929 and 1930 from Florida-grown peppermint. Only the physical and

chemical constants and the ketone content, as indicated by bisulfite assay, were determined at that time.

Since several of the peppermint oils of the years 1929–1932, inclusive, were still available, it was felt that a more detailed study of their composition and especially of the non-pulegone constituents should be undertaken. It is hoped that data provided by determining and reporting the other changes which accompanied the failure to develop menthol in these abnormal oils may prove of some value to others in their study of the biogenesis of peppermint oil and the genetics of the genus *Mentha*.

The question of the possibility of mistaken identity immediately suggests itself when the abnormal character of these oils is considered. However, the original root stock was obtained from the Bureau of Plant Industry, Washington, D. C., and in 1930 specimens of the plants then growing in the medicinal plant garden at the University of Florida were sent to Washington for verification. Reports from Messrs. Sievers and Lowman stated that no mistake was probable since the specimens from Florida

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Table I.—Peppermint Oils 1929–1932

Sample No.	Origin of Root Stock	Date Distilled	Green Herb (Lbs.)	Yield, Cc.	Yield, Lbs. per Acre
2	Washington, D. C.	6/25/29	525	1330	36.03
3	Washington, D. C.	7/ 7/30	1065	1620	25.40
4	Washington, D. C.	10/28/30	400	675	11.86
5	Washington, D. C.	8/ 7/31	55	115	...
6	Oregon	8/12/32	316	630	...
7	Washington, D. C.	9/26/29	682	1230	33.15

Sample No. 1 of this series, a Japanese Peppermint Oil, was not included in this investigation.

Table II.—Physical and Chemical Constants

Sample No.	$d_{25}^{25}$	$n_D^{20}$	$[\alpha]_D^{25}$	A. V.	E. V. (Calc. as Menthyl Acetate)	E. V. after Acetyl. (Calc. as Free Menthol)	Ketone Content, Per Cent
2	0.9275	1.4838	+13.65°	2.33	13.10 (4.63%)	36.49 (8.55%)	87
3	0.9251	1.4865	+15.81°	2.31	13.20 (4.67%)	19.70 (4.89%)	88
4	0.9311	1.4860	+16.96°	2.10	10.91 (3.87%)	43.95 (9.49%)	88
5	0.9298	1.4871	+15.63°	1.15	10.35 (3.66%)	30.23 (5.68%)	86
6	0.9192	1.4670	+ 0.24°	2.04	30.94 (10.94%)	138.00 (33.44%)	3
7	0.9351	1.4880	+17.41°	2.28	11.68 (4.13%)	35.58 (6.82%)	89

compared exactly with original stock growing in the Bureau's garden and producing oils of relatively high menthol content.

In this study a contemporary oil produced from peppermint grown from Oregon root stock is also included. The particulars with respect to the origin of the several samples are given in Table I.

#### EXPERIMENTAL

*Physical and Chemical Constants.*—For each of the samples these were redetermined and the values obtained set out in Table II. The E. V. after acetylation were determined according to the U. S. P. XI. In the latter, however, the calculation was carried out as recommended by Gildemeister and Hoffmann (3), *i. e.*, % free menthol = menthol in original oil  $\times (1 - (E_1/E_2))$ . The ketone content was determined by the neutral sulfite method.

*Apparent Ester Value, Pulegone.*—In 1929, Werner (4), working with the oil of *Pycnanthemum muticum* (*Koellia mutica* Michx.), an oil found to be rich in pulegone, observed that the presence of this constituent led to abnormally high ester and free alcohol values. Upon investigation he found that pulegone itself gave apparent ester values both before and after acetylation. To check this observation a sample of pulegone, regenerated from a sulfite addition product obtained with oil No. 2, was assayed by the U. S. P. XI method with the following results: E. V., 7.02, 7.39 (av. 7.21); E. V. after acetylation, 34.80, 33.14, 34.14, 33.19 (av. 33.82).

Consequently it would appear that menthyl acetate and free menthol contents as determined by the U. S. P. XI method (see Table II) cannot be relied upon in the case of oils high in pulegone content.

*Brignall Menthol Assay.*—Brignall (5) has recently devised a rapid method for determining free menthol which is claimed to avoid some of the errors of the official method. Its application to these pulegone-

containing oils was studied and the values so obtained are compared with those from the official method in Table III.

Table III.—Comparison of Brignall Method and U. S. P. XI Method

Sample No.	Percentage Free Menthol	
	Average by Brignall Method	Average by U. S. P. Method
2	9.01	8.55
3	2.46	4.89
4	5.60	9.49
5	6.83	5.68
6	26.08	33.44
7	2.58	6.82

Although the results of the Brignall determinations are generally lower than those obtained by the U. S. P. method, this may be due to the elimination of errors other than the error due to pulegone. This is illustrated particularly by the figures shown for oil No. 6, which contained only 3% pulegone. In an effort to determine the extent of the error due to pulegone, the study of the Brignall method was extended to known mixtures:

1. To 1.0315 Gm. of oil No. 6, 0.7039 Gm. of regenerated pulegone was added and the determination of free menthol carried out. Found: 26.54% (av., Table III, 26.08%).

2. A mixture was prepared containing menthol 30 parts, pulegone 30 parts, menthone 20 parts, and *d*-limonene 20 parts (all by weight). This was then assayed by the Brignall method and the results compared with determinations using a 30% w/w solution of menthol in *d*-limonene as a control. The following free menthol values were found:

For menthol (30%) in <i>d</i> -limonene..	30.74, 29.06
For menthol (30%)-pulegone-menthone mixture.....	27.28, 29.80

Thus in no instance in which pulegone was added did the Brignall method yield a result which was significantly higher than the control.

Table IV.—Re-fractionation of Oil No. 2

Fraction <sup>a</sup>	Vol., Cc.	B. P., °C., 5 Mm.	B. P., °C., 760 Mm.	$d_{44}^{25}$	$n_D^{20}$	$[\alpha]_D^{25}$	Ketone, Per Cent <sup>b</sup>
Fraction 2							
I	4	48-50	155.4	0.8542	1.4659	-16.0°	
II	5	55-58	169	0.8413	1.4712	-48.8°	
Fraction 3							
I	1	60	170.4	...	1.4774	...	
II	5	76-80	214.2	0.9227	1.4792	+18.8°	
III	2	84	211.4	0.9636	1.4849	...	
Fraction 4 (a)							
I	12	78-79	221	0.9291	1.4816	+23.2°	88
II	40	81	221	0.9287	1.4832	+22.3°	46
III	164	83	221	0.9311	1.4850	+21.0°	94
Fraction 4 (b)							
I	67	80	221	0.9384	1.4849	+21.0°	97
II	25	81.5	222	0.9388	1.4860	+16.0°	93
Residue (Original)							
I	11	82		0.9347	1.4843	+18.2°	94
II	32	87-88		0.9372	1.4860	+14.0°	88
III	3.5	94		...	1.4888	+6.6°	..
IV	3.0	101		...	1.4962	-8.0°	59

## Residue

<sup>a</sup> Fraction 1 (4 cc.) was received in the cold trap during the original fractionation and was not re-fractionated.<sup>b</sup> Neutral sulfite method.

Table V.—Re-fractionation of Oil No. 3

Fraction	Vol., Cc.	B. P., °C., 5 Mm.	B. P., °C., 760 Mm.	$d_{44}^{25}$	$n_D^{20}$	$[\alpha]_D^{25}$	Ketone, Per Cent
Fraction 1 (Received in cold trap, not re-fractionated)							
	3.4	Below 30		0.8581	1.4680	-26.8°	
I	2.5	38	157	0.8556	1.4490	-16.8°	
II	7.5	44	168-170	0.8402	1.4706	-55.2°	
Fraction 3							
I	310	83-85	220-223	0.9332	1.4859	+23.2°	98.5
Fraction 4							
I	13	91		0.9380	1.4892	+12.4°	86
II	5	91-114		0.9451	1.4989	-4.0°	38.3
III	3.6	114-138		0.9649	1.5025	-2.0°	

## Residue

*Fractional Distillation of Washington, D. C., Oils.*—Five hundred cc. of oil No. 2 was shaken with 5%  $\text{Na}_2\text{CO}_3$  to remove free acids, washed with water and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . It was then fractionated at 20 mm. using an 18-inch column packed with small Berl saddles and re-fractionated at 5 mm. In the re-fractionation either a 6-inch water-jacketed Vigreux 24-40 standard taper column or an 18-inch 24-40 standard taper column packed with Penn State rings was used, depending on the size of the original fraction. The results of re-fractionation are given in Table IV.

After removing free acids, 380 cc. of oil No. 3 (Table I) was fractionated at 5 mm. using the 18-inch column packed with Penn State rings and then re-fractionated at the same pressure, as indicated above. The data with respect to the fractions obtained are set out in Table V.

Referring to Table I, the remainder of oil No. 3 (200 cc.), oil No. 4 (500 cc.) and oil No. 7 (500 cc.) were each treated for several days with a large volume of 30% solution of sodium bisulfite until the ketone addition reaction appeared to be complete. The unabsorbed portions, after washing and drying,

Table VI.—Fractionation of Mixture of Unabsorbed Oils

Fraction	Vol., Cc.	B. P., °C., 20 Mm.	B. P., °C., 760 Mm.	$d_{44}^{25}$	$n_D^{20}$	$[\alpha]_D^{25}$	Free Alcohol, Per Cent <sup>a</sup>
1	4	58	168.5	0.8467	1.4679	-47.04°	...
2	4	63	171	0.8399	1.4675	-68.84°	...
3	6	81	201	0.8893	1.4559	+10.40°	14.04
4	4	88-89	208-210	0.9080	1.4622	+4.32°	44.10
5	4	...	209-211	0.9267	1.4750	+2.12°	39.93
Residue							

<sup>a</sup> Brignall Method (5) (calc. as menthol).

Table VII.—Fractionation of Oil No. 6

Fraction	Vol., Cc.	Per Cent of Whole	B. P., ° C., 20 Mm.	B. P., ° C., 760 Mm.	$d_{25}^{25}$	$n_D^{20}$	$[\alpha]_D^{25}$
1	6.3	2.74	Below 30 <sup>a</sup>	165	0.8647	1.4681	+23.68°
2	4.0	1.74	58-59	171	0.8855	1.4650	-13.00°
3	9.5	4.13	62-63	175	0.8804	1.4641	-26.64°
4	16.2	7.04	82-83	206.5	0.9542	1.4787	+75.89°
5	24.0	10.43	83-85	208-209	0.9373	1.4711	+47.50°
6	33.0	14.35	86-87	211-212	0.9046	1.4570	+9.88°
7	5.7	2.42	91	213-214	0.9029	1.4618	+16.16°
8	66.8	29.04	95-97	218	0.9091	1.4689	-16.16°
9	25.0	10.87	97-101	228.5	0.9184	1.4681	-16.96°
Residue	25.0	10.87	...	...	...	...	...
Total	216.8						

<sup>a</sup> Received in the cold trap.

Table VIII.—Comparison of Physical Constants of Separated Ketone with Those Recorded for Pulegon

	B. P., ° C.	$d$	$n_D$	$[\alpha]_D$
Averages found	220-223	$d_{25}^{25}$ 0.9329	$n_D^{20}$ 1.4850	+21.60°
Grignard and Savard (7)	84/6 mm.	$d_{15}^{15}$ 0.9346	$n_D$ 1.4894	$[\alpha]_D$ +21.00°
Heilbron (8)	224	$d_{15}^{15}$ 0.9370	$n_D^{15}$ 1.4880	$[\alpha]_D$ +28.23°
Kon (9)	109/19 mm.	$d_{15}^{15-9}$ 0.9381	$n_D^{15-9}$ 1.4881	
Schimmel and Co. (3)	224	$d_{15}$ 0.9405	$n_D$ 1.48796	+20.48°
Wallach (3)	221-223	$d$ 0.9360	$n$ 1.4846	

Table IX.—Fractionation of Pulegone (250 Cc.) from Oil No. 3

Fraction	Vol., Cc.	B. P., ° C., 20 Mm.	B. P., ° C., 760 Mm.	$d_{25}^{25}$	$n_D^{20}$	$[\alpha]_D^{25}$
1	2	95	209.5	0.9162	1.4672	+59.64°
2	2	99	216	0.9211	1.4697	...
3	4	99-101	220	0.9239	1.4727	+38.56°
4	4	101	222	0.9245	1.4786	+30.20°
5	8	102	217	0.9294	1.4836	+26.04°
6	4	102-103	222.5	0.9316	1.4849	+26.16°
7	6	104	222.5	0.9291	1.4811	+34.68°
8	18	105-106	223	0.9315	1.4853	+26.88°
9	87	105-106	224.5	0.9321	1.4681	+24.99°
10	75	106-107	222	0.9315	1.4870	+22.54°
Total	228					

were mixed together and 82 cc. of this pulegone-free mixture was fractionated at 20 mm. (Table VI). An efficient 4 $\frac{1}{2}$ -foot spiral screen packed column, described by Lecky and Ewell (6) was employed.

*Fractional Distillation of Oil No. 6 (Oregon).*—Two hundred and thirty cubic centimeters of this oil, the physical and chemical constants of which differed markedly from the Washington, D. C., oils (see Table II), was taken for fractionation at 20 mm. The 4 $\frac{1}{2}$ -foot spiral screen packed column was used and the fractions listed in Table VII were obtained.

*Fractional Distillation of Pulegone.*—In Table VIII the average physical characters of the several lots of ketone separated from the Washington, D. C., oils are compared with those recorded for pulegone by different authors. The ketone was obtained either by fractionation or by regeneration from bisulfite addition product, or both.

Each of the pulegone lots was fractionally distilled using the 4 $\frac{1}{2}$ -foot screen packed column mentioned above. The data presented in Table IX may be considered typical of such fractionations.

#### IDENTIFICATION OF CONSTITUENTS

*I. Terpenes and Other Low-Boiling Constituents.*—As indicated in Tables IV, V and VI, only small portions of the fractions containing these constituents were available from the Washington, D. C., oils.

The constants of fraction 2, I (Oil No. 2) and fraction 2, I (Oil No. 3) checked reasonably well with those reported for  $\alpha$ -pinene. (Gildemeister and Hoffmann (3) for 1- $\alpha$ -pinene: b. p. 153.5° to 154.5° C.;  $d_{16}^{16}$  0.8626;  $n_D^{13}$  1.4690;  $[\alpha]_D$  -43.8°. Heilbron (8): b. p. 155° to 156° C.;  $d_{20}$  0.8595;  $n_D^{20}$  1.47299;  $[\alpha]_D$  -47.2°.) A nitrosochloride (m. p. 103° to 105° C.) was prepared, which yielded a nitrol-piperidide (m. p. 121° to 122° C.). These derivatives were considered to have resulted from the presence of the inactive  $\alpha$ -pinene.

The fractions boiling 168° to 171° C. were examined for limonene and phellandrene but neither the tetrabromide nor the nitrosite could be obtained.

*Cineol:* Fraction 1 from the mixture of oils Nos. 3, 4 and 7 (see Table VI) smelled strongly of cineol and suffered a loss of 26% when shaken with 50% resorcin solution.

*1,3-Methylcyclohexanone:* During the fractionation of one of the lots of pulegone a small amount of oil was recovered from the cold trap. Its characters were determined as follows: m. p. 168° to 169° C.;  $d_{25}^{25}$  0.9121;  $n_D^{20}$  1.4480. It yielded a crystalline semicarbazone (m. p. 178° to 179° C.). Wallach (10) records for 1,3-methylcyclohexanone: b. p. 169° C.;  $d_{21}$  0.909;  $n_D$  1.4460;  $[\alpha]_D$  +12.5°; semicarbazone 180° C.

Cineol was also found to be present in the low-boiling fractions from oil No. 6 (Oregon). Fractions 2 and 3 (see Table VII) combined to the extent of 57% and 41%, respectively, with resorcin solution. In this case a small amount (less than 1 cc.) of a colorless oil was recovered from the combined resorcin addition mixtures. It smelled strongly of cineol and the following characters were determined: b. p. 171° to 172° C.;  $n_D^{20}$  1.4588. Simonsen (7) records for cineol: b. p. 174.4° C.;  $n_D^{15}$  1.45839. The quantity available did not permit the preparation of a derivative.

*II. Menthone and Free Menthol.*—With respect to the Washington, D. C., oils, the only fractions at all suggestive of either menthone or free menthol were obtained from the fractionation of the residue uncombined with bisulfite from the mixture of oils Nos. 3, 4 and 7. Fractions 3, 4 and 5 (see Table VI) possessed a similar penetrating odor suggestive of menthol, but masked by a sweetish, though somewhat musty smell. By the Brignall method their respective free alcohol contents, calculated as menthol, were 14.04%, 44.1% and 39.93%.

*Menthone:* The constants recorded in Table VI for fraction 3 approach those given (3) for menthone: b. p. 208° C.;  $d$  0.8940;  $n_D$  1.4496. This fraction yielded a semicarbazone which melted 181 to 182° C. Melting points ranging from 184° to 186° C. are reported for menthone semicarbazone by various authors.

*Menthol:* Fractions 4 and 5 (Table VI) gave phenylurethanes which crystallized from pentane in spear-shaped masses of slender crystals (m. p. 111° to 112° C.). Menthyl phenylurethane is usually given as 111° to 112° C. The reaction mixtures also gave smaller yields of a phenylurethane which appeared to be more soluble in pentane and crystallized as short translucent rods (m. p. 107° to 108° C.). Gildemeister and Hoffmann (3) cite 107° to 108° C. as the melting point of *d*-neomenthol phenylurethane. Menthol was also identified in these fractions through its 3,5-dinitrobenzoate, m. p. 152° to 153° C. For the same Cohen and Armes (11) give 153° to 154° C.

Oil No. 6 (Oregon), in contrast to the Washington oils, gave well-defined menthone and menthol fractions at the expected levels. From fractions 6 and 7 (Table VII) good yields of a semicarbazone (m. p. 187° C.) were obtained. When hydrolyzed by treatment with oxalic acid and steam distillation, a light yellow oil was obtained. It has a menthol-like odor and the following constants: b. p. 209° to 210° C.;  $d_{25}^{25}$  0.8900;  $n_D^{20}$  1.4509;  $[\alpha]_D^{25}$  -13.76. Thus menthone was established as a major constituent of these fractions. Menthol was separated by freezing out from fraction 8 and identified through its phenylurethane and 3,5-dinitrobenzoate. In this case an  $\alpha$ -naphthylurethane (m. p. 117° to 119° C.) was also prepared. Bickel and French (12) record 119° C.

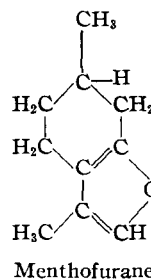
*III. Identification of 3,6-Dimethylcoumarone Tetrahydride (Menthofurane).*—Fractions 4 and 5, oil

No. 6 (Oregon), possessed boiling points almost within the menthone-menthol range and yet the observed rotations of +75.89° and +47.50°, respectively, pointed to the presence of some other constituent. These fractions apparently contained some menthone, however, and this was removed as the semicarbazone, leaving a thick, light brown oil.

Carles (13), in 1929, reported the isolation from peppermint oil of a new constituent possessing the following constants: b. p. 196° C./760 mm.;  $d_{15}^{15}$  0.965;  $n_D^{20}$  1.4807;  $[\alpha]_D +81^\circ$ . It was later identified by Wienhaus and Dewein (14) who derived its empirical formula,  $C_{10}H_{14}O$ , and established its constitution as 3,6-dimethylcoumarone tetrahydride (4:5:6:7). It was named "Menthofurane."

The specific gravity of 0.9542 found for the fraction having a plus rotation of +75.89° pointed to the possibility that 3,6-dimethylcoumarone tetrahydride was a constituent of this oil. It was also noted that colorless crystals tended to form above the surface of the liquid on the glass wall of the test-tube in which the fraction was stored. A small yield of similar crystals was obtained by oxidizing 2 cc. of the fraction with chromic acid. The melting point in each case was found to be 183° to 185° C. Wienhaus and Dewein state that the substance 3,6-dimethylcoumarone tetrahydride is oxidized in air to a colorless acid melting at 186° C.

*IV. Proof of Pulegone.*—The physical characters of the well-defined ketone fractions from the Washington, D. C., oils have been averaged in Table VIII and compared with those reported for pulegone. Pulegone was further characterized by preparation of derivatives and by hydrolysis.



*Pulegone semicarbazone:* Good yields of semicarbazone were obtained when these ketone fractions were treated with semicarbazide. The impure semicarbazone melted at 158° to 166° C. Recrystallization from alcohol failed to raise this above 171° C. However, when the procedure of Hugh, Kon and Linstead (15) was applied, the semicarbazone was obtained in the form of translucent crystals melting at 173° to 174° C. (Recorded for pulegone semicarbazone: Heilbron (8) 174° C., Gildemeister and Hoffmann (3) 167.5° to 168° C.)

*Pulegone 2,4-dinitrophenylhydrazone:* The method of Brady (16) was used in the preparation of this derivative. The red granular substance so obtained

was recrystallized from alcohol and was then found to melt rather indefinitely at 142° C. After recrystallization from pentane it was obtained in the form of dark red crystalline plates, m. p. 147° to 148° C. Campbell (17) gives 147° C. while several others record 142° C.

*Hydrolysis of pulegone:* Eighty-two cubic centimeters of regenerated pulegone (from oil No. 2) was hydrolyzed by boiling with an equal volume of 85% formic acid in an oil bath for 16 hours. The oil separated from this reaction mixture was dried and fractionated. A 17-cc. fraction had the following constants: b. p. 163.5° C.;  $d_{25}^{25}$  0.9190;  $n_D^{20}$  1.4456;  $[\alpha]_D^{25} +12.2$ ; m. p. (semicarbazone) 179° to 180° C. These results show good agreement with the values recorded by Wallach (see above) for 1,3-methylcyclohexanone.

Oil No. 6 (Oregon) was also found to contain a small amount of ketone other than menthone (see Table II). Pulegone was identified through its semicarbazone (m. p. 171° to 172° C.) as a minor constituent of fraction 9 from this oil.

*V. Identification of d-Isomenthone.*—During the preparation of pulegone semicarbazone, it was found that some other reaction product of relatively high melting point was always present in the first separation of crystalline material. After all, or the greater part, of the pulegone semicarbazone had been separated, excessive dilution of the reaction mixture always resulted in a small yield of white granular material which melted indefinitely between 125° and 150° C. The high-melting material appeared to be quite insoluble in alcohol and repeated washing with pentane and ether followed by digestion with boiling alcohol left it in the form of a residue (m. p. 256° to 260° C.). Thus the possibility of hydrazodicarbonamide (m. p. 245° to 246° C.) appeared to be excluded.

In 1907, Barrowcliffe (18), working with oil of American Pennyroyal (*Hedeoma pulegioides* (L.) Pers.), reported circumstances similar to those noted above. After regenerating the ketone from the lower-melting semicarbazone and determining its properties, he concluded *d*-isomenthone was a constituent of the oil under examination. While Barrowcliffe does not appear to have connected the low-melting semicarbazone with the material insoluble in hot alcohol, it is to be noted that Heilbron (8) records a melting point of 264° C. for *d*-isomenthone semicarbazone. It seems likely, therefore, that this ketone is capable of forming both  $\alpha$ - and  $\beta$ -semicarbazones.

*VI. Examination of High-Boiling Residues.*—In the case of the Washington, D. C. oils, the examination of the still residues boiling above the pulegone range (220° to 223° C.) was restricted by the relatively small bulk of such residues and the fact that a considerable amount of resinous material was present. This latter probably arose as a result of prolonged heating of the pulegone during fractionation.

The high-boiling residue from oil No. 6 (Oregon), while also small in quantity, was relatively free from

resin. Since the assay of the original oil had indicated the presence of 10.94% ester (calculated as menthyl acetate), this residue was saponified and the oil recovered from the saponification mixture was distilled over an oil bath at 760 mm. A small amount of an oil was obtained: b. p. 206° to 212° C.;  $d_{25}^{25}$  0.9020;  $n_D^{20}$  1.4683;  $[\alpha]_D -22.36$ . When assayed by the Brignall method, 75.42% of free alcohol (calculated as menthol) was found.

*Menthol* was identified in this case as the phenylurethane (m. p. 112° C.) and the 3,5-dinitrobenzoate (m. p. 154° C.).

#### DISCUSSION OF RESULTS

The abnormal character of the Washington, D. C., oils is apparent from their physical and chemical constants, as set out in Table II. The characters for the five samples studied may be averaged as follows:  $d_{25}^{25}$  0.9297;  $n_D^{20}$  1.4863;  $[\alpha]_D +15.89$ ; ester content (as menthyl acetate) 4.19%; free alcohol content (as menthol) 7.09%; ketone content 88%. It has been demonstrated, however, that ester and free alcohol contents determined by the U. S. P. XI method cannot be relied upon in the presence of large amounts of pulegone. The apparent ester value due to pulegone is explainable on the basis that undue resinification or polymerization is induced by prolonged heating with potassium hydroxide, some of the base being used up. In the case of the free alcohol determination, the formation of an enol acetate during acetylation where pulegone is present would appear to explain the higher ester value. Difficulty in judging the end-point in saponification mixtures was also a factor when the U. S. P. method was used.

When the Brignall method was applied to these same oils, an average of 5.30% free alcohol (calculated as menthol) was found. Furthermore when the quantity of menthol present was known and pulegone was added, the Brignall method was found to give a satisfactory result. The fact that saponification after acetylation is avoided in this procedure is believed to have been a decided advantage in the case of these pulegone-containing oils.

The oil produced from peppermint grown from Oregon root stock stood intermediate in respect to all its characters between the Washington, D. C., oils and U. S. P. stand-

ards. A free alcohol content (calculated as menthol) of 26.08% was found by the Brignall method, as compared to 33.44% by the U. S. P. XI method. Since any error due to pulegone must necessarily be very small in this case, there is evidence to support Brignall's contention that other errors are avoided in this method.

The low menthol content and high ketone content of the Washington, D. C., oils, as indicated by assay, was substantiated by the results of fractionation. The data in Tables IV and V hold no suggestion of either menthone or menthol. However, menthone was found in traces in Fraction 3, Table VI. In this case a total of 1200 cc. of oils Nos. 3, 4 and 7 was represented in the fractionation. Fractions 3, 4 and 5 from this same mixture also contained free alcohol. Brignall assays indicated 14.04, 44.1 and 39.93%, respectively (calculated as menthol). Menthol was identified in these fractions but the amount was well below even the average of 5.30% found in the original oils by the Brignall method. There was some evidence that traces of *d*-neomenthol also accompanied the menthol.

With oil No. 6 (Oregon), on the other hand, well-defined menthone and menthol fractions were obtained at the expected levels. Furthermore the total quantity of menthol separated by fractionation (see fractions 8 and 9, Table VII) was an approximation of the amount indicated by assay. 3,6-Dimethylcoumarone tetrahydride (or menthofurane) was also found to be associated with menthone and menthol in this oil. Its high dextro-rotation and the close approach of its boiling point to that of menthone are believed to have been responsible for the dextro-rotatory power observed for the menthone fractions (Fractions 6 and 7, Table VII). The low rotation of the menthol fractions, only  $-16^\circ$  approximately, may likewise have been due to contamination by menthofurane, although the presence of small amounts of *d*-neomenthol would influence this also.

Pulegone was identified as the major constituent of the Washington, D. C., oils and as a minor constituent of oil No. 6, which was produced from peppermint grown

on the same soil but from root stock of quite different origin (*i. e.*, Oregon). From the standpoint of biogenesis, the latter fact may hold equal significance with the former.

Fractional crystallization of the semicarbazones obtained from various pulegone fractions revealed that *d*-isomenthone was closely associated with pulegone in the Washington, D. C., oils. Furthermore, the relatively high degree of efficiency of the spiral screen packed column resulted in the separation from apparently pure pulegone of small amounts of oil with dextro-rotations ranging up to  $59.64^\circ$ . When these fractions were mixed and treated with semicarbazide, a white solid material (m. p.  $135^\circ$  to  $142^\circ$  C.) separated which resembled the low-melting semicarbazone obtained by fractional crystallization. Both of these reaction products resisted efforts toward recrystallization which is reported to be characteristic of *d*-isomenthone semicarbazone.

The examination of the low-boiling and high-boiling portions of the oils was generally less satisfactory because of the small amounts of such fractions available. Inactive  $\alpha$ -pinene was identified and another terpene of higher boiling range was also present. Cineol was also found to be a constituent of both the Washington, D. C., and Oregon oils. Identification of menthol in the saponification residue indicated that menthyl esters were present in the latter oil.

#### SUMMARY

Several abnormal peppermint oils produced during the years 1929-1932, inclusive, have been studied. These oils were distilled from plants grown from root stock originally obtained from Washington, D. C. A contemporary oil obtained from plants grown from Oregon root stock has also been examined.

The Washington oils have been found to consist mainly of pulegone with menthol and menthone present only in traces. The Oregon oil, although well below the official standard, contained a good proportion of menthol. It also contained a small amount of pulegone.

In connection with the quantitative determination of free menthol, it has been

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  $\alpha$ -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