

THE WATER-SOLUBLE VOLATILE CONSTITUENTS
OF AMERICAN PEPPERMINT OIL

by

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The Water-soluble Volatile Constituents of
American Peppermint Oil.

In 1917-1918, E.R. Miller cohobated the aqueous distillate resulting from the distillation of about 5500 pounds of fresh peppermint herb. From the cohobated distillate he obtained, by fractionation, about 1140 cc. of distillate which boiled below 80° C. This distillate was separated into fractions as follows:

68°-70°, 70°-74°, 74°-79°, Residue.

More than half of the distillate boiling below 80° proved to be ethyl alcohol. In addition to ethyl alcohol, the following substances were found and identified in the fractions: traces of formaldehyde and acetone, methyl alcohol, furfural, ammonia, methylamine, a small amount of one or more of the constituents of fusel oil.

Following up the work of Miller, R.E., Kremers in 1920, cohobated the aqueous distillate obtained in the distillation of wormwood, tansy, milfoil, and peppermint oils. The peppermint distillate used by Kremers represented 270 gallons of the original aqueous distillate obtained in the production of 5110 grams of peppermint oil. From this he obtained approximately 430 cc. of distillate boiling below 80° C. Kremers followed the methods of Miller and identified aldehydes (not specific), furfural, acetone, methyl alcohol,

ethyl alcohol, methylamine, and "sulphur compounds". His results differed from Miller's in that methyl, not ethyl, alcohol constituted the largest single constituent.

During the summer of 1921, 792000 grams or 1746 pounds of peppermint grown in the University of Wisconsin Medicinal Herb Garden were distilled with steam. 3297 grams of oil were obtained, 3105 grams by direct distillation and 192 grams by three cohobations. The aqueous distillate obtained by direct distillation was 242,242 cc. or 63.9 gallons. By three successive cohobations this was reduced to 69,212 cc. or 18.3 gallons respectively. The above work was done under the direction of Professor W.O. Richtman, who was in charge of the Pharmaceutical Herb Garden. The 1.5 gallons of aqueous distillate resulting from the third cohobation were turned over to the writer for examination.

Distillation of the Fresh Herb.

	Grams.
Amount of Herb Distilled	792,000
Amount of Oil Obtained	3,297
a. By direct distillation	3,105
b. By 1st, 2nd, and 3rd cohobation	192

The Aqueous Distillate

	C.C.
a. Direct Distillate	242,242
b. 1st Cohobation	69,212
c. 2nd Cohobation	19,775
d. 3rd Cohobation	5,650

Fractionation of the Aqueous Distillate Obtained from the
3rd Cohobation.

The total of 5650 cc of the aqueous distillate obtained from the 3rd cohobation was fractionated and collected in four fractions, as follows:

A. First Run:

1. Up to 95° C	24cc
2. 95° to 98°	1496cc
3. 98° to 100°	3284cc
4. 100° +	741cc
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	5545cc

The second and third fractions were further fractionated as follows:

B. Second Run.

I. Fraction 95° to 98° - 1496 cc.

1. Up to 95°	250 cc.
2. 95° to 97°	236 cc.
3. 97° to 98°	410 cc.
4. 98° to 99°	486 cc.
5. 99° to 100°	50 cc.
6. 100° +	55 cc.
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	1487 cc.

II. 98° to 100° - 3284 cc.

1. Up to 98°	145 cc.
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2. 98° to 99°	2859 cc.
3. 99° to 100°	104 cc.
4. 100° +	176 cc.
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	3284 cc.

The fractions from A and B up to 95° were combined with the fraction from B II up to 98° and then refractionated as below:

C. Third Run.

I. Up to 95° - 419 cc.

1. Up to 85°	33 cc.
2. 85° to 90°	35 cc.
3. 90° to 95°	100 cc.
4. 95° to 96°	20 cc.
5. 96° to 97°	35 cc.
6. 97° to 98°	165 cc.
7. 98° to 100°	10 cc.
8. 100° +	21 cc.
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	419 cc.

II. 98° to 99° - 2859 cc.

1. 97° to 98°	75 cc.
2. 98° to 99°	2494 cc.
3. 99° to 100°	190 cc.
4. 100° +	100 cc.
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	2859 cc.

D. Fourth Run.

I. Up to 85° - 33 cc.

1. Up to 75°	5 cc.
2. 75° to 78°	7 cc.
3. 78° to 79°	11 cc.
4. 79° +	10 cc.
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	33 cc.

II. 85° to 90° plus 10 cc. from D-I-4. - 45 cc.

1. Up to 80°	7 cc.
2. 80° to 83°	10 cc.
3. 83° to 85°	10 cc.
4. 85° +	18 cc.
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	45 cc.

III. 90° to 95° plus 18 cc from D-II-4 - 118 cc.

1. Up to 86°	20 cc.
2. 86° to 90°	20 cc.
3. 90° to 93°	3 cc.
4. 93° to 95°	10 cc.
5. 95° to 97°	20 cc.
6. 97° to 98°	15 cc.
7. 98° to 100°	20 cc.
8. 100° +	10 cc.
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	118 cc.

IV. 95° to 96° and 96° to 97° - 55 Cc.

1. Up to 93° 5 cc.

2. 93° to 95° 5 cc.

3. 95° to 97° 10 cc.

4. 97° to 98° 12 cc.

5. 98° + 23 cc.

55 cc.

V. 97° to 98° (C-I and B-I) 575 cc.

1. Up to 97° 30 cc.

2. 97° to 98° 300 cc.

3. 98° to 99° 150 cc.

4. 99° to 100° 50 cc.

5. 100° + 45 cc.

575 cc.

The combined results of the several fractionations are shown on the accompanying table.

Fractionation of the Aqueous Distillate Obtained from the
3rd Cohobation.

Fraction Number	Boiling Point.	Run # 1.	Run # 2.	Run. # 3.	Run # 4.	
1	Up to 75°				5 cc.	
2	75° - 78°	24 cc.	274 cc.	33 cc.	7 cc.	
3	78° - 79°				11 cc.	
4	79° - 80°				7 cc.	
5	80° - 83°				10 cc.	
6	83° - 85°				10 cc.	
7	85° - 86°				35 cc.	20 cc.
8	86° - 90°				20 cc.	
9	90° - 93°				100 cc.	8 cc.
10	93° - 95°				15 cc.	
11	95° - 96°				1496 cc.	236 cc.
12	96° - 97°	35cc				
13	97° - 98°	555 cc.	650 cc.	402 cc.		
14	98° - 99°	3284 cc.	3345 cc.	2980 cc.	30 cc.	3130
15	99° - 100°		154 cc.	354 cc.	394	
16	100° +	741 cc.	972 cc.	1093 cc.	1148 cc	
17	Total	5545 cc.	5536 cc.	5526 cc.	5513 cc	

Chemical Study of the Fractions.

Aldehydes.

I. Detection.

The presence of aldehydes in the various fractions was shown by the use of magenta solution, commonly known as Schiff's reagent. By the addition of a few drops of each of the fractions to test tubes containing two or three cubic centimeters of Schiff's reagent, positive tests for aldehydes were obtained from all fractions boiling below 80° .

Confirmative tests for aldehydes were made on these fractions by the use of the silver mirror tests. Only the two lower fractions, -75° and 75° to 78° , reduced the ammoniacal silver nitrate solution readily.

II. Identification.

1. Formaldehyde was shown to be present by the following tests:

a. The Hydrochloric Acid Test.

One cubic centimeter of hydrochloric acid containing a trace of iron was added to 5 cc. of milk and 6 drops of the fraction boiling below 75° . The mixture was heated to boiling. This test is best carried out in a porcelain casserole as an aid to color detection. The solution turned to a reddish-

pink color, indicating the presence of formaldehyde.

b. Shrewbury- Knapp Test.

A reagent prepared by mixing 1.6 cc. of a normal solution of nitric acid and 100 cc. of concentrated hydrochloric acid produces a violet color with very minute quantities of formaldehyde. The reagent must, however, be freshly prepared.

Ten cc. of this solution was added to five cc. of milk containing 4 drops of the fraction - 75° in a test tube. The tube was placed in a water bath at a constant temperature of 50° for 10 minutes, and then rapidly cooled to 15°. The milk turned purple, giving a decidedly positive test.

c. Hehner's Milk Test.

Milk containing formaldehyde produces, with strong sulphuric acid, a purple- violet color. The acid must contain a trace of iron which may be added in the form of ferric chloride.

About 10 cc. of milk containing 4 drops of the fraction -75° was transferred to a test tube. 5 cc. of pure sulphuric acid

containing a drop of ferric chloride solution was added in such a manner as to underlay the milk with the acid. Just before adding the acid, a few crystals of potassium sulphate were added for the purpose of bringing about better results. A purple violet color was seen just below the line of junction. This reaction was rather faint and not nearly so conclusive as tests "a" or "b".

d. Hehner's Phenol Test.

If to an aqueous solution of formaldehyde, one drop of a dilute aqueous solution of phenol be added, and the mixture be poured upon some strong sulphuric acid in a test tube, a bright crimson zone appears at the point of contact of the two liquids. Only a trace of phenol should be used, and this should be mixed with the solution to be tested before it is poured upon the sulphuric acid.

This test was decidedly positive. The positive results given by these several tests corroborate the results given by Miller and Kremers and establish beyond a doubt the presence of formaldehyde in the aqueous distillate.

2. Furfural.

The furfural test consists in treating aniline-acetate paper with portion of the fraction to be tested. The aniline-acetate paper was made by dipping filter paper into a mixture of aniline and acetic acid in the cold. The sample to be tested was heated in a test tube and a piece of aniline acetate paper was inserted. The vapors turned the paper a bright pink, showing the presence of furfural.

Upon experimentation, it was found that it was not necessary to heat the sample. One drop poured upon the test paper was sufficient to produce the pink color.

Fractions above 98° gave a negative test for furfural. Fractions 95° to 98° were most positive and the reaction graded down to 75°, below which no reaction occurred.

Ketones.

I. Identification.

Acetone.

a. Lieber's Iodoform Test.

Acetone gives the iodoform reaction in the cold, thus differing from ethyl alcohol which gives this reaction only when heated. To 2 cc. of the liquid to be tested, 3 to 5 drops of a 10 % sodium hydroxide solution were added, and then, drop by drop, half normal iodine solution until very faintly yellow. In the presence of acetone, iodoform should separate at once. A reaction for acetone was given, but judging from the slowness with which the iodoform formed, there can be only a small amount of acetone present.

b. Legal's Nitroprusside Test.

To 5 cc. of the liquid to be tested, 5 drops of freshly prepared sodium nitroprusside are added, and then 1 cc. of 10 % sodium hydroxide solution. In the presence of acetone, the liquid assumes an orange tint, which fades to a clear yellow in 15 or 20 minutes. If the experiment be repeated and the solution be made just distinctly acid with acetic acid immediately after the addition of the alkali, a purplish-

red color will develop in the presence of acetone, This color remains practically unchanged for 15 or 20 minutes. The comparative persistence of this purple color (it slowly changes- becoming more blue) serves to distinguish acetone from its homologues.

In carrying out this test, the orange-yellow color was obtained but in the second part of the test only a faint purple-red was developed.

This test, in conjunction with Lieberman's Iodoform Test, would tend to prove that there are but traces of acetone present. The above results were obtained from fractions below 78°C .

Alcohols.

I. Identification.

1. Methyl Alcohol.

a. Methyl Salicylate Test.

The fraction below 75°, when heated with salicylic acid and sulphuric acid, gave the odor of wintergreen oil -- methyl salicylate. This test was tried several times with no apparant positive results. All the test tubes were allowed to stand for about one-half hour, at the end of which time they were emptied. As the liquid was poured from test tubes, a strong odor of methyl salicylate was noticed. This odor was even more pronounced when about 10 cc. of water was poured into the emptied test tubes. Evidently, some other constituent was present in the heated mixture, which was sufficiently strong to mash, or cover up, the odor of wintergreen oil.

b. Formaldehyde Test.

Because of the small amount of the lowest fraction, it was impossible to try oxidizing the methyl alcohol to formaldehyde by means of the heated copper spiral.

2. Ethyl Alcohol.

a. Ethyl Acetate Test or Acetic Acid Test.

A test for ethyl alcohol was obtained in the fractions 78° to 79° , and -80° . The first test tried was the acetic acid test. When part of the fraction was heated with acetic acid and sulphuric acid, a very distinct odor of ethyl acetate was produced.

b. Iodoform Test.

A second positive test was the iodoform test. A portion of the fractions were heated with iodine and sodium carbonate. This yielded a yellow precipitate with a decided odor of iodoform. The amount of iodoform obtained this way was not sufficient to recrystallize from alcohol and determine the melting point. A microscopic slide, however, of the yellow precipitate was prepared and it was found that these crystals were identical with iodoform crystals.

This iodoform test is more satisfactory when sodium hydroxide is substituted for sodium carbonate. The only difference, then, between the iodoform tests for acetone and

ethyl alcohol is that iodoform will form from acetone in the cold, and with ethyl alcohol it is necessary to heat the mixture.

c. Benzoic Acid Test.

The benzoic acid test was not successful. This test consists in producing the odor of ethyl benzoate when benzoic acid and ethyl alcohol are brought together. Perhaps the original odor of the fraction was so strong as to completely cover the delicate odor of the ethyl benzoate.

Nitrogen Compounds.

I Primary Amine or Amino Group in the Benzene Nucleus.

I. Detection:

a. Carbylamine Test.

The presence of a primary amine was shown by obtaining the positive carbylamine test which consists in heating together an alcoholic solution of sodium hydroxide, chloroform, and the fraction suspected of containing the primary amine. In the presence of the primary amine, the odor of isonitrile becomes apparent. This test was run on several fractions, but in each case the results were somewhat doubtful, owing, no doubt, to the very small amount of material available. Two or three drops from each fraction were then taken, and the test tried again. This time the results were very positive.

2. Identification.

Because of the small quantity of material, it was impossible to identify the amine.

Sulphur Compounds.

I. Dimethyl Sulphide.

Peppermint oil is reported to contain some dimethyl sulphide, particularly in the so-called "Vorlauf". If this be the case, it ought certainly to be found in the aqueous distillate, since, on account of its volatility, the dimethyl sulphide collects in the first portions on rectification of the oil. The lower fractions were accordingly tested for dimethyl sulphide, giving positive results.

1. Identification.

The test consists in pouring some of the distillate or fraction, or an aqueous solution of mercuric chloride. In a short time, the formation of a soft skin is observed at the zone of contact of the two liquids.

Summary.

The aqueous distillate resulting from the distillation of peppermint oil was fractionated, and the following substances were identified as being among its constituents:

1. Aldehydes
 - a. Formaldehyde
 - b. Furfural
2. Ketones
 - a. Acetone
3. Alcohols
 - a. Methyl alcohol
 - b. Ethyl alcohol
4. Nitrogen Compounds
 - a. A primary amine
5. Sulphur Compounds
 - a. Dimethyl sulphide.

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Approved *Mellie Wakeman*-----

-----*Just in Plum Case*-----