OIL OF ACHILLEA MILLEFOLIUM, L. 1922.*

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The evaluation of the economic possibilities of a native volatile-oil-yielding medicinal plant is not rational until the variability of the oil has been investigated over a period of years and until its behavior under cultivation has been observed. For these reasons, and also because this particular oil is the best source of the blue hydrocarbon azulene, the Wisconsin Pharmaceutical Experiment Station has been growing an increasing area of *Achillea millefolium, L.*, commonly known as milfoil or yarrow. The data here recorded for an oil distilled during July, 1922, are the first afforded by the cultivated material. Inasmuch as previous researches[2,3] have established the composition of the oil fairly well, the principal attention was given to unsettled problems, *i. e.*, to the "thujone fraction" and to azulene.

The plants were harvested from a mixed stand; some were two years old, others were set out in the fall of 1921, and still others were transplanted in the spring of 1922. However, all plants were at the flowering stage. The cultivation and distillation were supervised by Prof. W. O. Richtmann of the Station. The yield of oil was 780 Gm. from the primary distillation and an additional 420 Gm. from the cohobation of the aqueous distillate. The latter represents an increase of 53.3 per cent. The total yield of oil was equal to 0.1715 per cent. of the herb distilled.

The oil possessed its characteristic azulene blue color and its usual odor. The following values were determined.

	Original oil.	Cohobated oil.
d_{25}	0.8980	0.9410
Ester No.	22.0	14.9
Ester No. after acetylation	70.9	115.7
Per cent. ester	7.8	5.3
Per cent. combined alcohol	6.1	4.2
Per cent. free alcohol	14.8	31.1
Per cent. total alcohol, $C_{10}H_{20}O$	20.9	35.3

Acids.—The free acids were removed from the oil by two extractions with 10 per cent sodium bicarbonate solution, 100 cc. and 50 cc., respectively. The separated aqueous layers were combined, filtered to remove suspended oil and acidified with an excess of 25 per cent sulphuric acid. The organic acids were then removed by steam distillation. They were recovered in the form of their Na-salts by evaporation of the neutralized distillate. The amount was too small for investigation.

Aldehydes.—Following the removal of the free acid, the oil was examined for aldehydes. Tiemann's sulphite-bicarbonate method for separating citral and citronellal (*Ber.*, 32, 822 (1899)) took out only a trace of oil. However, a 30 per cent sodium bisulphite solution yielded a small quantity of a crystalline product from which was regenerated an aldehyde having a lemon-like odor. A few milligrams of semicarbazone were obtained m. p. 72°; m. p. after recrystallization 72–75° C.

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* Contribution from the Wisconsin Pharmaceutical Experiment Station.

[1] National Research Council Fellow in Chemistry.

[2] E. R. Miller, "The Chemistry of the Oil of Milfoil," *Bull.*, 785, U. of Wis.

[3] Kremers, R. E., "The Chemistry of the Volatile Oil of Milfoil," JOUR. A. PH. A., 10, 252 (1921).

The trace of aldehyde regenerated from the bisulphite mother liquor possessed a furfural-like odor. This yielded a semicarbazone that could not be purified.

FRACTIONATION.

In order to remove any possible destructive action of dissolved inorganic substances on azulene during the subsequent fractionation, the oils were steam distilled. The higher boiling, dark blue fractions came over slowly, but completely, leaving only a green, tarry residue. The steam-distilled fractions were dried over Na_2SO_4 and were fractionated four times. The lower boiling substances yielded the following series:

B. p., ° C.	Vol., cc.	d_{25}.
-160	38	0.856
160- 65	130	0.859
165- 70	59	0.863
170- 75	45	0.868
175- 80	33	0.876
180- 85	27	0.891
185- 90	31	0.907
190- 95	37	0.917
195- 00	75	0.933
200- 05	62	0.935
205- 10	60 separated borneol	0.951
210 plus°	residue	

The cohobated oil fractionated as follows:

° C.	Cc.
-185	40
185- 95	45
195- 00	65
200° plus	residue

The residual fractions, which were colored dark blue, were united and worked up for azulene. The other fractions were appropriately combined.

Examination of Ketone Fraction.—Since thujone has been previously identified in milfoil oil, its removal was attempted as follows. After as much borneol as possible had been frozen out of fractions 200–210°, fractions 190–210° were combined, in all 220 cc. To these were added 60 cc. of alcohol and 300 cc. of 33 p. c. bisulphite solution. The mixture was shaken frequently. At the end of two weeks, however, no addition compound had separated. Hence the oil was recovered by steam distillation. No ketone was regenerated from the aqueous bisulphite residue by neutralizing with Na_2CO_3.

The rectified oil was assayed for alcohols. After acetylation in the usual manner, saponification revealed an ester number of 72.8, corresponding to 21.2 per cent bornyl acetate. The total oil was then acetylated: 200 cc. of oil, 50 cc. acetic anhydride and 20 Gm. sodium acetate were heated together on a water-bath for 4 hours. The excess of anhydride was then decomposed by water, and the oil was washed free of acid by saturated NaCl solution. The oil was fractionated *in vacuo* four times resulting in the following series:

B. p. 28 ° C.	Vol., cc.	d_{23}.	n.	α degrees.
-100	57	0.921	1.4585	−14
100– 05	44	0.937	1.4595	−20.6
105– 10	11	...	1.4620	−22.6
110– 20	33	0.956	1.4640	...
120°	10	residue		

Fractions boiling —105° were oxidized by permanganate, ref. Wallach, *Ann.*, 353, 211 (1907), to see whether any Fenchone was present.

Oil = 100 cc.
$KMnO_4$ = 230 Gm.
H_2O = 3750 cc. at 40°

The mixture was thoroughly shaken for 40 minutes, during which time a pronouncedly exothermic reaction took place. After standing over night, the oxides had settled, leaving a colorless aqueous solution. Needle-like crystals, presumably borneol, had sublimed on to the walls of the flask. The soluble salts were removed by filtration and their solution was concentrated on a water-bath to a small volume. The free acids were precipitated by dilute mineral acid and crystallized after standing some days. M. p. 78–9° C. Semicarbazone m. p. 191–2° C. These constants agree with those recorded for B—thuja ketocarbonic acid.

The acetylated fractions were saponified by refluxing them in alcohol solution with KOH for three hours. The alcohol was then largely removed by distillation and the oil precipitated by water. The product crystallized on cooling and consisted essentially of borneol.

The isolation of azulene was accomplished by the previously published technique (*J. Am. Chem. Soc.*, 45, 717 (1923)). It should, however, be reported that a repetition of the permanganate oxidation confirmed previous results. A small yield of non-volatile acid giving a phthalein reaction was again obtained. In addition, acetic acid was positively identified among the volatile acids by its conversion into acetanilide, m. p. 113–14° C.

SUMMARY.

(1) The foregoing investigation revealed that the cultivated milfoil oil obtained in 1922 had a higher density and a greater percentage of borneol than the native 1916 and 1919 oils (3) but compared very well with those obtained by Miller in 1924 (2).

(2) The constituents of the oil appeared to be identical with those of the oils previously examined by the writer. Thujone was again the only ketone identified but the failure to isolate it through the bisulphite addition product is puzzling. Fenchone was not found.

(3) The previously observed behavior of azulene toward permanganate oxidation was confirmed, and acetic acid was definitely characterized as a product.