Gas Chromatographic Analysis of the Essential Oil from *Achillea millefolium* L.

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Gas chromatography with a differential flame-ionization detector was used with polyethylene glycol adipate on Chromosorb W AW 60/80 column. Thirteen components of the essential oil were identified and quantitatively determined.

IN THE PAST 200 years the essential oil from *Achillea* species has been a subject of interest to many investigators. The earliest recorded production of the volatile oil was in 1719 by Hoffman (1), whereas Bley (2) is officially considered to be the first one to produce the oil. Many investigators directed their work towards the study of azulene in the essential oil since it is responsible for the blue coloration of the oil (2–7). Oswiecimska (8) has reported that the presence of azulene or azulene precursors in the various species of *Achillea* is related to the number of chromosomes. Oswiecimska has found that the tetraploid plants produce azulene, whereas the hexaploid and octoploid plants do not. The authors have concentrated their work on *Achillea millefolium L.* which is the hexaploid variety and therefore it is azulene free (9).

EXPERIMENTAL

Plant Material Used—The plants used were a clone which has been started from seeds of a wild-growing specimen in Bemis Woods, Forest Preserves, Chicago, Ill. The seeds were germinated in the greenhouse of the University of Illinois Drug and Horticultural Experiment Station, Downers Grove, Ill. Later generations were obtained by vegetative propagation of shoots, thus assuring a pure strain for all observations.

Stems, leaves, and inflorescences were determined for the yield of the volatile oil and it was found that the anthodia (flower heads) produce the highest amounts of the oil. The following yields of oil were obtained per 100 g. of air-dried plant parts: Stems—0.18 ml; leaves—0.41 ml.; and anthodia—1.67 ml. The essential oil free of azulene is light yellow in color.

Extraction—The essential oil was obtained by steam distillation using a Clevenger apparatus. One hundred grams of anthodia were placed in a 5000-ml. round-bottom flask and 2000 ml. of water was added. The distillation was completed in 3 hr. From 1400 g. of anthodia 23.4 ml. of essential oil was obtained.

The distillate was saturated with anhydrous NaCl and then taken up in ether. The ether extract was then washed with an aqueous solution of $NaHCO_3$ and this was followed with an aqueous solution of NaOH and finally washed with distilled water until the extract was neutral. The ether was distilled off under reduced pressure at 32°, and the essential oil was stored over anhydrous Na_2SO_4 and refrigerated.

Apparatus—The oil was analyzed using a gas chromatograph (Perkin-Elmer model 881), with a differential flame-ionization detector. A recorder (Sargent SR) was employed, which was equipped with disk chart integrator model 204. Helium was used as a carrier gas, and the flow rate was maintained at 15 ml./min., which the chart speed of the recorder was 1.27 cm./min. and in the course of analysis was changed to 2.54 cm./min. as indicated on the chromatogram (Fig. 1). The gas chromatograph was equipped with automatic temperature programming.

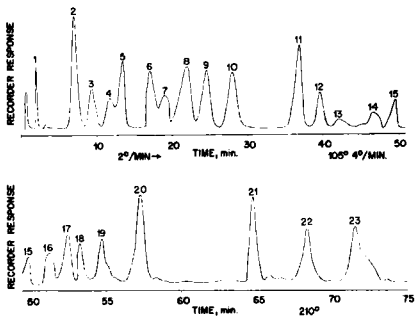

Fig. 1—Gas chromatographic separation of components of Achillea *oil on polyethylene glycol adipate. The attenuation settings are as follows: ×1K—1, 2, 8, 11, 16, 18; ×2K—3, 4, 5, 19; ×200—6, 7, 20–23; ×500—9, 10, 12–14; ×10K—15; ×5K—17. (For operating conditions see Table II.)*

Column Preparation—Two 152.4 cm. (6 ft.) columns were packed with 15% polyethylene glycol adipate as the stationary liquid phase. Polyethylene glycol adipate[1] was supported on Chromosorb W AW 60/80 mesh. One column served as reference and another as sensing. Columns were made of glass, with 0.71 cm. (0.28 in.) o.d. and 0.191 cm. (0.075 in.) i.d. and were cylindrically coiled.

Operating Conditions—Dual detector was used. A 4.5-μl. sample was used. Sample was injected at 55°. The temperature was kept constant for 15 min. After that time the programming was started at a rate of 2°/min. which was changed to 4°/min. when a temperature of 110° was reached. With the elution of Component 15 the chart speed was changed from 1.27 cm. to 2.54 cm./min. Maximum temperature at which 15% polyethylene glycol adipate column was operated was 210°.

Oil Sample and Standard Compounds—For purposes of identification, the retention times and relative retention times were compared with a known pure standard sample. This provided a tentative identification of most of the compounds. Table I lists the retention times, and relative retention times of standard compounds.

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[1] Reoplex 400.

Table I—Retention Times and Relative Retention Times of Standard Compounds on Polyethylene Glycol Adipate at a Flow Rate of 15 ml./min.[a]

Standard Compd.	Retention Times, min.	Relative Retention Times
Menthol	2.0	0.20
(+)-α-Pinene	7.0	0.70
DL-Camphene	9.8	1.00
(−)-β-Pinene	12.3	1.21
L-Limonene	13.8	1.40
Cineole	21.9	2.30
p-Cymene	36.5	3.80
L-Borneol	41.8	4.40
Isovaleric acid	46.9	4.90
1-(−)-Camphor	49.5	5.20
Isobutyl acetate	51.9	5.41
Furfuryl alcohol	54.8	5.71
Terpineol	59.4	6.21

[a] DL-Camphene = 1.00

This procedure was followed by enrichment method in which known compounds were added individually to the sample chromatographed and compared in each instance to the oil alone. In each instance, on addition of standard compound, the height of the tentatively identified peak was increased. Table II lists relative retention times of individual components as compared with standard compounds.

Table II—Comparison of Relative Retention Times of the Components of *Achillea* Oil with Standard Compounds[a]

Peak No.	Compd.	Unknown	Known
1	Menthol	0.20	0.20
2	(+)-α-Pinene	0.70	0.70
3	DL-Camphene	1.00	1.00
4	(−)-β-Pinene	1.21	1.21
5	L-Limonene	1.40	1.4
6	Unidentified	1.80	—
7	Unidentified	2.01	—
8	Cineole	2.30	2.30
9	Unidentified	2.60	—
10	Unidentified	2.91	—
11	*p*-Cymene	3.81	3.80
12	Unidentified	4.11	—
13	L-Borneol	4.40	4.40
14	Isovaleric acid	4.90	4.90
15	1-(−)-Camphor	5.20	5.20
16	Isobutyl acetate	5.50	5.41
17	Furfuryl alcohol	5.80	5.71
18	Unidentified	5.91	—
19	Terpineol	6.21	6.21
20	Unidentified	6.71	—
21	Unidentified	8.31	—
22	Unidentified	9.10	—
23	Unidentified	9.71	—

[a] Stationary phase—polyethylene glycol adipate; helium flow rate—15 ml./min.; DL-Camphene = 1.00.

Table III—Percent Yield of Components of *Achillea* Oil

Component	Percent
Menthol	1.04
(+)-α-Pinene	8.21
DL-Camphene	5.98
(−)-β-Pinene	5.49
L-Limonene	10.70
Cineole	9.70
p-Cymene	7.30
L-Borneol	0.41
Isovaleric acid	0.81
1-(−)-Camphor	20.61
Isobutyl acetate	1.41
Furfuryl alcohol	11.41
Terpineol	4.30
Unidentified	12.63
Total	100.00

The percentage yield of each tentatively identified component was determined and is presented in Table III.

DISCUSSION

The gas chromatographic analysis indicates the presence of 23 compounds of which 13 were tentatively identified. The remaining 10 components are present in about 12.5% of the total amount of compounds. An analysis of *Achillea fragrantissima* was made by Shalaby and Richter (11) and it revealed the presence of 17 compounds of which identifications were indicated in 14 cases. It has been found that *A. millefolium* has 11 compounds not found in *A. fragrantissima* and that the latter has 12 compounds not detected in *A. millefolium*.

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Keyphrases

Achillea millefolium essential oil—analysis
Oil, essential, *A. millefolium*—constituents
GLC—separation, identity