Chromatographic Investigation of the Essential Oil of *Achillea fragrantissima*

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Achillea fragrantissima oil was obtained by steam distillation in a yield of 0.83 per cent (v/w)—d_{23}^{23} 0.9001, $[\alpha]_D^{20}$ + 8.25°, n_D^{23} 1.4640. It was light yellow and had a fresh aromatic odor. The oil was free from azulene, proazulene, aldehydes, and acids; but it contained the following: terpene hydrocarbons—α-pinene, *d*-myrcene, and sabinene; esters of formic, acetic, and butyric acids (the alcoholic parts of the esters were *l*-linalool, α-terpineol, *n*-hexen(3)-ol(1), and a small amount of nerol); ketones—ethyl *n*-amylketone and a short-chain unsaturated, unidentified ketone; phenols—eugenol and carvacrol; alcohols—*l*-linalool, *n*-hexen(3)-ol(1), 3-nonanol, and the possible presence of methanol, ethanol, and *n*-butanol is indicated.

ALTHOUGH MANY of the *Achillea* species have been investigated extensively, there have been no reports on the chemical constituents of *A. fragrantissima* (Forsk.) Sch. Bip., a xerophyte growing in the Egyptian desert.

The present work was carried out to determine the physical constants of the oil, to investigate the azulene and proazulene present, to separate the other oil components, and to identify the terpene hydrocarbons, esters, carbonyl compounds, phenols, and alcohols.

EXPERIMENTAL

The plant material used in the present investigation was collected by A. F. Shalaby from Wadi Hoff near Helwan, about 25 Km. South of Cairo, and was determined systematically (1).

Volatile Oil

The plant material, cut into small pieces, was subjected to water distillation in B.P. apparatus. The oil yield, 7 ml., was 0.83% (v/w) on the fresh-weight basis. The oil was neutral to pH paper. On cooling to −23°, there was no deposit from it. On treating the oil with EP-reagent (2), a negative result was obtained, an indication of the absence of azulenes.

The oil was pale yellow with a fresh aromatic odor. After drying over anhydrous sodium sulfate, the oil had the following physical constants: d_{23}^{23} 0.9001; n_D^{23} 1.4640; $[\alpha]_D^{20}$ + 8.25°.

Chromatographic Separation of the Oil Components

Thin-Layer Chromatogram.—The oil (1:9 in benzol) was spotted on a chromatoplate covered with Kieselgel G (3), and the chromatograph was developed with benzol:ethyl acetate (95:5). The developed plate was dried at room temperature, sprayed with antimony pentachloride, and dried at 105°. Dark spots formed with terpene hydrocarbons, esters, and alcohols, and a reddish spot with phenols (Fig. 1). The plate was then sprayed with 2,4-dinitrophenylhydrazine (DNPH), and a yellow spot formed with the carbonyl compounds.

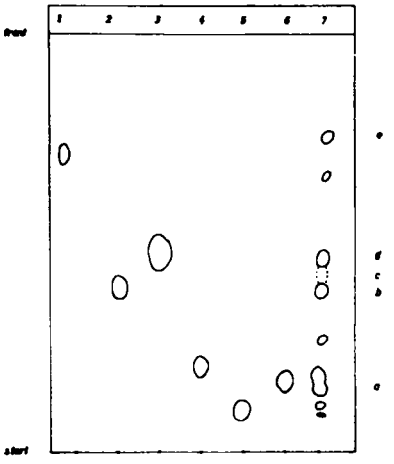

Fig. 1.—Chromatoplate of 1, α-pinene; 2, ethyl *n*-amylketone; 3, terpinyl acetate; 4, *l*-linalool; 5, *n*-hexen(3)-ol(1); 6, 3-nonanol; 7, achillea oil; a, alcohols; b, ketones; c, phenols; d, ethers; e, terpenes.

Column Chromatography.—The different components of the oil were separated on a silica gel column as follows: 0.5 ml. of oil was put on top of a silica gel[1] column. The packed column was 26.5 cm. high, with a diameter of 0.8 cm. About 100 ml. of petroleum ether (b.p. 30–50°) was added to the column, and twelve 7.5-ml. fractions were collected. These fractions contained the terpene hydrocarbons. The column was then eluted with benzol–petroleum ether (1:1), and 81 fractions were collected. A plate chromatogram was developed with each fraction, using the aforementioned system. For detection of the terpene hydrocarbons, esters, alcohols, and phenols, the plate was sprayed with antimony pentachloride reagent. For detection of ketones, the plate was then sprayed with DNPH. The results are shown in Fig. 2 and indicated in Table I.

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[1] Kieselgel Merck, 0.2–0.5 mm., activated at 150° for 3 hours.

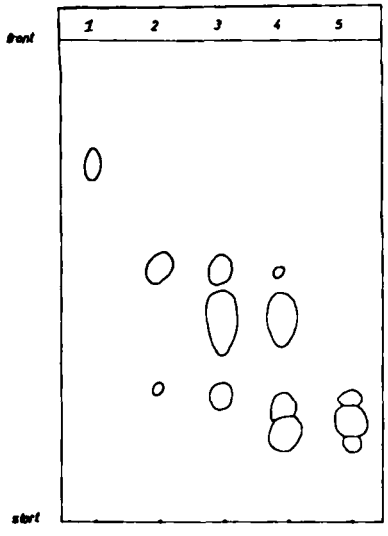

Fig. 2.—Plate chromatogram of different fractions from *A. fragrantissima* oil obtained by separation on kieselgel column: 1, fractions 1–12: terpenes; 2, fractions 13–16: esters and alcohols; 3, fractions 17–20: esters, ketones, and alcohols; 4, fractions 21–37: esters, ketones and alcohols; 5, fractions 38–93: alcohols.

Terpene Hydrocarbons

Gas Chromatographic Analysis.—Details of the two columns used for identification of the terpene hydrocarbons fraction, liquid phase, and operating conditions are summarized in Table II. The pure terpene fractions (from 1–12, Table I) were evaporated *in vacuo* at room temperature, and 10 μl. of the residual terpenes was injected on each of the two columns. Figures 3 and 4 show the results obtained with experiments *A* and *B*, respectively, which indicated the presence of α-pinene, *d*-myrcene, and sabinene. These results were also confirmed by references and by mixing them with achillea terpenes.

TABLE I.—CONTENT OF COLLECTED FRACTIONS[a]

Fraction No.	Terpenes	Esters	Ketones	Alcohols
1–12	+++	...	...	...
13–16	...	+++	...	+
17–20	...	+++	+++	++
21–37	...	+	++	++
38–93	...	...	...	+++

[a] Amounts of the substances: +, small; ++, moderate; +++, large.

Esters

To obtain the ester fraction free from other constituents, another column was prepared as described previously but using 1 ml. of oil. The prepared column was eluted with petroleum ether (b.p. 30–50°) and 33 fractions of 8 ml. each were obtained. The first 20 fractions contained the terpene hydrocarbons, and fractions 21 to 33 contained the esters. The ester fractions were mixed, and the petroleum ether was distilled off. The acidic and alcoholic parts of the esters were identified as follows.

Acidic Part.—The acids of the separated esters were identified in the form of hydroxamates by treating part of the ester with alcoholic hydroxylamine hydrochloride (4). The hydroxamate derivatives were then chromatographed in the presence of test substances on Schleicher & Schüll 2048 MGl paper. Development was done in the system of amyl alcohol–acetic acid–water (4:1:5), using the ascending technique. The developed chromatogram was dried, then the spots were detected with alcoholic solution of 0.1% $FeCl_3$. Formic, acetic, and butyric acids were identified (see Fig. 5).

Alcoholic Part.—The other part of the ester fraction was saponified with 5 ml. of 5% KOH in methanol at room temperature. After 2 hours, the mixture was diluted with 50 ml. of water, then shaken with ether to extract the alcohols. The ether phase was washed with water, then dried with anhydrous sodium sulfate. The ether was distilled, and the residual alcohols were identified by the following methods.

Paper Chromatogram.—Ascending chromatography (5) utilized Ederol 208/P paper, with the system of methyl isopropyl ketone–*n*-heptane (5:20 v/v), and detection was made in a saturated jar with osmium tetroxide reagent. Three spots were identified as linalool, terpineol, and nerol.

Gas Chromatography.—The operating conditions are summarized in Table II. The result (Fig. 6) confirmed the presence of *l*-linalool, α-terpineol, *n*-hexen(3)-ol(1), and a small amount of nerol.

Phenols

Two milliliters of oil in 10 ml. of petroleum ether (b.p. 30–50°) were shaken with 5% NaOH solution. The sodium phenolate solution was acidified with sulfuric acid, the phenols were extracted with ether, the ether phase was dried with anhydrous sodium sulfate. The ether was then removed, and the residual phenols were investigated by the following methods.

Paper Chromatogram.—The ascending procedure utilized Ederol 208/P paper and the following two solvent systems: (*a*) *n*-hexane–*n*-heptane–acetic

TABLE II.—OPERATING CONDITIONS[a]

Conditions	Terpene Hydrocarbons Expt. *A*	Terpene Hydrocarbons Expt. *B*	Alcohols of Esters and Free Alcohols
Column liquid phase	Reoplex 400	β,β′-Oxydipropionitrile	Reoplex 400
Ratio liquid:support (w/w)	20:100	15:100	20:100
Column temperature (°C.)	75.0	65.0	145.0
Hydrogen flow rate (ml./min.)	60.0	60.0	50.0
Gas inlet pressure (ata)	0.25	1.5	0.5
ma.	250	250	200
Philips writer (mv.)	2.5	2.5	2.5

[a] The following conditions apply to all experiments: apparatus, Gasofract 300 with Katharometer; solid support, Chromo sorb W; column, 3-mm. length, 4-mm. diameter, copper coil; paper speed, 0.5 cm./minute; sample size, 10 μl.

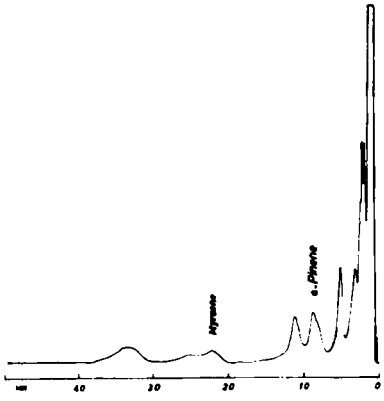

Fig. 3.—Gas chromatographic separation of the monoterpene hydrocarbons of achillea oil on Reoplex 400. (For operating conditions see Table II.)

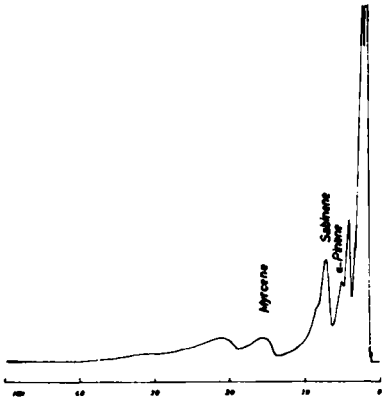

Fig. 4.—Gas chromatographic separation of the monoterpene hydrocarbons of achillea oil on β,β'-oxydipropionitrile. (For operating conditions see Table II.)

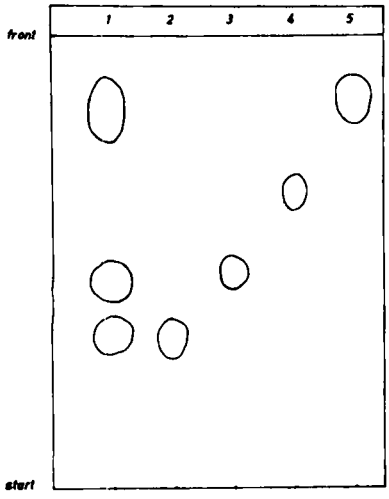

Fig. 5.—Paper chromatogram of 1, hydroxamate derivatives of the acidic part of the esters; 2, formyl hydroxamate; 3, acetyl hydroxamate; 4, propionyl hydroxamate; 5, butyryl hydroxamate.

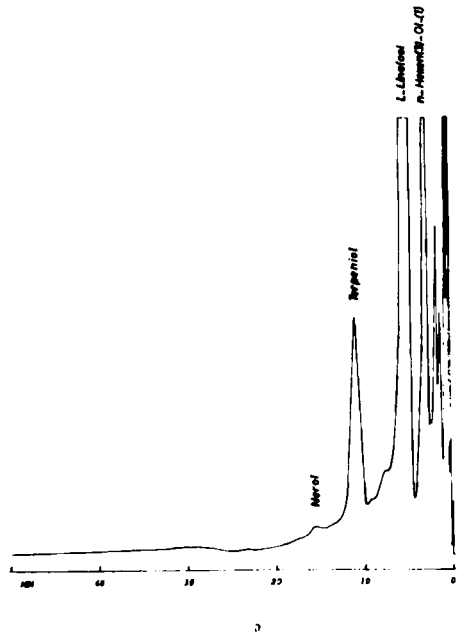

Fig. 6.—Gas chromatographic separation of the alcoholic part of the ester fraction of achillea oil on Reoplex 400. (For operating conditions see Table II.)

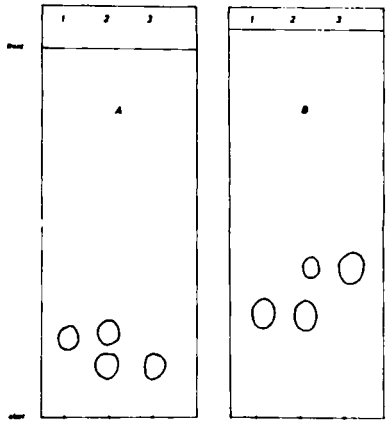

Fig. 7.—Paper chromatogram of 1, eugnol; 2, achillea phenols; 3, carvacrol. Solvent in A: *n*-hexane–*n*-heptane–acetic acid (15:15:2 v/v). Solvent in B: methyl isopropyl ketone—*n*-heptane (5:20 v/v).

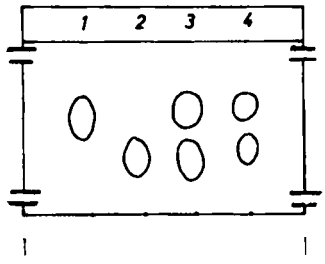

Fig. 8.—Part of a chromatoplate of 1, eugenol; 2, carvacrol; 3, achillea phenols; 4, mixture of achillea phenols, eugenol, and carvacrol.

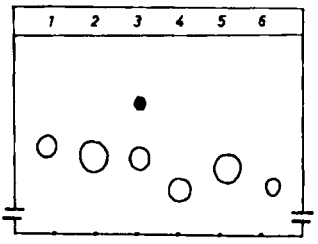

Fig. 9.—Part of a chromatoplate of the ketone-hydrazone derivatives of 1, 3-octanone; 2, ethyl-*n*-amylketone; 3, achillea ketones; 4, 3-decanone; 5, 3-nonanone; 6, 2-decanone; ●, unsaturated ketone.

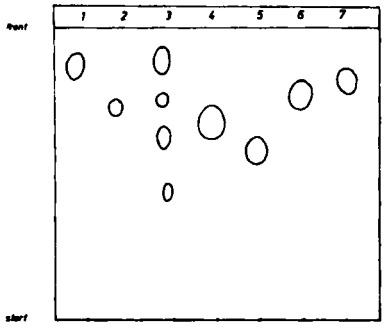

Fig. 10.—Paper chromatogram of the alcohols 1, *l*-linalool; 2, citronellol; 3, achillea alcohols; 4, farnesol; 5, geraniol; 6, terpineol; 7, santalol.

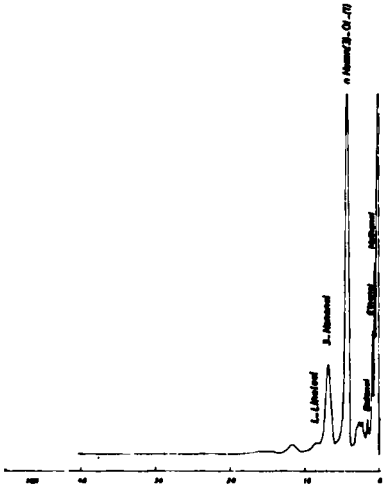

Fig. 11.—Gas chromatographic separation of the free alcohols in achillea oil on Reoplex 400. (For operating conditions see Table II.)

acid (15:15:2 v/v); (*b*) methyl isopropyl ketone–*n*-heptane (5:20 v/v). The antimony pentachloride reagent yielded a brown color with eugenol and a red-violet with carvacrol (Fig. 7).

Plate Chromatogram.—A chromatoplate covered with Kieselgel G was used with chloroform as the developing solvent. The results are indicated in Fig. 8.

Ketones

The ketones were identified in the form of 2,4-DNPH-derivatives (6). The crystalline derivatives were dissolved in benzol, and the solutions were spotted on a plate covered with silica gel which was impregnated with 5% paraffin in benzene. The solvent system of dimethylformamide–methanol–water (4:1:1) was used for development (7). Two ketonic hydrazones were detected (Fig. 9): a short-chain unsaturated ketone (+v result with OsO_4) with higher R_f value and ethyl *n*-amyl ketone.

Free Alcohols

Phthalate derivatives had been separated by Ruzicka (8), and Sabetay (9) separated the alcohols by means of 30% NaOH. The pure alcohols were identified in this work as follows.

Paper Chromatogram.—The method described in the investigation of the alcohols of the esters was used with the free alcohols. The result is indicated in Fig. 10, where it appears that there are at least three alcohols, and that one of them is *l*-linalool.

Gas Chromatography.—Gas chromatography, using the operating conditions summarized in Table II, indicated the presence of *l*-linalool, *n*-hexen(3)-ol(1), 3-nonanol, and the possible presence of methyl, ethyl, and butyl alcohols (Fig. 11).

DISCUSSION

Species other than *A. millefolium*, which does contain azulene and proazulene in its oil, have been reported to contain no azulene—*viz.*, *A. ageratum* (10), *A. atrata* (11), *A. clavenae* (12), *A. moschata* (11), *A. micrantha* (13, 14), *A. filipendulina* (15), and *A. coronopifolia* (16).

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