Note

The Constituents of the Essential Oil from *Erigeron annuus**

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Erigeron annuus (Japanese name, "Himejiyon") is an annual-biennial herb which grows wild in various areas of North America, Europe and Japan. This plant is used in folk medicine as an hypoglycemic drug, and pimecoic acid, quercetin and apigenin-7-glucoside were isolated from the flower by Imai.¹⁾ The acetylenic compounds in this plant have been thoroughly investigated²⁾ and the isolation of matricaria ester and lachnophyllum ester has been reported,³⁾ but the terpenoid, phenol and fatty acid constituents have not so far been studied. This paper describes the volatile constituents from the flower, leaves, stalk and root of *E. annuus* collected in the suburbs of Higashiosaka-shi in Japan.

The neutral fraction of the essential oil of *E. annuus* was analyzed by various spectrometric techniques, the 29 components listed in Table I being identified. The relative concentrations were calculated on the basis of GLC peak areas. The flower part contains germacrene-D and lachnophyllum ester as main components and β -elemene, β -caryophyllene, *trans*- β -farnesene, γ -muurolene, δ -cadinene, γ -cadinene, α -cadinol and matricaria ester as minor components. In addition, there are 6 monoterpenes, 12 sesquiterpene hydrocarbons, 3 sesquiterpene alcohol, 2 acetylenic compounds and 4 miscellaneous compounds. Among the most noteworthy features of this oil are the presence of germacrene-D, β -caryophyllene, β -guaiene and α -himachalene and a rather large quantity of acetylenic compounds. The macrocyclic sesquiterpene hydrocarbons consisted of 4 unsaturated compounds (α -himachalene, β -caryophyllene, germacrene-D, β -guaiene), and these represented 32.7% of the flower oil.

Twelve sesquiterpene hydrocarbons which included 3 tricyclic compounds, 6 bicyclic compounds, 2 monocyclic compounds and 1 acyclic compound amounted to 45.4% of the total flower oil. Table I lists sesquiterpene compounds that amounted to 48.2% (flower), 59.7% (leaver), 40.7% (stalk), and 5.4% (root) of the

each oil. Germacrene-D, β -elemene and β -caryophyllene, are the major compounds in this class. Acetylenic compounds amounted to 35.9% (flower), 28.5% (leaves), 31.6% (stalk), and 93.5% (root) of the each oil. With regard to flower and leaves lachnophyllum ester was the major compound in the acetylenic compounds (flower, 89.6%; leaves, 73.3%). On the other hand, with regard to stalk and root part, matricaria ester was the major compound in them (stalk, 52.8%; root, 69.5%). Thus, the oils from flower, leaves, stalk and root are mainly composed of sesquiterpenes and acetylene compounds with smaller quantities of several monoterpenes (0.9%, trace, 0.2% and 0.0%, respectively), phenols (0.6%, 1.6%, 4.8% and trace, respectively), miscellaneous compounds (0.6%, trace, 4.3% and 0.0% respectively) and fatty acids (each trace). In conformity with flower oil, the neutral volatile fraction of leaf oil contained predominantly sesquiterpene hydrocarbons, while oxygenated compounds of terpenoid and non-terpenoid origin were present in minor quantity only. The neutral material from stalk and root oil, on the other hand, had a considerably lower concentration of sesquiterpene hydrocarbons and a greater number of oxygenated compounds. The most striking difference between the four part oils, however, was observed in the varying concentrations of germacrene-D. This compound, which was by far the most abundant volatile constituent of flower and leaves, was present in trace amounts only in the root part. The acidic part examined after methylation was considerably less complex than the neutral fraction. As expected, all C_4 - C_{18} straight-chain saturated fatty acids were found in *E. annuus*, palmitic acid being the main constituent.

EXPERIMENTAL

IR spectra were taken in liquid films, and the NMR spectra were measured on a 60 MHz apparatus with tetramethylsilane as internal standard in CCl_4 . For measuring the MS spectra, a single focus apparatus was used with an ion accelerating voltage of 3500 V, an ionization voltage of 70 eV and an ionization chamber temperature of 210°C. Analytical GLC was performed on a Yanaco-G8 instrument equipped with a flame ionization detector. The temperature of the injection block was kept at 220°C and that of the detector at 250°C. Stainless steel columns (2.25 m $\times$ 3 mm) packed with 20% Carbowax 20 M on 80~100 mesh Chromosorb W were used. The temperature was programmed from 70 to 200°C at 2°C/min. Helium flow was maintained at 30 ml/min. Preparative GLC was performed with Yanaco-G800 instrument containing a thermal conductivity detector.

Plant material and essential oil. The plant was collected at the unoccupied ground in Higashiosaka-

* Studies on Chemical Constituents of Naturalized plants of Japan Part VI, For Part V, IV, III, II and I; see ref. 9, 8, 7, 6 and 5 respectively.

TABLE I. CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL OF *E. annuus*

Compound	Method of identification	Flower (%)	Leaves (%)	Stalk (%)	Root (%)
<i>Neutral fraction</i>					
(Mono-terpenes)					
α -Pinene	MS, IR, Rt	+			
Camphene	MS, IR, Rt	+			
β -Pinene	MS, IR, Rt	+			
α -Phellandrene	MS, IR, Rt	0.7			
Limonene	MS, IR, Rt	+	+	0.2	
<i>p</i> -Cymene	MS, IR, Rt	0.2	+	0.2	
(Sesqui-terpenes)					
α -Cubebene	MS, IR	0.1	0.3		
α -Copaene	MS, IR, Rt	0.2	0.5		
β -Bourbonene	MS, RI	0.3	0.2	1.1	
β -Elemene	MS, IR, NMR, Rt	7.0	13.7	10.2	0.5
α -Himachalene	MS, IR	+	+	+	
β -Caryophyllene	MS, IR, NMR, Rt	2.5	3.1	3.0	
<i>trans</i> - β -Farnesene	MS, IR, Rt	+	+	+	
γ -Muurolene	MS, IR, NMR, Rt	2.1	+	2.9	
Germacrene-D	MS, IR, NMR, Rt	30.0	23.9	8.9	
δ -Cadinene	MS, IR, Rt	0.5	0.4	0.3	
γ -Cadinene	MS, IR, Rt	2.5	4.9	2.2	
β -Guaiane	MS, IR	0.2	0.7	1.1	
β -Eudesmol	MS, IR, Rt	0.3	1.0	4.2	0.5
Nerolidol	MS, IR, NMR, Rt	0.4	7.9	3.8	
α -Cadinol	MS, IR, Rt	2.1	3.1	3.0	4.4
(Acetylenic compounds)					
Lachnophyllum ester	MS, IR, NMR	32.2	20.9	14.9	28.5
Matricaria ester	MS, IR, NMR	3.7	7.6	16.7	65.0
(Miscellaneous compounds)					
$C_{10}H_{22}$	MS, IR, Rt	+			
$C_{12}H_{26}$	MS, IR, Rt	+			
Perillene	MS, IR, Rt	0.3			
<i>cis</i> -3-Hexen-1-ol	MS, IR, NMR, Rt	0.3	+	4.3	
<i>Phenolic fraction</i>					
<i>o</i> -Cresol	MS, IR, Rt	+	+	0.6	+
<i>p</i> -Cresol	MS, IR, Rt	+	1.6	0.5	+
Eugenol	MS, IR, Rt	+	+	0.6	+
Thymol	MS, IR, NMR, Rt	0.6	+	3.0	+
<i>Acidic fraction</i>					
Butyric acid	MS, Rt	+	+	+	+
Caproic acid	MS, Rt	+	+	+	+
Heptanoic acid	MS, Rt	+			+
Caprylic acid	MS, Rt	+	+	+	+
Nonanoic acid	MS, Rt	+			
Capric acid	MS, Rt	+	+	+	+
Undecanoic acid	MS, Rt	+	+		+
Lauric acid	MS, Rt	+	+	+	+
Tridecanoic acid	MS, Rt	+			
Myristic acid	MS, Rt	+	+	+	+
Pentadecanoic acid	MS, Rt	+	+	+	+
Palmitic acid	MS, Rt	+	+	+	+
Stearic acid	MS, Rt	+	+	+	+
Oleic acid	MS, Rt	+	+	+	+
Linoleic acid	MS, Rt	+	+	+	+

+, trace amount; Rt, retention time

shi on June 1975, it was divided into four parts; flower, leaves, stalk and root. After steam distillation of each part, essential oils of each part (flower, 0.223%; leaves, 0.105%; stalk, 0.069%; root, 0.035%) were obtained by the extraction of the distillate with diethyl ether and evaporation of solvent from the extract under N_2 . The essential oils had the following characteristics:

	d_4^{25}	n_D^{25}	$[\alpha]_D^{25}$	A.V.	S.V.
Flower	0.9163	1.5142	-13.94°	8.47	74.27
Leaves	0.9201	1.5012	-27.03°	7.41	28.09
Stalk	0.9221	1.4841	-17.51°	6.85	35.36
Root	0.9198	1.5554	-12.74°	5.78	140.76

Every one of the essential oils (flower, 10.0 g; leaves, 12.5 g; stalk, 8.3 g; root, 5.6 g) was at first dissolved in ether, and then was added to the 5%- Na_2CO_3 solution. After shaking, the aqueous layer (acidic fraction) was separated, then acidified with dil-HCl, and extracted with ether. After the evaporation of the solvent *in vacuo*, the residue was obtained as yellow liquid (flower, trace; leaves, 0.1 g; stalk, trace; root, trace). Furthermore, ethereal layer (Na_2CO_3 insoluble fraction) was treated with 5%-NaOH solution, and separated into NaOH soluble fraction (phenolic fraction) and neutral fraction. The phenolic fraction was acidified and then extracted with ether to give yellow oil (flower, trace; leaves, 0.2 g; stalk, 0.3 g; root, trace).

Neutral fraction. Neutral fraction (flower, 9.8 g; leaves, 12.1 g; stalk, 7.9 g; root, 5.4 g) was chromatographed on activated alumina (a 2.5 cm $\times$ 55 cm glass tube) with *n*-hexane, benzene, ethyl ether, ethyl acetate and methanol, and divided into 5 fractions. Each of the fractions was subsequently subjected to elution chromatography and/or preparative GLC (3 m $\times$ 3 mm stainless steel separation column packed with 15% SE-30 and/or 15% PEG-20M on Celite-545) for the separation of individual components. The components thus isolated in sufficient amount were identified by IR, NMR, MS,⁴⁾ and retention time (R_t) of GLC with those of authentic samples and samples isolated from plants according to our previous papers.⁵⁻⁹⁾

Phenolic fraction. The phenolic compounds were isolated in the pure state by means of preparative GLC. Identification of the compounds was attained by comparing their MS, IR, NMR and R_t with those of authentic samples obtained commercially or synthetically.

Acidic fraction. The acidic fraction from the essential oil was treated with ethereal CH_2N_2 overnight and subsequently examined by GC-MS.

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