H-10), 2.02 (3H, *s*, $MeCO_2$), 4.71 (*d*, $J=7$ Hz, anomeric H), 5.28 (1H, *d*, $J=4$ Hz, H-1), 7.41 (1H, *d*, $J=1$ Hz, H-3).

Loganin pentaacetate (**4**) *from compound* **2**. Compound **2** (35 mg) was methylated and acetylated as in the case of **1**, to afford **4**, colourless needles from EtOH mp 135–138°; $[\alpha]_D^{19}$ −76.2° ($CHCl_3$; *c* 0.78); EIMS *m/z* (rel. int.): 600 $[M]^+$ (3), 569 (3), 541 (4), 331 (90), 253 (34), 193 (77), 169 (100), 109 (90); IR ν_{max}^{KBr} cm^{-1}: 2950, 1755, 1645, 1370, 1230, 1050, 910; UV λ_{max}^{EtOH} nm (log ε): 232 (4.18); ^1H NMR ($CDCl_3$): δ 1.03 (3H, *d*, $J=6$ Hz, H-10), 1.92, 2.01, 2.04 (×2), 2.10 (each 3H, *s*, $MeCO_2$), 3.70 (3H, *s*) 7.28 (1H, *s*, H-3). Identical with an authentic sample as well as **4** derived from **1**.

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ESSENTIAL OIL OF *CONYZA CANADENSIS*

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Key Word Index—*Conyza canadensis*; Asteraceae; horseweed; phytochemistry; sesquiterpenes; acetylenes; matricaria esters; bergamotene.

Abstract—GC/MS of the essential oil from horseweed (*Conyza canadensis*) was used to identify three matricaria ester isomers, lachnophylum ester, and two related lactones plus a new ethyl ester of matricaria acid. In addition, eight mono- and 10 sesquiterpenes were resolved and identified. The composition of the sesquiterpene fraction shows seasonal variation indicating a flow of material from β-*trans*-farnesene via several intermediates to the final main product bergamotene in agreement with current biosynthetic pathways.

INTRODUCTION

This study was done to improve the characterization of the essential oil of horseweed (*Conyza canadensis*). The essential oil of numerous members of the Asteraceae have been characterized. However, a thorough and comprehensive analysis of horseweed essential oil has not been carried out using current technology.

In 1952, Guenther [1] reported horseweed, on steam distillation, yielded a colourless or slightly yellow oil which contained *d*-limonene and matricaria methyl ester. Later, Ogg *et al.* [2] confirmed the presence of *d*-limonene and matricaria ester and identified 11 other compounds, including several sesquiterpenes, by GC/MS. Matricaria ester was obtained crystalline in *ca* 20% yield from the oil. Improved analytical techniques have led to the discovery of geometric isomers of matricaria ester and related acetylenic compounds in horseweed. In 1979, Bohlmann and Jakupovic [3] reported the presence of a total of six of these acetylenic compounds. Most reports of horseweed essential oil are concerned only with the matricaria ester and related compounds. In particular, a detailed examination of the sesquiterpene content of horseweed oil using current techniques has not been reported.

RESULTS AND DISCUSSION

Oil yields ranged from 1.35% (O.D.) for juvenile plants to 1.55% (O.D.) for mature plants. Chromatograms of the raw horseweed oil indicated about 25 compounds were present at a concentration above 0.1%. The identities of these compounds are listed in Table 1, in which approximate concentrations in the mature oil are also given. GC/MS of the oil gave very good results, and

Table 1. Composition (%) of the essential oil of *C. canadensis*

Monoterpenes†	%	Kovats' Index	Mode of ID
α-Thujene	0.4	1036	a,b
β-Pinene	4.05	1124	a,b
Myrcene	2.91	1156	a,b
d-Limonene	67.25	1206	a,b
cis-Ocimene	trace	1228	a,b
trans-Ocimene	1.00	1250	a,b
Cosmene	0.89	—	c
Cosmene isomer	trace	—	c
Sesquiterpenes†			
trans-Caryophyllene	0.17	1580	a,b
α-*cis*-Bergamotene	4.31	1602	c
trans-b-Farnesene	0.83	1663	a,b
cis-β-Farnesene	0.26	1681	a,b
β-Himachalene	0.83	1685	c
β-Cubebene	0.87	1693	c
β-*trans*-Bergamotene	0.50	1707	c
β-Bisabolene	0.14	1713	c
δ-Cadinene	0.12	1730	c
α-Curcumene	0.57	1735	c
Acetylenes			
Matricaria methyl ester			
cis, cis	9.19		c
cis, trans	0.78		c
trans, trans	trace		c
Matricaria ethyl ester			
unkown confif.	trace		c
Lacnophylum methyl ester			
trans	1.03		c
Matricaria lactone			
cis	trace		b
Lacnophylum lactone	trace		b

*(a) Identified by co-chromatography with authentic sample. (b) Identified by comparison of GC/MS data with those of authentic sample. (c) Identified by comparison of GC/MS data with literature data.

†Kovats' Indices reported for SE-30 column.

‡Kovats' Indices reported for DX-4 column.

prefractionation of the raw oil into hydrocarbons and oxygenated compounds using deactivated silica gel was found to be an excellent supplement to working with the whole oil.

Monoterpenes

The monoterpenes found are the hydrocarbons α-thujene, β-pinene, myrcene, *d*-limonene, *cis*- and *trans*-ocimene. Ogg *et al.* [2] had suggested the presence of only one ocimene isomer, but it is now apparent that both isomers are present. Two unidentified compounds of M_r 134 were also found and are believed to be cosmene and an isomer of cosmene. Further work is being done to determine the exact structure. Ogg *et al.* [2] also reported menthol, menthone, and carvone. These could not be found even in trace amounts. They are major components in peppermint and spearmint oil and may have occurred as contaminants in the plant sample or equipment used.

Sesquiterpenes

Ogg *et al.* [2] also reported the presence of the sesquiterpenes α-curcumene, α-farnesene and δ-cadenol in horseweed oil. We have verified α-curcumene; however, two isomers of β-farnesene were found rather than α-farnesene, and δ-cadenol could not be found. A total 10 sesquiterpenes have now been identified (Table 1), and all of these originate from the *trans*-farnesyl pyrophosphate.

Analysis of the oil at different times during the growing season shows that substantial changes occur. Data for oil collected in June, July and September are summarized in Table 2. The main change is a decline in farnesene and an increase in bergamotene. This change in composition fits well with known biosynthetic pathways for sesquiterpene formation [4])

Table 2. Seasonal variation of sesquiterpene components of horseweed essential oil*

Compound	June	July	September
trans-Caryophyllene	5.1	2.7	2.7
α-*cis*-Bergamotene	24.5	23.2	47.7
trans-β-Farnesene	23.4	12.4	3.3
cis-β-Farnesene	6.7	11.8	8.0
β-Himachalene	1.1	7.1	1.3
β-Cubebene	24.3	19.8	25.8
β-*trans*-Bergamotene	2.9	9.7	4.6
β-Bisabolene	1.1	2.7	1.3
δ-Cadinene	3.0	3.6	0.6
α-Curcumene	2.5	1.7	1.3

*Expressed as a percentage of the sesquiterpene fraction of the essential oil.

The polar oil fraction was determined to contain mainly three isomers of matricaria methyl ester and the related acetylenic compounds lachnophylum ester, lachnophylum lactone, and matricaria lactone. These compounds were previously reported by Bohlmann *et al.* [3]. The presence of these compounds was confirmed by GC/MS. In addition to the isomers of matricaria methyl ester, GC/MS analysis also revealed a trace amount of matricaria ethyl ester.

EXPERIMENTAL

Plant material. Initial samples of *Conyza canadensis* (L.) Cronq. were obtained in August 1984 from Union Gap, Washington. Samples for the seasonal variation study were collected at the Center for Urban Horticulture, Seattle, Washington. Plants were collected over the period of June to September 1986 and identified by W.H.H. (voucher specimens deposited in the University of Washington herbarium). Three levels of plant maturity were examined: (i) plants without flower buds, (ii) plants with flower buds that had not yet bloomed, and (iii) plants that had bloomed.

Extraction and prefractionation. The essential oil was removed from the plant material by steam distillation. The whole oil obtained from steam distillation was prefractionated using polyethylene glycol (Carbowax 20M) deactivated silica gel. Hydrocarbons were eluted with hexane and polar, oxygenated compounds were eluted with MeOH [5].

Synthesis. Matricaria methyl ester (II) was saponified to matricaria acid (IX) following the method of ref. [6]. The acid was then converted to matricaria lactone following the procedure outlined in ref. [7]. The two isomers of ocimene were synthesized photochemically from α-pinene as reported in ref. [8].

Analytical GC. The hydrocarbon and oxygenated oil fractions were analysed separately using a Carbowax 20M, SE-30, or DX-4 fused silica capillary column. Thd DX-4 (J&W Scientific, Inc., Rancho Cordova, CA) column gave the best overall resolution: monoterpene, sesquiterpene, and acetylenic fractions were resolved as distinct groupings of related compounds.

Two GC techniques were utilized to aid in identification of oil components. First, when available, R_ts of known terpenoids were compared to the elution time of unknowns. Identification was made on the basis of co-chromatography with standard compounds. Kovats' retention indices obtained using even and odd *n*-alkanes were also used to identify unknown terpenoids. The *n*-alkane standards were added to aliquots of oil and concentrations were adjusted to maintain a Gaussian peak distribution and avoid column overloading. Calculated Kovats' indices were then compared to published lists of indices obtained for Carbowax columns [9].

GC/MS. Analysis of the oil was also carried out using GC/MS techniques. Compounds were identified by comparison of unknown spectra with published spectra of known compounds [10–12]. Fragmentation patterns were used to determine molecular structure when reference spectra were unavailable.

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