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GAS–LIQUID CHROMATOGRAPHY OF TERPENES—XII
SEASONAL VARIATION IN THE VOLATILE OIL FROM *TANACETUM VULGARE* L.*

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Abstract—The seasonal variation in the composition of the volatile oil from locally grown wild tansy plants was determined. Some plants were found to produce 80–90 per cent *l*-thujone, whereas others gave the normal content (70–85 per cent) of *d*-isothujone throughout the growing season. Significant variations in the content of the minor components were found to occur only in very young plants, when appreciable amounts of ϵ-, γ- and δ-cadinene and an unidentified labile (possibly C_5) alcohol were detected.

INTRODUCTION

RECENTLY, a gas–liquid chromatographic (GLC) analysis of the oil of tansy, the volatile oil from the leaves and flowers of *Tanacetum vulgare* L., was carried out by one of us.[1] It was shown that the chemical composition of the oil from local wild tansy plants differed somewhat from that of the commercial oil. Most noteworthy was the lack of camphor and a fairly high content of 1:8-cineole, *l*-thujone, *l*-carvone, and a sesquiterpene (peak 26). From a biosynthetic point of view it was of interest to determine whether these variations reflect on biosynthetic pathways or interrelationships between constituents of the oil. Alternatively, it is possible that such differences are due to genetic differences between individual plants. The GLC technique allows a quantitative comparison of the composition of very small amounts of oil[2] and it is possible to examine the oil from individual shoots emanating from the same clone (root stock). A study has now been made of the composition of the oil of tansy plants at different stages of growth to determine whether the synthesis of one or more components was particularly rapid during any part of the growing season.

RESULTS

Initial results showed that the oil of some of the wild plants contained a large amount of *d*-isothujone with only a few per cent of *l*-thujone. Others, in contrast, gave an oil containing 80–90 per cent *l*-thujone and only little *d*-isothujone. A few of these plants were transplanted to an experimental plot and the composition of the oil from each plant was determined at fairly regular intervals before, during, and after flowering (Tables 1 and 2). In late fall (1963) parts of the root stocks were transferred to a greenhouse. When the oil from very young shoots of these transferred plants was analyzed, some significant differences in composition were detected. Consequently, the new growth (1964) of the plants left outside was investigated

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[1] E. VON RUDLOFF, *Can. J. Chem.* **41**, 1 (1963).
[2] E. VON RUDLOFF, *Phytochem.* **1**, 195 (1962).

TABLE 1. VARIATION IN THE PER CENT COMPOSITION OF THE MAJOR COMPONENTS OF THE VOLATILE OIL OF TANSY PLANTS (1963)

Date	7/8	9/8	12/8	14 8	16/8	19·8	21/8	23 8	28 8	30·8	4.9
Plant A											
Component											
Sabinene	0·5	0·5	t*	t	t	t	t	0 5	t	t	0·5
Cineole	2	3·5	2·5	2	2	2	4	4	1	2	2·5
Thujone	82	82	86	85	84	84	85	84	91	89	89
Isothujone	2	2	2 5	1·5	2·5	3·5	3	3	2	2	3
Peak 19	1·5	1	1	1	0 5	1	0 5	0·5	t	0 5	t
ε-Cadinene + carvone	8	7·5	4	6	7·5	5	4	4	4 5	3	3·5
γ- + δ-Cadinene	1·5	1 5	1	1 5	1	1 5	1	0·5	t	t	t
Plant B											
Component											
Sabinene	2	2·5	1 5	3·5	4	0·5	1	1	0 5	1	1·5
Cineole	7	8·5	5	13·5	2	7	10·5	5	4	6	7
Thujone	0·5	1·5	1	1	2	2	1	1	1·5	2	1·5
Isothujone	76	76	80	70	73	79	75	77	78	77	82
Peak 20	1·5	1·5	1·5	1 5	0·5	2	2	2	2 5	2	1·5
Borneol	1	1	1	1	1	1·5	1	1	1 5	1	1
ε-Cadinene + carvone	5	3	7	6	7·5	3·5	5·5	3·5	4	2 5	2·5
γ- + δ-Cadinene	1·5	1·5	1	1·5	1·5	2	2	2·5	2 5	2	1·5
Plant C											
Component											
Sabinene		1*		1	1	0·5		0 5	1	1	1
Cineole		4		3	4	5		4	3	1	3
Thujone		80		85	84	86		86	87	90	89
Isothujone		2·5		2	2	3		4	4	3	4
Peak 19		1		0 5	1	0·5		0·5	0·5	1	0·5
ε-Cadinene + carvone		6		6	4	3		4	3	4	1
γ- + δ-Cadinene		1		1	1	0·5		0 5	0 5	t	t
Plant D											
Component											
Sabinene		4·5		4·5	2·5	2		2	2	2·5	2·5
Cineole		3		3	7	6		6	4	4	2·5
Thujone		1·5		1·5	1	2		2	2	2	2·5
Isothujone		73		73	76	74		77	78	78	81
Peak 19		1		1	0·5	1		1	1	0·5	t
Peak 20		1		1	2	2		1	1	1	1
Borneol		1		1	1·5	1·5		0 5	0 5	1	1·5
ε-Cadinene + carvone		9·5		7 5	5 5	5		4	3	3	4
γ- + δ-Cadinene		1		1	t	1·5		0·5	1	1	1 5
Plant E											
Component											
Sabinene		--		3	3	--		2	5	1	0 5
Cineole		--		5	6	--		4	1·5	4	0·5
Thujone		--		1	1	--		1·5	2	2·5	2
Isothujone		--		79	75	--		78	75	78	87
ε-Cadinene + carvone		--		4	4	--		3 5	5	5 5	2·5
γ- + δ-Cadinene		--		1	2	--		1·5	2	1·5	2

* t = trace.

TABLE 2. COMPOSITION OF THE VOLATILE OIL (MAJOR COMPONENTS) OF THE FLOWERS AND OF SINGLE SHOOTS OF MATURE TANSY PLANTS

	Plant B = 22/8/63		Plant D = 11/9/63		
	Flower:		Shoot No:		
	Yellow	Brown	1	2	3
Component					
Sabinene	1	1	3	2	0·5
Cineole	7·5	10·5	5	6	1
Thujone	1	1	1	2	2
Isothujone	80	79	80	73	75
Peak 19	2	1·5	0·5	0·5	1
Peak 20	1·5	t*	0·5	1	1·5
Borneol	1·5	1	1	1	2
ϵ-Cadinene + carvone	2	1	4	5·5	3
γ- + δ-Cadinene	1·5	2	1	1·5	2

* t = trace.

further (Table 3). Since the yield of oil from very young single shoots is very small a more sensitive instrument was used for the GLC analysis.

Previously it was shown that the oil of tansy contained at least 26 different terpenes, most of which were present in amounts of less than 1 per cent.[1] Direct comparison of chroma-

TABLE 3. VARIATION IN THE PER CENT COMPOSITION OF THE MAJOR COMPONENTS OF THE VOLATILE OIL FROM YOUNG TANSY PLANTS (1964)

Date	11/5	19/5	25/5	1/6	5/6	11/6	15/6	18/6
Plant A								
Component								
Sabinene	0·5	0·5	0·5	0·5	0·5	1	0·5	0·5
Labile alcohol	1·5	2	2	1·5	1·5	1·5	1	1
Cineole	0·5	0·5	1	1	1	2	2	2
Thujone	73	75	77	78	80	78	80	79
Isothujone	4	5	4	3	5	4	3	3
Terpinen-4-ol	1	1	1	1	1	0·5	1	1
ϵ-Cadinene	6	8	5	4	2	1	1	1
Carvone	2	1·5	3	3	4	4	4	5
γ- + δ-Cadinene	1·5	3	1·5	1	0·5	0·5	0·5	0·5
Total oxygenated sesquiterpenes	5·5	4	4	3	2	1·5	1	1
Plant B								
Component								
Sabinene	—	2·5	3	2·5	2	4	3	3·5
Labile alcohol	—	7	5·5	3	2	2	1	1
Cineole	—	1	1·5	1	1	2·5	3	4
Thujone	—	2	2	1	1	2	1	1
Isothujone	—	72	74	75	78	78	76	77
Terpinen-4-ol	1	1	1	1	1	1	0·5	1
ϵ-Cadinene	—	6	4	8	5·5	3	1·5	1
Carvone	—	1·5	2·5	2	1	2	3	3
γ- + δ-Cadinene	—	2	1	2	2·5	1	1	1·5
Total oxygenated sesquiterpenes	—	5	4	3·5	4	2	1·5	1

tographic charts showed that these minor peaks varied considerably in size from one oil sample to another. Since the accuracy of determination drops markedly with peak size[3] these variations may be due to the analytical technique rather than to biological variation. Therefore, only the components recorded in amounts of 1 per cent and more were considered in this study. These were *d*-sabinene (peak 5*), 1:8-cineole and unknown alcohol (peak 9), *l*-thujone (peak 14), *d*-isothujone (peak 15), peaks 19 and 20, borneol (peak 22), carvone and ε-cadinene (peak 23), and γ- and δ-cadinene (peak 26).

The oil from very young shoots showed the presence of increased amounts of peaks 9, 23, and 26, as well as eight peaks in the oxygenated sesquiterpene range in 0·5–2 per cent amounts not previously observed.[1] By combining the oil from many young plants sufficient oil was obtained to attempt isolation of some of these components. The oil was divided into hydrocarbons and oxygenated terpenes by chromatography on modified silicic acid.[4,5] During this operation most of peak 9 was lost. Comparison of retention data of the unknown in peak 9 on a number of different columns (see Table 4) led to the following conclusions:

(1) On the polyethylene glycol (PEG 20M) and Apiezon N (A–N) columns the unknown behaved like a C_5 alcohol. (2) On the rapeseed oil (RO), ethylene glycol bis-(propionitrile) (EGPN) and fluorinated silicone (QF-1) columns the retention times were considerably higher than the saturated C_5 alcohols, indicating the presence of a double bond. (3) Since carbonyl compounds are very strongly retained on QF-1,[1] the compound appears to be an alcohol or a hydrocarbon. The data obtained with the PEG 20M and A–N columns exclude a hydrocarbon. Thus, the unknown appears to be a C_5 alcohol with a somewhat more polar double bond than β,β-dimethyl allyl alcohol. Attempts to isolate the unknown failed. It was also noticed that more than half of this compound was lost when the oil was left standing in the dark at cold temperatures for 3 days.

From the mixture of hydrocarbons fairly pure ε-cadinene (part of peak 23), a mixture of γ- and δ-cadinene (peak 26) and small amounts of β-elemene (part of peak 18) were isolated. A trace of an unidentified azulene was also detected. Therefore, during the early stages of growth, cineole (peak 9) is accompanied by substantial amounts of the labile alcohol, terpinen-4-ol (peak 18) by β-elemene, and carvone (peak 23) by ε-cadinene. The amounts of oxygenated sesquiterpenes were too small for isolation of individual components, nor were the retention times of any one similar to those of α- and δ-cadinol, α-, β- and γ-eudesmol, or cedrol. In the mature plants (1963) all of these newly identified components were present in less than 1 per cent amounts.

DISCUSSION

The most significant conclusion which can be drawn from the results shown in Table 1 is that two different types of tansy plants were found near Saskatoon, Saskatchewan. The oil from plants A and C (Table 1) had a very high content of *l*-thujone (80–90 per cent) with only 1–4 per cent of *d*-isothujone being recorded. In contrast, plants B, D, and E produced an oil with a "normal" content of isothujone (73–87 per cent: commercial oil of tansy contains about 70 per cent[1]) and only minor amounts of thujone. At no time during this investigation did a plant with high thujone content revert to the production of normal amounts of isothujone. Inspection of the plants showed no recognizable morphological

* Peak numbering according to elution on the polyethylene glycol column [1]

[3] J. JANAK, *J. Chromatog.* **3**, 308 (1960).
[4] E. KUGLER and E. SZ. KOVATS, *Helv. Chim. Acta* **46**, 1480 (1963).
[5] E. VON RUDLOFF and F. M. COLEMAN, *Can. J. Chem.* **42**, 1890 (1964).

difference and on the basis of botanical taxa the plants must be considered to belong to the same species. It follows that the oil from the wild plants investigated previously[1] must have been obtained from both thujone- and isothujone-producing plants. It is noteworthy that none of the local plants produced an oil with more than 1 per cent of camphor whereas the commercial oil analyzed previously[1] contained 13·9 per cent of this ketone.

The thujone-producing plants gave oils with a consistently lower content of sabinene (Tables 1 and 3) and the amounts of 1:8 cineole, γ- and δ-cadinene also tended to be lower than those found in the normal isothujone-producing plants. Comparison of the composition of the oils from shoots of the same plant bearing flowers of varied maturity (yellow to brown flowers) showed no significant difference (Table 2). When three shoots were cut at the same time at the end of the growing season (Table 2) the variation in the composition of the oil was as large as the overall variation recorded for the summer growth (compare with Table 1). The variation from one plant to another, even of the same type, was higher than the overall variation within a single plant.

Battaile and Loomis[6] have presented evidence that *de novo* synthesis of terpenes in mint plants occurs only in very young tissues and that terpene interconversion takes place in older ones. Earlier[7] it had been shown that during the growth of mint plants the content of menthol increases with concomitant decrease in menthone. It is also accepted that the maximum oil content is reached near the maturity of mint plants. In the present study on tansy plants the yield of oil was from 0·1–0·2 per cent and no exceptionally large variation was noticed during the main growing season. During early summer growth it was not possible to obtain an accurate measure of the yield of oil because of the very small amounts involved, but in the large-scale experiment 0·07 per cent oil were obtained. Since no marked change in the composition of the oil was recorded before, during, and after flowering, the terpene synthesis in tansy plants must differ markedly from that in mint plants.

Initial results with oil from very young shoots (5–15 cm high) of tansy plants had shown significantly larger relative amounts of peak 9 (1:8-cineole and the labile alcohol), ϵ-, γ- and δ-cadinene, as well as several oxygenated sesquiterpenes. This was confirmed by analysis of the oil from young plants growing in the experimental plot (June 1964, Table 3). Thus, it appears that the biosynthesis of sesquiterpenes, and possibly a labile C_5 alcohol, is very marked in the young shoots of tansy plants. Their production diminishes as the plants mature and the only noticeable change which occurs later in the growing season (July and August) is a slight increase in the thujone or isothujone content. The mature local plants form an excellent source of these two ketones.

EXPERIMENTAL

Temperature-programmed GLC runs were obtained first (1963) with an Aerograph model 700 Autoprep instrument and later (1964) with an F & M model 500 instrument. A typical chromatogram on a 6 ft × ¼ in. O.D. polyethylene glycol (PEG 20M) column was shown previously.[1] Each oil sample was also analyzed on a similar Apiezon-N (A–N) column (1:6 w/w on Chromosorb W, 60–80 mesh). Retention times were determined as described before[1] in isothermal runs on similar 6 ft PEG 20M, A–N, polyethylene glycol adipate polyester (APEG), ethylene glycol bis-(propionitrile) (EGPN), fluorinated silicone (QF-1), and 10 ft rapeseed oil (RO) columns (see also Table 4). The per cent composition was deter-

[6] J. Battaile and W. D. Loomis, *Biochim. Biophys. Acta* **51**, 545 (1961).
[7] E. Guenther in *The Essential Oils*, Vol. III, pp. 595–6, D. Van Nostrand, Inc., New York (1949).

mined by measurement of the area under the peaks (triangulation method) recorded on the PEG 20M column. The error was ±3 per cent for large peaks, ±3-10 per cent for medium peaks and ±10 per cent and higher for small peaks.[3] Since thujone and isothujone were incompletely separated in these runs, the error for each was higher, but the total amount of both was within ±2-3 per cent. The results obtained from duplicate runs were averaged and are shown in Tables 1 to 3. To distinguish between the labile alcohol and 1:8-cineole, as well as between carvone and ε-cadinene, the chromatograms obtained on the A–N column were analyzed quantitatively for these peaks and the thujones.

Shoots from each plant were harvested during mid-morning, except the flowers listed in Table 2, which were cut in the afternoon. The shoots (5-50 g fresh weight) were macerated with 10–100 ml water in a Waring blendor and then steam distilled until 50–200 ml distillate was recovered. The distillate was saturated with sodium chloride and then extracted with three portions of petrol ("Skellysolve F", b.p. 40–60°). The extract was dried over anyhdrous sodium sulphate, transferred quantitatively to a small flask with a long neck, and then placed on a steam-bath. Evaporation of the solvent was continued for 1 to 3 min, after visual examination showed the end of petrol distillation.[2] Based on the fresh weight of plants (1963) the yield of oil ranged from 0·1–0·2 per cent. Where the weight of a very young shoot (1964) was small and the yield of oil sometimes less than 10 mg, the evaporation was stopped before all solvent had distilled off.

Single shoots from plants A to E were cut every second or third day during August and September 1963. Aliquots (3–5 μl) of the volatile oil from each shoot were analyzed on the PEG 20M and A–N columns (non-linear temperature-programmed from 65° to 210° in 40 min; Aerograph "Autoprep"). The quantitative results obtained at each harvest for the five plants are shown in Table 1. Analysis of the oils from shoots harvested in 1964 was carried out on similar columns using linear temperature programming (65° to 210° at 4°/min; F & M model 500). The results obtained for the oil from single shoots from plants A and B are shown in Table 3.

Identification of individual components was carried out by direct comparison with the GLC data obtained previously.[1] In addition, the volatile oil (1·66 g) from young shoots (2·4 kg, June 1964) was chromatographed on silicic acid (25 g) modified with polyethylene glycol (PEG 20M).[4,5] Elution with petrol gave a mixture of hydrocarbons (0·13 g) and subsequent elution with methanol gave a mixture of oxygenated terpenes (1·41 g). GLC analysis of each fraction indicated that the separation was almost quantitative.

Sesquiterpenes

Aliquots (10 μl) of the hydrocarbon mixture were chromatographed on the PEG 20M column (100° to 220° at 4°/min). Peaks 18, 23, and 26 were present in sufficient amounts to permit isolation in mg quantities. Comparison of i.r. spectra showed these peaks to correspond to β-elemene, ε-cadinene and a mixture of γ- and δ-cadinene respectively. These assignments corresponded with the retention data. The optical rotation of ε-cadinene was $[\alpha]_D^{26} -162$ (*c* 0·9, $CHCl_3$) and that of the mixture of γ- and δ-cadinene −8 (*c* 0·7, $CHCl_3$). Dehydrogenation with palladium-on-charcoal (30 per cent) for 1 hr at 300-310° gave a mixture of hydrocarbons in which cadalene predominated (GLC analysis).

Unknown Labile Alcohol

GLC analysis of the mixture of oxygenated terpenes showed that the labile alcohol (part of peak 9) was not recovered from modified silicic acid. Attempts to isolate this com-

ponent by preparative GLC on the A–N column also failed. The component was therefore characterized by relative retention times (limonene = 1·00) on the PEG 20M, A–N, RO, EGPN, and QF-1 columns. The values obtained for the unknown constituent, as well as isoamyl alcohol, n-amyl alcohol, n-hexanol, *cis*-hex-3-en-1-ol, α-pinene, sabinene, 1:8-cineole, and β,β-dimethyl allyl alcohol are shown in Table 4. The latter was prepared by lithium aluminium hydride reduction of β,β-dimethyl acrylic acid.

TABLE 4. RELATIVE RETENTION TIMES (LIMONENE = 1·00) OF UNKNOWN LABILE CONSTITUENT AND A VARIETY OF ALCOHOLS AND TERPENES

Column	PEG 20M[1]	A–N[2]	RO[3]	EGPN[4]	QF-1[5]
Temperature °C	65	65	90	70	55
Flow rate (ml He/min)	100	102	170	170	170
t_R Limonene (min)[6]	9·8	16·6	16·2	9·0	7·0
Unknown	1·03	0·15*	0·57*	(3·90)*	0·97*
Isoamyl alcohol	0·94	0·09	0·24	1·58	0·30
n-Amyl alcohol	1·25	0·12	0·32	2·10	0·48
β,β-Dimethyl allyl alcohol	0·97	0·12	0·37	3·95	0·63
n-Hexanol	2·52	0·27	0·66	3·70	0·94
cis-Hex-3-en-1-ol	3·06	0·22	0·63	5·10	0·93
1:8-Cineole	1·05	0·88	0·96	1·67	1·60
Sabinene	0·62	0·55	0·64	0·70	0·54
α-Pinene	0·29	0·40	0·44	0·28	0·93

[1] Polyethylene glycol (Carbowax 20M); 15 per cent on Gaschrom P (60–80 mesh); 6 ft × ¼ in. O.D.

[2] Apiezon N; 10 per cent on Gaschrom P (60–80 mesh); 6 ft × $\frac{3}{16}$ in.

[3] Rapeseed oil; 10 per cent on Anakrom ABS (60–70 mesh); 10 ft × ¼ in.

[4] Ethylene glycol bis-(propionitrile); 15 per cent on Chromosorb W (60–80 mesh); 6 ft × ¼ in.

[5] Fluorinated silicone polymer; 15 per cent on Gaschrom P (60–80 mesh); 6 ft × ¼ in.

[6] Retention time of limonene measured from time of injection to initial emergence of peak.

* Broad peak (decomposing?).

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