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Note

Gas chromatographic characterization of frequently occurring sesquiterpenes in essential oils

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The aim of this work was to determine the retention factors, relative to quaiazulene, for some of the most commonly encountered sesquiterpenes in essential oils, *e.g.* caryophyllene, cadinene, farnesene and bisabolene[1], using two stationary phases of different polarities. This should facilitate the identification of these compounds in natural materials, as the standard deviation calculated from the relative retention factors of a given sesquiterpene in five different oils did not exceed $6 \cdot 10^{-3}$.

EXPERIMENTAL

The separations were carried out on a Jeol JGC 1100 gas chromatograph equipped with a flame ionization detector, a Jeol IR-251 A recorder and a Digint 21 integrator (Chinoin). The injector and detector were held at 200 and 240°C, respectively, and the electrometer sensitivity range was $8 \cdot 10^{-10}$ A/mV. Nitrogen was used as carrier gas, at a flow-rate of 38 ml/min.

Column 1 was a glass spiral (3 m × 2.3 mm I.D.), coated with 3% OV-17 on 100–120 mesh Gas-Chrom and programmed from 60 to 230°C at 8°C/min. The efficiency relative to linalool was 1825 plates/m at McReynolds' constants of 119, 158, 162, 243 and 202[2]. Column 2 was a glass spiral (3 m × 3.4 mm I.D.) coated with 1.5% SP-2250 + 0.95% SP-2401 (mixed SP-phases) on 100–120 mesh Supelcoport, and programmed from 60 to 230°C at 8°C/min. The efficiency relative to linalool was 1776 plates/m at McReynolds' constants of 129, 189, 238, 330 and 244. (The constants for the mixed SP-phases were calculated from those of the pure components[2].)

Samples of 0.1–0.5 µl of solutions containing 10–20 mg/ml of essential oil in chloroform or hexane were injected. The retention times were measured with an accuracy of 0.1 sec; the relative retention factors were determined from three to five parallel measurements, and the column temperature variation was ± 1°C. The sesquiterpenes present in the essential oils were identified either by mass spectrometry (MS) or, more frequently, by gas chromatography (GC)–MS or GC–infrared (IR) techniques. In a few cases (farnesene isomers, α-bisabolol. azulenes) the compounds were isolated by preparative GC or thin-layer chromatography (TLC) before identification by MS or IR[3–5].

The raw materials were sesquiterpene-rich Hungarian commercial oils (chamomile, yarrow, basil, and wormwood oils) and also the volatile oil of *Cannabis sativa* L.var. Mexico, grown in Hungary.

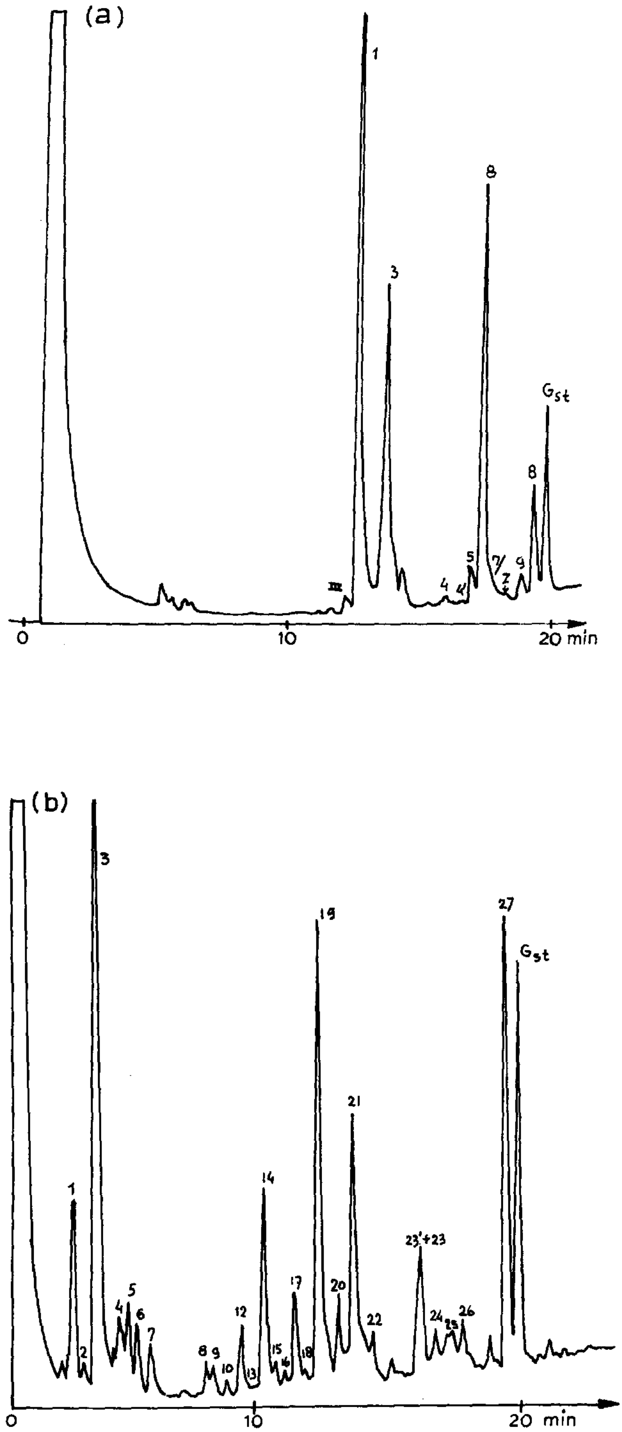
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Fig. 1.

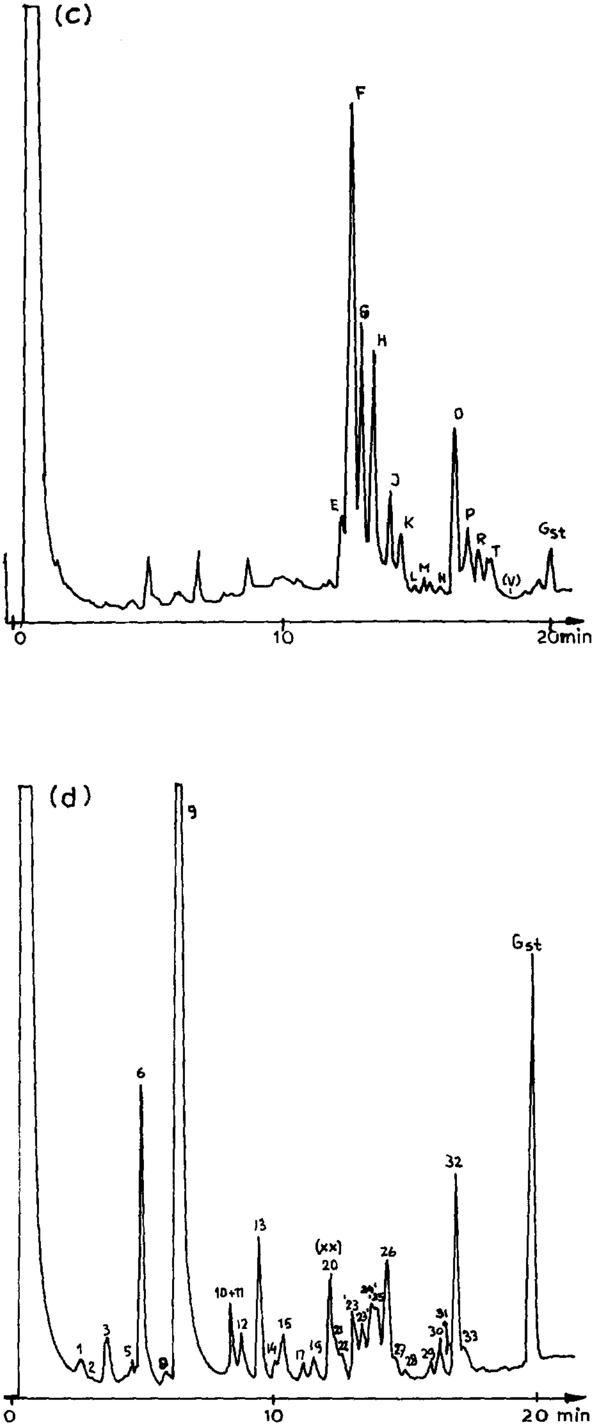

Fig. 1.

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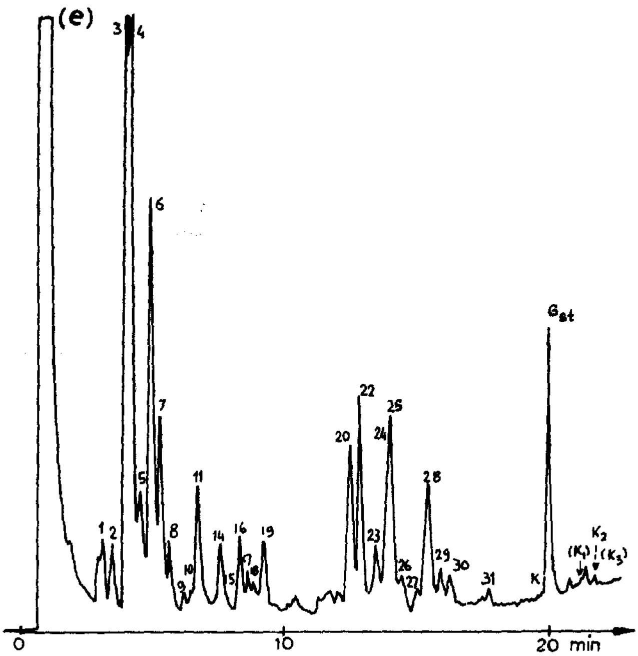

Fig. 1. Gas chromatograms of essential oils on column 1. For conditions, see Experimental. For peak identification see Tables I and II. (a) Chamomile oil; (b) yarrow oil (1–18 are monoterpenes); (c) cannabis oil (S and V are not present in the leaf oil, only in the flower oil); (d) basil oil (1–19 are monoterpenes and phenylpropane derivatives; 23 and 31 are not present in freshly distilled oils; XX, 24 and 25 are present only in freshly distilled oils; (e) wormwood oil (K_1, K_3 are present only in the oils obtained by special distillation[5]; 1–19 are monoterpenes).

RESULTS AND DISCUSSION

As isolated representative sesquiterpenes were not available for the determination of retention factors, we could not prepare a standard series of compounds similar to that previously described for the monoterpene series[2]. Therefore, the retention factors were calculated directly from the t_R values of the sesquiterpenes identified in the five essential oils mentioned above (Fig. 1a–e).

Tables I and II list the retention factors found for the sesquiterpenes studied, for each stationary phase used, relative to that of guaiazulene as standard ($F_G \cdot 10^3 = 1000$). Corresponding standard deviation values, which are in the range $1 \cdot 10^{-3}$–$6 \cdot 10^{-3}$, and retention factors of selected C_{15}–C_{20} *n*-alkanes are included in these Tables to demonstrate the reproducibility and to facilitate the use of this identification procedure.

During the GC separations, it was observed that the sesquiterpene hydrocarbons (azulenes excepted) were eluted first, from 160 to 180°C, followed by the oxygen-containing sesquiterpenes, from 180 to 210°C, and finally by the quaiane-type sesquiterpenes, from 210 to 230°C. Better separations were sometimes achieved on the polar mixture of SP-phases, *e.g.* the separation of compound 22 from *trans-β*-farnesene in basil and wormwood oils; of spathulenol from caryophyllenol-I and caryophyllene epoxide; of compound 23 from 23′ in yarrow oil.

TABLE I

RETENTION FACTORS OF SESQUITERPENES RELATIVE TO GUAIAZULENE STANDARD ON 3% OV-17 STATIONARY PHASE IN VARIOUS ESSENTIAL OILS

n-Alkane series	*Sesquiterpenes*					*Retention factor, $F_G \cdot 10^3$*	*Standard deviation, $\Delta S \cdot 10^3$*
	Chamomile oil	*Yarrow oil*	*Cannabis oil*	*Basil oil*	*Wormwood oil*		
			E α-bergamotene	20 α-Bergamotene		606	2.65
				XX α-Cedrene		606	
C_{15}	III M^+ 204	19 β-Caryophyllene	F β-Caryophyllene	21	20 β-Caryophyllene	612	4.06
	1 *trans*-β-Farnesene		G *trans*-β-Farnesene	22	22	634	4.35
		20 α-Humulene	H α-humulene	23 M^+ 204		652	4.21
				23	23	661	2.08
	2	21 γ-Muurolene		24 γ-Muurolene		677	4.34
				24′	24	684	2.83
C_{16}	3 α-Farnesene		I α-Farnesene	25	25	692	2.49
				25 M^+ 204		700	
		22 γ-Cadinene	K γ-Elemene	26 γ-Cadinene	26	709	3.12
			L α-Gurjunene	27		732	
			M β-Bisabolene	28	27 β-Bisabolene	751	5.0
					28	766	
C_{17}						776	
			N M^+ 220		29	790	
	4 Spathulenol	23′+ 23 Caryophyllenol-I	O Caryophyllene epoxide	29	30 Caryophyllene epoxide	808	6.0
				30 M^+ 204		820	
	4′ M^+ 238			31		833	
		24 M^+ 222	P M^+ 222	32 M^+ 204		843	4.55
C_{18}	5 Bisabolol-oxide II					854	
		25 M^+ 222	R M^+ 220			864	
	6 α-Bisabolol		S α-Bisabolol	33 α-Bisabolol	31 α-Bisabolol	875	5.0
		26 M^+ 220	T M^+ 220			890	
	7 Bisabolone-oxide					900	
C_{19}	Z		V M^+ 222			925	
	9 Bisabolol-oxide I					952	
	8 Chamazulene	27 Chamazulene			K Chamazulene	977	2.41
C_{20}			Guaiazulene standard			1000	
					K_1 Methylchamazulene	1052	
					K_2 M^+ 214	1083	
					K_3 Ethylchamazulene	1110	

TABLE II

RETENTION FACTORS OF SESQUITERPENES RELATIVE TO GUAIAZULENE STANDARD ON 1.5% SP-2250 PLUS 0.95% SP-2401 STATIONARY PHASE IN VARIOUS ESSENTIAL OILS

n-Alkane series	*1Sesquiterpenes*					*Retention factor* $F_G \cdot 10^3$	*Standard deviation* $\Delta S \cdot 10^3$
	Chamomile oil	*Yarrow oil*	*Cannabis oil*	*Basil oil*	*Wormwood oil*		
			E α-Bergamotene	20 α-Bergamotene		607	5.0
				XX α-Cedrene		607	
C_{15}	III M^+ 204	19 β-Caryophyllene	F β-Caryophyllene	21	20 β-Caryophyllene	624	5.0
				22	22	630	
	1 *trans*-β-Farnesene		G *trans*-β-Farnesene			641	
	2	20 α-Humulene	H α-Humulene	23 M^+ 204	23	663	1.71
				23		668	
		21 γ-Muurolene		24 γ-Muurolene	24	682	1.9
				24′		686	
C_{16}	3 α-Farnesene		I α-Farnesene	25	25	691	4.73
				25 M^+ 204		700	
		22 γ-Cadinene	K γ-Elemene	26 γ-Cadinene	26	713	4.09
			L α-Gurjunene	27		739	
			M β-Bisabolene	28	27 β-Bisabolene	755	4.93
					28	770	
C_{17}						787	
			N M^+ 220	29	29	802	4.06
	4 Spathulenol	23′				810	
		23 Caryophyllenol-I	O Caryophyllene epoxide	30 M^+ 204	30 Caryophyllene epoxide	830	3.03
	4′ M^+ 238			31		837	
		24 M^+ 222	P M^+ 222	32 M^+ 204		846	3.2
C_{18}	5 Bisabolol-oxide II		R M^+ 220			864	
	6 α-Bisabolol	25 M^+ 222	S α-Bisabolol	33 α-Bisabolol	31 α-Bisabolol	878	4.0
	7 Bisabolone-oxide	26 M^+ 220	T M^+ 220			892	2.3
C_{19}	7′ C_{20}		V M^+ 222			925	
	9 Bisabolol-oxide I					955	
	8 Chamazulene	27 Chamazulene			K Chamazulene	976	1.0
	Guaiazulene standard					1000	
					K_1 Methylchamazu-lene	1051	
					K_2 M^+ 214	1082	
					K_3 Ethylchamazulene	1113	

The OV-17 and mixed SP stationary phases proved to be much more favourable than PEG 20M (which is widely mentioned in the literature), because the sesquiterpenes and monoterpenes do not overlap during separation. It fact, it may be difficult to separate mono- from sesquiterpenes on PEG 20M, even on a capillary column[6].

Besides their chromatographic significance, the results given in Table I and II also provide some new information regarding the qualitative composition of the essential oils studied. Thus, the occurrence of α-farnesene in Hungarian chamomile and cannabis oils, of α-bisabolol in basil and wormwood oils, and of α-humulene, γ-cadinene, γ-muurolene and caryophyllenol-I in yarrow oil, was not previously known[6–19].

CONCLUSION

The present collection of retention data of sesquiterpenes should facilitate the qualitative analysis of essential oils. In this respect, it complements the similar data previously published for the monoterpene series[2]. These results may also help in the selection of a suitable stationary phase for separating known sesquiterpenes, and represent a possible starting point for the analytical investigation of essential oils of unknown origin.

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