Journal of Chromatography, 449 (1988) 312–316
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 747

Note

Characterisation of volatile oil constituents with relatively long gas chromatographic retention times on two stationary phases

T. J. BETTS
School of Pharmacy, Curtin University of Technology, GPO Box U 1987, Perth, W. Australia 6001 (Australia)
(First received April 20th, 1988; revised manuscript received June 20th, 1988)

The new spectral-recording gas chromatographic (GC) detectors making computerised analyses of their records are the "state-of-the-art" in GC instrumentation. However, they are quite expensive, and many laboratories have to make do with cheaper, more traditional detectors. Even with these new luxury detectors it is valuable to have another method to confirm the spectral identification, especially if the computer conclusion does not have a high probability.

The flame ionisation detector is a universal, quickly set up GC detector for organic solutes, but unless molar response factors are considered, it is unable to distinguish between different chemical classes of solutes it detects.

Even before the invention of this detector attempts were made to use two stationary phases for the identification of substances. In 1956, different straight line plots for alkanes, alkanols and alkanones were obtained[1] by subtracting logarithmic expressions of retention times on a triethyleneglycol column from those on an ester (both polar!). Many papers appeared relating solute structure and retention values using two phases, for example that of Lewis *et al.*[2]. Merritt and Walsh found[3] that the only "very good" pairing was of two polyethyleneglycols for distinguishing homologous series. However, they then recorded[4] that the "only pair (of columns) which showed a change in order of elution of (five mixed) components" was polyethyleneglycol with a silicone. Smith *et al.*[5], and later Ladon[6], studied hydrocarbons only, on polar and non-polar (squalane) columns, and the lattermost could identify unknown peaks mathematically.

In 1970, Breckler and Betts[7] investigated the use of relative retention times ($t_{R,rel}$) to linalol on packed columns, as a guide to such distinction for the most volatile constituents of volatile oils. Betts[8] reviewed this work, where substances of low polarity like limonene, cineole and menthyl acetate showed higher $t_{R,rel}$ values on the low-polarity methyl polysiloxane column than on polar columns. More polar solutes such as anethole, citral and geraniol gave the opposite effect, with $t_{R,rel}$ values on the low polarity column being well below the results for polar columns. It was also possible to detect an alcohol, as unlike other polar solutes it would give higher $t_{R,rel}$ values on polyethylene glycol stationary phase (with its matching hydroxy groups) than on polyester. Five groups of volatile oil constitutents with $t_{R,rel}$ values usually in the range 0.24–4.00 could be distinguished by this method. Betts[9] applied this concept to capillary columns in 1984.

0021-9673/88/$03.50

This work has now been extended to volatile oil constituents with relatively long retention times such as polysubstituted aromatics and sesquiterpenes. Lemberkovics and Verzar-Petri[10] appreciated there was a problem in identifying frequently occurring sesquiterpenes in volatile oils, but complicated their experimental situation by apparently mixing two polysiloxanes in one packed column.

Betts[8] found that a standard giving best results was a terpene alcohol, and as the previously used standard linalol has a short retention time, geraniol, which has the longest retention time of any monoterpenol, was here selected as standard.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 5790A gas chromatograph was applied, fitted with a flame ionisation detector used at 200°C, capillary control unit and splitter injection port used at 200°C. Hewlett-Packard 3390A and 3380A recorder/integrators were used.

Two Hewlett-Packard fused-silica capillaries approximately 25 m × 0.21 mm I.D. containing: (a) Carbowax 20M (polyethylene glycol), or (b) high-performance cross-linked methyl silicone, both used isothermally at 135°C, were used.

Helium was used as the mobile phase gas at about 1 ml min^{-1}, and as make-up gas for the detector.

Materials

The materials investigated are indicated in Table I.

Method

Several old volatile oils were examined on the two capillary columns using repeated injections from an initially filled and emptied microsyringe. The earlier injections gave "percentage composition" of the peaks from recorder percentage area measurements, and the subsequent injections gave shorter, more reliable retention times, particularly for the major oil components. For each capillary, the retention time of geraniol was approximately 1.45 min.

RESULTS AND DISCUSSION

Results are given in Table I, based on the shortest retention time obtained by using traces of solutes. It is apparent that $t_{R,rel}$ ratios for polar against non-polar phase (P/N) can give an indication of the chemical class of a volatile oil solute. From the substances studied, if the ratio is less than 0.30, it is a sesquiterpene hydrocarbon. If P/N is 0.40–0.95, the substance is most likely a terpenoid, being a sesquiterpenoid if the retention times are high. The aromatics anethole and safrole are exceptionally in this range. If the ratio is greater than 0.95, the solute is an aromatic substance, being an ether if the P/N is less than 1.6, but an aldehyde or phenol if it is greater, the lattermost being more likely with higher ratios. Although not intended as part of these studies, it was observed that the monoterpene hydrocarbon limonene had P/N 0.67 (with very short retention time). It can be concluded that methyl eugenol in terms of P/N is close in polarity to geraniol, although its retention times on both stationary phases are about 70% greater than those of this standard.

TABLE I

RELATIVE RETENTION TIMES (GERANIOL = 100) AT 135°C

Mobile phase: helium at flow-rate about 1 ml min^{-1} at detector exit, giving t_R for geraniol (Sigma) 1.35–1.55 min on both capillaries.

Volatile oil constituent (and source)	$t_{R,rel}$ *vs. geraniol*		*P/N*	*Chemical nature and comment*
	Polar (P): Carbowax 20M	*Non-polar (N): methyl silicone polysiloxane, cross-linked*		
Caryophyllene (Koch-Light)	0.52	2.22	0.23	Sesquiterpene hydrocarbon
Zingiberene (Ginger oil Kelkar)	0.67	2.92	0.23	Sesquiterpene hydrocarbon
Curcumene (Ginger oil Kelkar)	0.76	3.31	0.23	Sesquiterpene hydrocarbon
Eudesmol (Plaimar research)	3.62	5.79	0.63	Sesquiterpenoid mixture, main peak. Very long t_R
Terpinen-4-ol (Dragoco)	0.52	0.80	0.65	Monoterpenoid-detected in Nutmeg oil
Carvone (Koch-Light)	0.68	0.99	0.69	Monoterpenoid
Perillal (Koch-Light)	0.82	1.09	0.75	Monoterpenoid
Anethole (Sigma)	0.88	1.15	0.76	Aromatic ether, monomethoxy
Citronellol (BDH)	0.77	0.92	0.84	Monoterpenoid
Farnesol (Aldrich)	6.92	8.14	0.85	Sesquiterpenoid (acyclic). Extremely long t_R
Safrole (Fritzsche)	1.00	1.15	0.87	Aromatic ether, methylene dioxy (detected in Nutmeg oil)
Santalol (Sandawood oil Izumi)	6.04	6.46	0.93	Sesquiterpenoid. Extremely long t_R
Methyleugenol (Fritzsche)	1.65	1.75	0.94	Aromatic ether, dimethoxy
Elemicin (synthesised)	3.58	3.46	1.03	Aromatic ether, trimethoxy
Myristicin (Nutmeg oil Bush Boake Allen)	3.83	2.86	1.34	Aromatic ether, methylenedioxy monomethoxy (detected in Parsley seed oil)
Cinnamal (Cinnamon oil)	1.73	1.06	1.63	Aromatic aldehyde
Eugenol (Rampre)	2.82	1.50	1.88	Phenol
Thymol (Sigma)	3.15	1.14	2.76	Phenol

Application to Chenopodium oil

A sample of Chenopodium oil (Felton, Grimwade and Bickford, Perth, Australia) exhibited a main polar phase capillary peak (67 and 62% of total peak areas determined on two runs) at t_R 1.15 min or less, which if related to the main non-polar peak (60% and 59%) at t_R 1.52 min gave P/N of 0.71/1.00 = 0.71. This indicated a terpenoid, namely the peroxide ascaridole, present in appropriate quantity for a good specimen[11]. The results taken from the printouts are given in Table II.

TABLE II

RESULTS TAKEN FROM HP 3390A PRINTOUTS FROM GC RUNS OF CHENOPODIUM OIL

Run No. 23, Chenopodium oil (FGB) Area%, POLAR PHASE, geraniol t_R 1.53 min					*Run No. 3, Chenopodium oil (FGB) Area%, NON-POLAR PHASE, geraniol t_R 1.52 min*				
t_R	*Area*	*Type**	*AR/HT***	*Area%*	t_R	*Area*	*Type**	*AR/HT***	*Area%*
0.52	377080	D BB	0.023	17.587	0.42	1634	PV	0.030	0.158
0.63	7533	BP	0.045	0.351	0.47	1999	D VB	0.025	0.193
0.75	1817	PV	0.043	0.085	0.78	215720	PB	0.029	20.865
0.81	3802	VV	0.026	0.177	0.93	12353	PV	0.038	1.195
0.86	9235	VV	0.030	0.431	1.01	7885	VB	0.054	0.763
0.91	3441	VV	0.029	0.161	1.20	4346	PV	0.032	0.420
0.94	4369	D VP	0.034	0.204	1.24	6223	D VP	0.047	0.602
1.15	1433000	PB	0.057	66.834	1.40	8388	PV	0.062	0.811
1.24	1553	D BP	0.024	0.072	1.52	610280	VV	0.041	59.028
1.30	13308	PV	0.035	0.621	1.58	8470	D VV	0.030	0.819
1.39	4415	VV	0.037	0.206	1.64	6998	VV	0.033	0.677
1.46	4019	VV	0.032	0.187	1.74	25134	VV	0.038	2.431
1.51	12019	VV	0.043	0.561	1.89	74367	VB	0.037	7.193
1.66	111140	VV	0.039	5.184	2.09	2209	BP	0.035	0.214
1.71	4905	D VB	0.027	0.229	2.21	2262	PV	0.060	0.219
2.08	12695	PV	0.041	0.592	2.27	3886	VV	0.039	0.376
2.42	4072	BV	0.044	0.190	2.33	7832	VV	0.039	0.758
2.50	10832	VB	0.048	0.505	2.64	28541	PB	0.042	2.761
3.40	11239	PV	0.083	0.524	3.03	1698	BB	0.051	0.164
3.65	28914	VV	0.065	1.349	3.25	3672	PB	0.074	0.355
3.78	9245	VB	0.064	0.431					
4.90	42483	PB	0.036	1.981					
5.26	13252	BV	0.104	0.618					
6.85	13599	BB	0.105	0.634					
7.62	6144	PB	0.126	0.287					

* The integrator provides an opinion about the type of peak recorded. B = Peak begins or ends on baseline (BB is thus the best); D = distorted peak; P = penetration of baseline (reset); V = valley between closely emerging peaks rather than baseline (VV indicates incomplete peak resolution on both sides).

** AR/HT expresses the width of the peak at half its height, in min. Retatively high values indicate a less efficient match of stationary phase and solute.

Ignoring an early monoterpene hydrocarbon peak (about 20%), a polar peak was noticed at t_R 1.66 min or less, giving $t_{R,rel}$ 1.06 (5 and 6%). A corresponding non-polar peak, t_R 1.89 (7 and 7%) gave $t_{R,rel}$ 1.24. This yielded P/N = 0.85 and it could, from t_R, be an aromatic. Such provisional identification can assist a computerised search of spectal data on file.

A late polar peak (2 and 2%), t_R 4.90 min or less with $t_{R,rel}$ 3.16, if matched with the non-polar t_R 1.74 min, $t_{R,rel}$ 1.14 (2 and 3%) indicated by P/N = 2.77 a phenol, in fact thymol. This is a reported degradation product of ascaridole[12]. If the late polar peak was "matched" with the non-polar one at t_R 2.64 min, a phenol was again indicated, although not thymol.

Application to Pimento oil

A sample of "Ol Pimentae" (Faulding, Perth, Australia) gave eugenol as its major constituent, about 70% of total peak areas, an appropriate value[13].

The second peak in terms of area percentage (12 and 10%) was $t_{R,rel}$ 3.07 on the polar capillary and 2.61 (11 and 11%) on the other, giving P/N = 1.18, indicating an aromatic ether which is not methyl eugenol, a reported constituent[13].

The peak $t_{R,rel}$ 0.53 (4 and 6%) on polar phase, if matched with 2.18 (4 and 4%) non-polar, gave P/N = 0.24, a sesquiterpene hydrocarbon, probably β-caryophyllene, which has been reported at 4.2% in the oil[13].

The long $t_{R,rel}$ 6.07 (1.5 and 1.3%) on the polar capillary could be related to the non-polar peak 2.99 (1.6 and 1.6%). This gave P/N of 2.03, suggesting a phenol other than eugenol. It is possibly chavicol, which has been reported[13].

Application to Eucalyptus rudis oil

An oil distilled from fresh leaves of *Eucalyptus rudis* in these laboratories in 1974 was examined. It was yellow and mobile, somewhat viscous. The main component cineole was present to the extent of just over 50% peak area on both columns, and some cineole had probably been lost during storage.

Another early peak at about 11% peak areas showed $t_{R,rel}$ values of 0.65 and 0.84 on the capillaries, giving P/N of 0.77 for a monoterpene hydrocarbon. A similar peak area (10, 11, and 14%) was observed at $t_{R,rel}$ 2.12 on the polar phase, and 4.28 non-polar (12, 13 and 13%) with P/N 0.50 for a sesquiterpenoid (as retention times were large). This same substance has been detected here in oils distilled from *E. cinerea*, *E. globulus*, *E. nicholii* and *E. viminalis*.

A polar phase peak consistently of 4% total area on both capillaries was noted at $t_{R,rel}$ of 0.54 and 2.38, giving P/N of 0.23 for a sesquiterpene hydrocarbon. Another peak on the polar phase, $t_{R,rel}$ 0.98 (3, 3 and 4%) could be related to 3.89 non-polar (2, 3 and 4%) with P/N of 0.25 indicating another sesquiterpene hydrocarbon.

Peaks of 2% total area on both capillaries with the same $t_{R,rel}$ of 1.66 gave, of course, P/N = 1.00, suggesting an aromatic.

REFERENCES

1 G. J. Pierotti, C. H. Deal, E. L. Derr and P. E. Porter, *J. Am. Chem. Soc.*, 78 (1956) 2989.
2 J. S. Lewis, H. W. Patton and W. I. Kaye, *Anal. Chem.*, 28 (1956) 1370.
3 C. Merritt and J. T. Walsh, *Anal. Chem.*, 34 (1962) 903.
4 C. Merritt and J. T. Walsh, *Anal. Chem.*, 34 (1962) 908.
5 B. Smith, R. Ohlson and G. Larson, *Acta Chem. Scand.*, 17 (1963) 436.
6 A. W. Ladon, *J. Chromatogr.*, 99 (1974) 203.
7 P. N. Breckler and T. J. Betts, *J. Chromatogr.*, 53 (1970) 163.
8 T. J. Betts, *Aust. J. Pharm., Suppl.*, 52 (1971) 57.
9 T. J. Betts, *J. Chromatogr.*, 294 (1984) 370.
10 E. Lemberkovics and G. Verzar-Petri, *Essential Oils and Aromatic Plants*, Martinus Nijhoff/Dr. W. Junk, Dordrecht, The Netherlands, 1985, p. 103.
11 E. K. Nelson, *J. Am. Chem. Soc.*, 33 (1911) 1405.
12 E. K. Nelson, *J. Am. Chem. Soc.*, 35 (1913) 84.
13 J. Nabney and F. V. Robinson, *Flav. Ind.*, 3 (1972) 50.