ESSENTIAL OILS AND THEIR CONSTITUENTS

XXXII. GAS CHROMATOGRAPHY OF SESQUITERPENE HYDROCARBONS*

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INTRODUCTION

In contrast to numerous publications reporting systematically correlated gas chromatographic data for monoterpenes[2-4] no study of comparable magnitude has so far appeared in the literature concerning similar criteria of identity for sesquiterpene hydrocarbons, whose preparation and authentication are delicate and difficult operations. It is the purpose of this paper to present such data, to illustrate their value for the analysis of essential oils and related products and to interpret characteristic gas chromatographic parameters assembled for some of the isolates in terms of molecular structure.

EXPERIMENTAL

Apparatus and procedures

Apparatus and gas chromatographic methods were described previously[5]. In addition to Reoplex 400 (10 %) deposited on acid-washed chromosorb W, silicone rubber SE-30 (10 %) and silicone nitrile XE-60 (5 %) were used as substrate. Monoterpenes were examined at 100° and sesquiterpene hydrocarbons at 150°. Carrier gas (helium) flow rate was maintained at 75 ($\pm$ 1) ml/min. Three stable aromatic hydrocarbons, namely naphthalene, 2-methylnaphthalene and acenaphthene, which are commercially available (purity 99 %), were used as internal standards.

Isolation of sesquiterpene hydrocarbons

Since these compounds are commercially unavailable, they were isolated from authenticated essential oils by careful rectification and column and/or preparative gas chromatography of specific fractions. Some of the products were courteously supplied by other researchers (see Table I). Their identity was in all instances confirmed by comparison of their infrared spectra with those of genuine reference standards or published data. Column chromatography was generally carried out using neutral grade I alumina (Woelm) (20 times the weight of the sample) as adsorbent and petroleum ether as eluant.

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* For Part XXXI, see ref. 1.

TABLE I

ISOLATION OF SESQUITERPENE HYDROCARBONS

No.	Compound	Source and method of isolation
	Acyclic	
1	β-Farnesene	Farnesol. Column (neutral grade I alumina) and gas chromatography (silicone nitrile XE-6o) of dehydration product[6]
	Monocyclic	
2	α-Bisabolene	Oil of opoponax. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-6o
3	β-Bisabolene	
4	γ-Bisabolene	
5	Curcumene	Oil of *Curcuma aromatica*. Column chromatography
6	β-Elemene	Oil of bois de rose. Fractional distillation, column chromatography of high-boiling fraction followed by gas chromatography. Liquid phase: Reoplex 400[7]
7	α-Humulene	Oil of *Zingiber zerumbet* Smith. Column chromatography followed by fractional distillation[8]
8	β-Humulene	Oil of copaiba balsam. Gas chromatography. Liquid phase: Reoplex 400[6]
9	α-Zingiberene	Oil of ginger. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-6o[9]
10	β-Zingiberene	
	Bicyclic	
11	α-Bergamotene	Courtesy Dr. R. B. BATES, University of Arizona, Tucson, Ariz., U.S.A.
12	β-Bergamotene	
13	α-Bulnesene	Oil of patchouli. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-6o
14	γ-Cadinene	Oil of ylang ylang, fraction III. Column chromatography, followed by gas chromatography. Liquid phase: silicone nitrile XE-6o
15	δ-Cadinene	
16	Caryophyllene	Oil of copaiba balsam. Gas chromatography. Liquid phase: Reoplex 400[6]
17	Isocaryophyllene	Courtesy Dr. E. WARNHOFF, University of Western Ontario, London, Ont., Canada
18	α-Guaiene	Oil of patchouli. Column chromatography followed by gas chromatography of petroleum ether eluate. Liquid phase: silicone nitrile XE-6o
19	α-Himachalene	Courtesy Dr. R. B. BATES, University of Arizona, Tucson, Ariz., U.S.A.
20	β-Himachalene	
21	β-Santalene	Oil of East Indian sandalwood. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-6o
22	epi-β-Santalene	
23	α-Selinene	Oil of bois de rose. Fractional distillation, column chromatography of high-boiling fraction followed by gas chromatography. Liquid phase: Reoplex 400[7]
24	β-Selinene	Oil of celery seed. Column chromatography
	Tricyclic	
25	Aromadendrene	Oil of *Eucalyptus globulus*. Column chromatography following removal of cineole by treatment with resorcinol[10]
26	Cedrene	Oil of red cedarwood. Gas chromatography. Liquid phase: Reoplex 400
27	α-Gurjunene	Oil of gurjun balsam. Column chromatography followed by gas chromatography. Liquid phase: Reoplex 400
28	β-Gurjunene	
29	Longifolene	Oil of *Pinus longifolia*. Fractional distillation followed by column chromatography
30	Isolongifolene	Courtesy Dr. S. DEV, National Chemical Laboratory, Poona, India

TABLE I (*continued*)

No.	Compound	Source and method of isolation
31	β-Patchoulene	Oil of patchouli. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
32	α-Santalene	Oil of East Indian sandalwood. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
33	Ylangene	Oil of ylang ylang, Fraction III. Column chromatography followed by gas chromatography. Liquid phase: silicone nitrile XE-60
	Tetracyclic	
34	Longicyclene	Courtesy Dr. S. DEV, National Chemical Laboratory, Poona, India

DISCUSSION

Performance of internal standards

Experimental data illustrating the gas chromatographic behaviour of the reference standards used are shown in Table II. Naphthalene was found to be most suitable when using Reoplex 400 as the liquid phase, 2-methylnaphthalene performed best when using silicone nitrile XE-60 as the substrate, and acenaphthene was applied to greatest advantage when silicone gum SE-30 served as the stationary phase.

Selectivity of column substrates

Valuable information regarding substrate performance is conventionally obtained by plotting logarithms of relative retention times (RRT) *versus* boiling points for compounds comprising a series of structurally related molecules. Due to lack of boiling point data (valid at 760 mm) such correlations can not be applied to sesquiterpenes. Boiling points at reduced pressures (10 ± 1 mm), available for fourteen of the compounds studied (see Table III), were plotted against log relative retention times, in order to judge the selectivity of the columns employed. A fairly straight line relationship was obtained only for the SE-30 column as shown in Fig. 1. Ses-
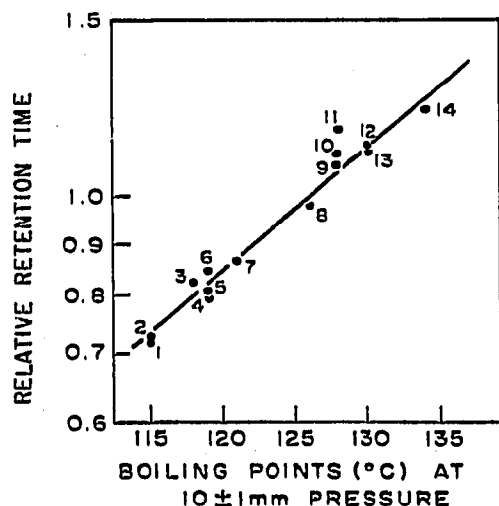

Fig. 1. Relation between boiling points and relative retention times of sesquiterpenes. Column: silicone rubber SE-30; retention data from Table V.

TABLE II

RELATIVE RETENTION TIMES OF REFERENCE STANDARDS
(Column temperature: 150°, Helium: 75 ml/min)

Compound	Reoplex 400 10%	Silicone nitrile XE-60 5%	Silicone gum SE-30 10%
Naphthalene	1.00	0.66	0.29
2-Methylnaphthalene	1.55	1.00	0.46
Acenaphthene	—	2.31	1.00

TABLE III

BOILING POINTS OF SESQUITERPENE HYDROCARBONS AT REDUCED PRESSURE (10 ± 1 mm)[*]

No.	Compound	Boiling point (°C)	No.	Compound	Boiling point (°C)
1	β-Elemene	115	8	β-Santalene	126
2	α-Gurjunene	115	9	α-Selinene	128
3	Cedrene	118	10	α-Zingiberene	128
4	β-Gurjunene	119	11	β-Zingiberene	
5	α-Santalene	119	12	β-Bisabolene	130–131
6	Caryophyllene	119	13	γ-Bisabolene	
7	Aromadendrene	121	14	γ-Cadinene	134

[*] Literature values, see ref. 11.

TABLE IV

SEPARATION FACTORS FOR SOME SESQUITERPENES ON 10% AND 20% REOPLEX 400 COLUMNS

No.	Sesquiterpene pair	Retention time (min)		Separation factor	
		10% column	20% column	10% column	20% column
1	Caryophyllene	9.95	13.75	1.26	1.31
	α-Humulene	12.55	18.10		
2	Caryophyllene	9.95	13.75	1.24	1.30
	β-Humulene	12.35	18.00		
3	epi-β-Santalene	9.50	14.00	1.05	1.06
	β-Santalene	10.00	14.80		
4	Longifolene	8.40	12.30	1.24	1.29
	Longicyclene	6.75	9.50		
5	α-Himachalene	10.95	15.70	1.23	1.33
	β-Himachalene	13.45	19.45		

quiterpenes displaying only minor differences with regard to boiling points were, however, more effectively separated by the XE-60 as well as the Reoplex 400 column. In some instances their order of emergence was even reversed.

Separation of peaks was not markedly improved when higher liquid phase concentrations were used. A 20 % Reoplex column, for example, brought about only slightly better separation of sesquiterpene hydrocarbon pairs than the 10 % column (Table IV). In practice the small gain in column effectiveness thus realized was offset by correspondingly longer retention times.

Resolution of sesquiterpene hydrocarbon mixtures occurring in nature

The resolution of sesquiterpene mixtures depends primarily on the vapour pressure and polarizabilities of the individual components. Hence, the nature of different substrates markedly affects column performance. This study has shown that Reoplex 400 and silicone nitrile XE-60 are substrates of choice for separating compounds of close boiling points but different polarizabilities, while silicone rubber SE-30 is most suitable for separating compounds of similar polarizabilities but different boiling points. Thus, Reoplex and silicone nitrile resolve the caryophyllene fraction of oil of copaiba balsam more effectively than silicone rubber as shown in Figs. 2, A, B and C. On the other hand silicone rubber SE-30 resolves β-santalene and *epi*-β-santalene, two epimeric constituents of oil of sandalwood, more effectively than silicone nitrile, no resolution whatever being obtained with Reoplex 400 (see Figs. 2, D, E and F). An example showing better performance of silicone nitrile XE-60 than either SE-30 or Reoplex 400 is the resolution of the sesquiterpene fraction of oil of ginger as illustrated in Fig. 2, G, H and I.

Application of experimental results to identification of essential oil constituents

The data shown in Table V are of value for the analysis of complex mixtures of sesquiterpenes, isolated from essential oils or obtained by synthesis. They allow for the recognition of constituents by comparison of their retention times on two or three columns of different polarities. Supplemented by infrared and coupled gas–thin-layer chromatographic analysis[12] of effluent fractions, the technique described affords a valuable tool for the examination and characterization of complex natural and synthetic compositions. Adopting this approach the authors established the occurrence of β-humulene in the caryophyllene fraction of oil of copaiba balsam[5] and are now reporting the occurrence of ylangene, α-bergamotene and δ-cadinene in this oil for the first time (see Fig. 2).

Dependence of retention time on structural features

Since the advent of gas chromatography, numerous attempts have been made to correlate gas chromatographic data of compounds with molecular structures. A method, first used by MARTIN and co-workers[13], consists of plotting the logarithms of retention volumes obtained with one stationary phase against those obtained with another stationary phase of different polarity. Of special interest in this regard are the studies by ZUBYK AND CONNER[14] and KLOUWEN AND TER HEIDE[4] concerning the correlation of gas chromatographic data with structural features of cyclic and acyclic monoterpenes.

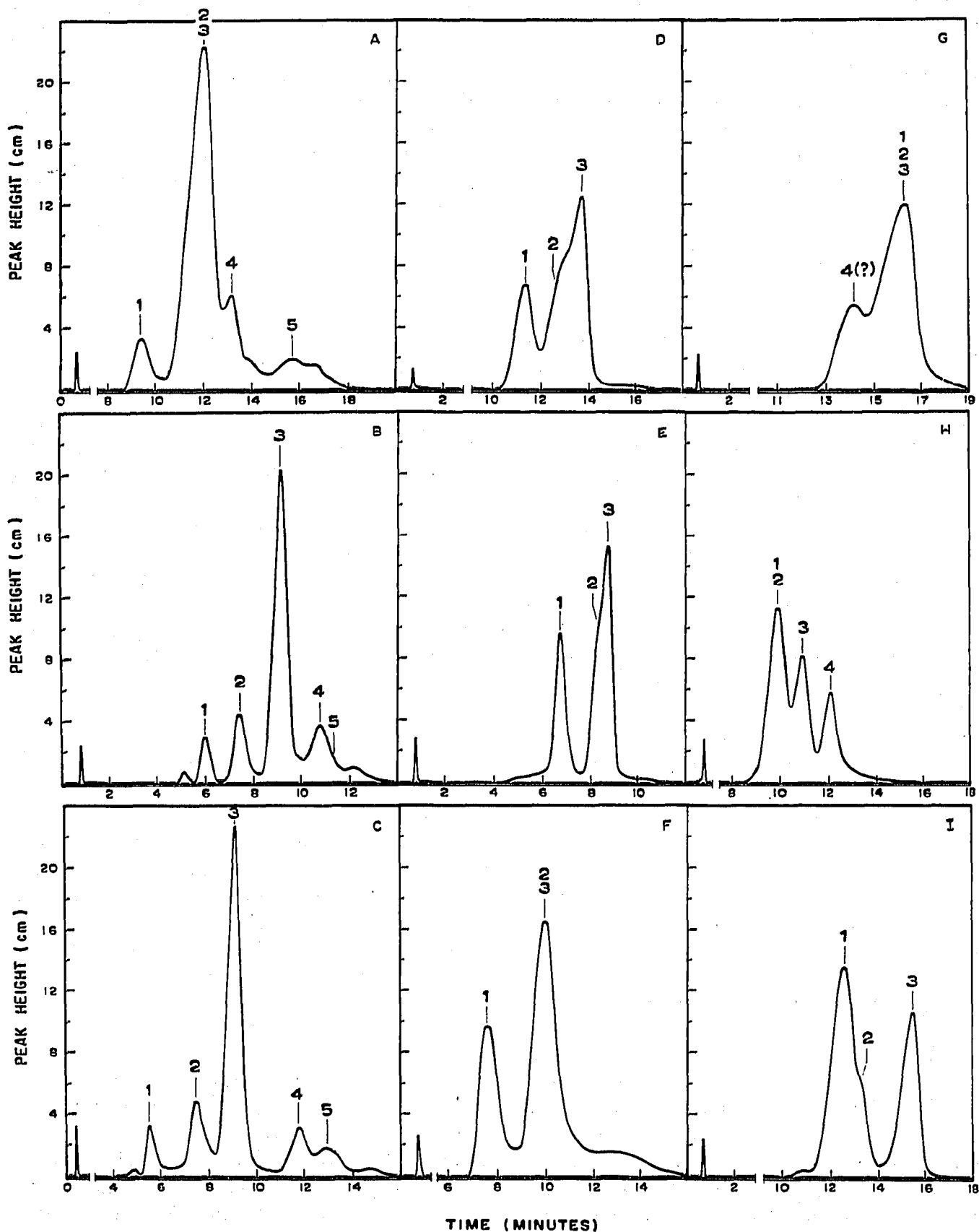The present investigation indicates that such broad correlations do not apply

Fig. 2. Separation of sesquiterpene mixtures. Samples: A, B and C = caryophyllene fraction from copaiba balsam; D, E and F = sandalwood oil sesquiterpenes; G, H and I = ginger oil sesquiterpenes. Constituents of A, B and C: 1 = ylangene, 2 = α-bergamotene, 3 = caryophyllene, 4 = β-humulene, 5 = δ-cadinene; D, E and F: 1 = α-santalene, 2 = *epi*-β-santalene, 3 = β-santalene; G, H and I: 1 = α- and β-zingiberene, 2 = β-bisabolene, 3 = curcumene, 4 = farnesene. Columns for A, D and G: silicone rubber SE-30 (10%); B, E and H: silicone nitrile XE-60 (5%); C, F and I: Reoplex 400 (10%). Temperature: 150°. Helium flow: 75 ml/min.

TABLE V

RELATIVE RETENTION DATA FOR SESQUITERPENES

No.	Sesquiterpene	Relative retention times *		
		SE-30 10%	XE-60 5%	Reoplex-400 10%
	Acyclic			
1	β-Farnesene	0.920	0.718	0.681
	Monocyclic			
2	α-Bisabolene	0.791	0.602	0.526
3	β-Bisabolene	1.120	0.885	0.939
4	γ-Bisabolene	1.113	0.850	0.867
5	Curcumene	1.113	0.975	1.132
6	β-Elemene	0.722	0.627	0.563
7	α-Humulene	0.936	0.883	0.838
8	β-Humulene	0.941	0.912	0.822
9	α-Zingiberene	1.100	0.875	0.889
10	β-Zingiberene	1.165	0.893	0.903
	Bicyclic			
11	α-Bergamotene	0.847	0.631	0.539
12	β-Bergamotene	0.880	0.692	0.623
13	α-Bulnesene	1.157	0.938	0.921
14	γ-Cadinene	1.060	0.958	1.078
15	δ-Cadinene	1.100	0.942	1.039
16	Caryophyllene	0.846	0.779	0.664
17	Isocaryophyllene	0.802	0.673	0.565
18	α-Guaiene	0.875	0.652	0.576
19	α-Himachalene	0.965	0.764	0.729
20	β-Himachalene	1.211	0.950	0.895
21	β-Santalene	0.981	0.759	0.667
22	epi-β-Santalene	0.926	0.733	0.633
23	α-Selinene	1.076	0.991	0.998
24	β-Selinene	1.060	0.948	0.960
	Tricyclic			
25	Aromadendrene	0.869	0.719	0.658
26	Cedrene	0.826	0.659	0.553
27	α-Gurjunene	0.727	0.607	0.468
28	β-Gurjunene	0.797	0.696	0.599
29	Longifolene	0.773	0.672	0.560
30	Isolongifolene	0.757	0.600	0.482
31	β-Patchoulene	0.719	0.527	0.411
32	α-Santalene	0.810	0.586	0.507
33	Ylangene	0.700	0.517	0.405
	Tetracyclic			
34	Longicyclene	0.706	0.566	0.450

* Reference standards for SE-30: acenaphthene; XE-60: methylnaphthalene; Reoplex 400: naphthalene.

A

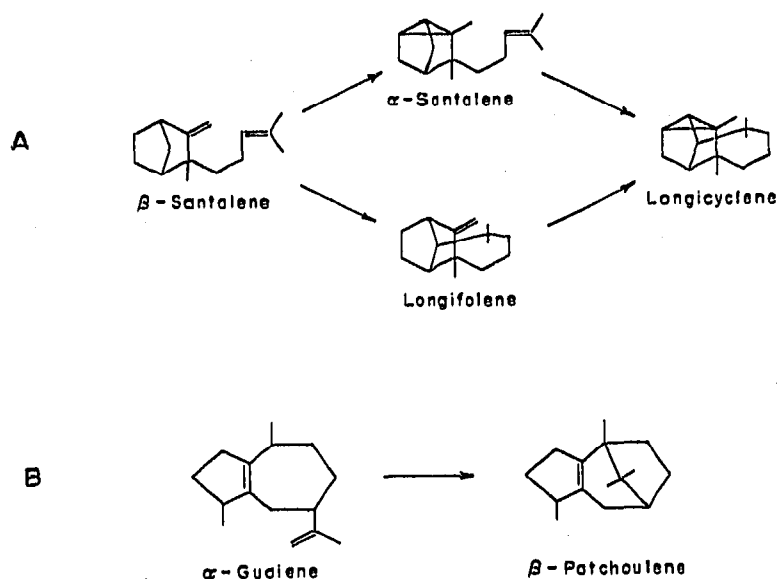β - Santalene → α - Santalene → Longicyclene
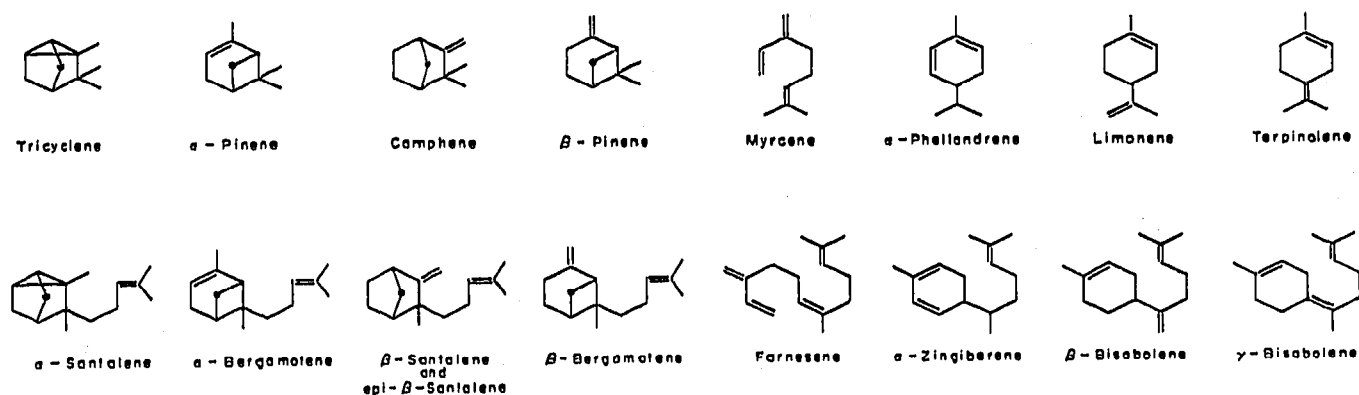
β - Santalene → Longifolene → Longicyclene

B

α - Guaiene → β - Patchoulene

Fig. 3. Cyclisation of sesquiterpenes.

Tricyclene   α - Pinene   Camphene   β - Pinene   Myrcene   α - Phellandrene   Limonene   Terpinolene

α - Santalene   α - Bergamotene   β - Santalene and epi-β-Santalene   β - Bergamotene   Farnesene   α - Zingiberene   β - Bisabolene   γ - Bisabolene

Fig. 4. Structural relationships between monoterpenes and sesquiterpenes examined by gas chromatography.
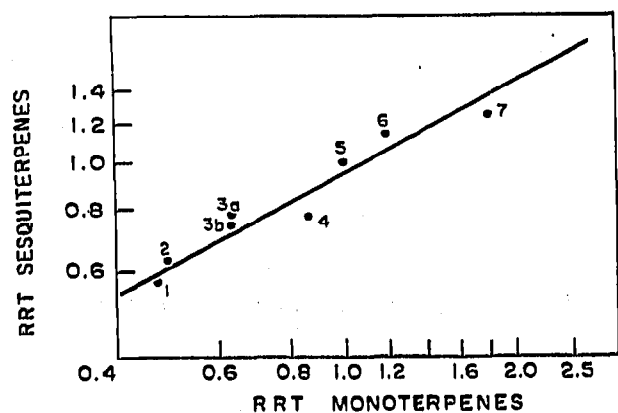
Fig. 5. Correlation of relative retention times of structurally related monoterpenes and sesquiterpenes.

to sesquiterpene hydrocarbons. However, careful examination of experimental data obtained for a number of compounds closely related in structure reveals some interesting relationships.

(*1*) *Aromatic structures*. Aromatisation of a ring possessing two conjugated double bonds leads to an increase in retention time. Thus, curcumene is held more strongly than α- or β-zingiberene by any of the three columns used (Table V).

(*2*) *Effect of cyclisation*. As shown in Fig. 3A distinct structural relationships exist between α-santalene, β-santalene, longifolene and longicyclene. β-Santalene may be visualised to undergo cyclisation involving either of the two unsaturated centres of the molecule and leading to the formation of α-santalene (formation of a cyclopropyl ring) or longifolene (formation of a 7-membered ring). Cyclisation of either of these two compounds may in turn lead to formation of the tetracyclic sesquiterpene, longicyclene. Each of these cyclisation steps is associated with a decrease in retention time. Thus on all three columns α-santalene and longifolene display relative retention times markedly lower than those of β-santalene, and longicyclene is less firmly held by either column than any of its precursors. Similarly β-patchoulene exhibits a shorter relative retention time than α-guaiene (see Table V and Fig. 3B).

(*3*) *Comparison of retention time data of monoterpene and sesquiterpene hydrocarbons*. Sesquiterpenes differ from monoterpenes in possessing an additional isoprene unit (C_5H_8). For example, α-santalene may be visualised to form by the addition of an isoprene unit to one of the geminal methyl groups of tricyclene (see Fig. 4). Relative retention times of some sesquiterpenes and their corresponding monoterpenes are recorded in Table VI.

TABLE VI

RELATIVE RETENTION TIMES FOR SOME SESQUITERPENE HYDROCARBONS AND THEIR CORRESPONDING MONOTERPENES[*]

No.	Sesquiterpene	RRT 150°	Monoterpene[**]	RRT 100°
1	α-Santalene	0.57	Tricyclene	0.47
2	α-Bergamotene	0.63	α-Pinene	0.49
3a	epi-β-Santalene	0.74		
3b	β-Santalene	0.78	Camphene	0.63
4	β-Bergamotene	0.76	β-Pinene	0.76
5	β-Farnesene	0.77	Myrcene	0.86
6	α-Zingiberene	1.00	α-Phellandrene	1.00
7	β-Bisabolene	1.15	Limonene	1.19
8	γ-Bisabolene	1.25	Terpinolene	1.82

[*] Column: Reoplex 400 (20%) on acid washed Chromosorb W.
[**] Compounds available in author's reference collection.

The results obtained demonstrate that the emergence of sesquiterpene hydrocarbons parallels that of the corresponding monoterpenes. When plotting logarithms of relative retention times of the sesquiterpene hydrocarbons *versus* those of the corresponding monoterpenes a fairly linear relationship is obtained (see Fig. 5). The experimental data, therefore, suggest that the contribution of an isoprene unit toward the retention time of a sesquiterpene hydrocarbon is always of the same order of magnitude.

Relative retention times of a number of sesquiterpene hydrocarbons occurring in essential oils were recently reported by LUKES AND KOMERS[15] using columns other than those reported in the present study. Deductions contemplated by these authors concerning relationships between molecular structure and retention data on different stationary phases will be awaited with keen interest.

SUMMARY

Characteristic gas chromatographic criteria of identity are reported for 34 authenticated sesquiterpene hydrocarbons, using Reoplex 400, silicone nitrile XE-60 and silicone rubber SE-30 as column substrates and naphthalene, 2-methylnaphthalene and acenaphthene as internal reference standards. The value of the experimental data is demonstrated by application to the analysis of essential oils and correlation with characteristic structural features of some sesquiterpenes. The occurrence of ylangene, α-bergamotene and δ-cadinene in oil of copaiba balsam is reported for the first time.

REFERENCES

1 I. C. NIGAM AND L. LEVI, *J. Pharm. Sci.*, 54 (1965) 1823.
2 H. P. BURCHFIELD AND E. E. STORRS, *Biochemical Applications of Gas Chromatography*, Academic Press, New York, 1962, p. 382.
3 E. GUENTHER, K. KULKA AND J. A. ROGERS, Jr., *Anal. Chem.*, 35 (1963) 39R.
4 M. H. KLOUWEN AND R. TER HEIDE, *J. Chromatog.*, 7 (1962) 297.
5 I. C. NIGAM AND L. LEVI, *Can. J. Chem.*, 40 (1962) 2083.
6 A. BHATI, *Perfumery Essent. Oil Record*, 54 (1963) 376.
7 I. C. NIGAM AND L. LEVI, *Perfumery Essent. Oil Record*, 54 (1963) 814.
8 I. C. NIGAM AND L. LEVI, *Can. J. Chem.*, 41 (1963) 1726.
9 M. C. NIGAM, I. C. NIGAM, L. LEVI AND K. L. HANDA, *Can. J. Chem.*, 42 (1964) 2610.
10 E. GUENTHER, *The Essential Oils*, Vol. II, Van Nostrand, New York, 1949, p. 708.
11 J. SIMONSEN AND D. H. R. BARTON, *The Terpenes*, Vol. III, Cambridge University Press, Cambridge, 1952; E. GUENTHER, *The Essential Oils*, Vol. II, Van Nostrand, New York, 1949.
12 I. C. NIGAM, M. SAHASRABUDHE AND L. LEVI, *Can. J. Chem.*, 41 (1963) 1535.
13 A. T. JAMES, A. J. P. MARTIN AND G. H. SMITH, *Biochem. J.*, 52 (1952) 238.
14 W. J. ZUBYK AND A. Z. CONNER, *Anal. Chem.*, 32 (1960) 912.
15 V. LUKES AND R. KOMERS, *Collection Czech. Chem. Commun.*, 29 (1964) 1599.