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Analysis of some synthetic insect pheromones by gas–liquid chromatography

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ABSTRACT

The isomeric composition of insect pheromones belonging to primary olefinic alcohols and their derivatives was analysed by gas–liquid chromatography. The chromatographic behaviour of geometric isomers on liquid crystalline stationary phases was studied. The effectiveness and selectivity of the chromatographic columns and the Kováts retention indices of (*E,Z*)- and (*Z,Z*)-7,9-dodecadienyl acetates were determined and their dependence on temperature was investigated. Geometric and positional isomers of primary aliphatic alcohols and acetates with one and two double bonds can be separated on capillary columns with 1,2,3-tris-(β-cyanethoxy)propane, diethylene glycol succinate and cholesteryl *p*-methoxybenzoate.

INTRODUCTION

Many insect pheromones are primary olefinic alcohols, aldehydes or acetates containing 10–20 carbon atoms. Species specificity is conferred by variations of the structure, with differences in functional group, chain length, or changes in the position or geometry of the double bonds. Rigorously defined mixtures of isomers or geometrical purity of a synthetic product are often vital for maximum activity of pheromones. A technique capable of providing precise geometric definition is requied for the effective identification and utilization of pheromones.

The difficulty of separation of these isomers varies depending on the position and configuration of the olefinic bond and separations may become complex when diunsaturated compounds are involved. Capillary columns with high-polarity stationary phases have made possible the analysis of geometrical isomers of insect pheromones[1], but it is difficult to obtain good capillary columns with such stationary phases. Several workers have used liquid crystalline stationary phases, which exhibit unique selectivity towards geometric isomers[2–4].

This paper deals with liquid crystals as stationary phases for the efficient gas chromatographic analysis of unsaturated insect pheromones and the development of procedures for the separation of geometric and positional isomers by capillary gas chromatography.

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EXPERIMENTAL

A Tsvet Model 100 gas chomatograph equipped with a flame ionization detector and packed glass columns (3 m × 3 mm I.D.) was used. The liquid crystals (Reakhim) were deposited on the support from a chloroform solution, the solvent being evaporated in a rotary vacuum evaporater. The packing was then dried and screened. The packed columns were conditioned at 200°C and with a carrier gas (nitrogen) flow-rate of 20 ml/min for 7 h before use. The characteristics of the liquid crystals and columns prepared are given in Table I and II, respectively.

A Chrom Model 41 gas chromatograph equipped with a flame ionization detector, a stream splitter with a splitting ratio of 1:100 and a stainless-steel capillary column (50 m × 0.25 mm I.D.) coated with 1,2,3-tris(β-cyanethoxy)propane (TRIS) by the dynamic method was used[5]. The carrier gas was nitrogen at a flow-rate of 7.5 ml/min and the column temperature was 160°C.

A Biochrom Model I gas chromatograph equipped with a flame ionization detector, a stream splitter with a splitting ratio of 1:100 and glass capillary columns (50 m × 0.25 mm I.D.) was also used. The glass columns were coated with diethylene glycol succinate (DEGS) and cholesteryl *p*-methoxybenzoate (CMB) by the static method[6].

Efficiency, selectivity and retention data tests were carried out using a mixture of (*E,Z*)- and (*Z,Z*)-7,9-dodecadienyl acetates. Isomers were prepared in our institute.

TABLE I

CHARACTERISTICS OF STATIONARY PHASES

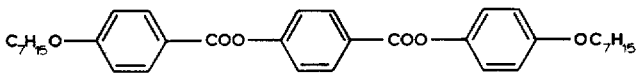
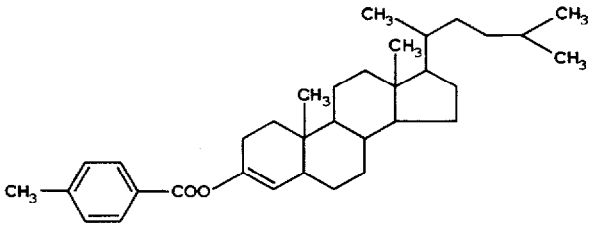
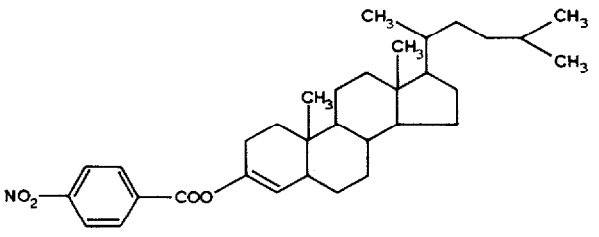
Phase	*Formula*	*Mesophase range (°C)*
I		120–195
II		178–230
III		185–235

TABLE II

CHARACTERISTICS OF CHROMATOGRAPHIC COLUMNS

Column	*Stationary phase*	*Support*	*Amount of stationary phase on support (%)*	*Column length (m) × I.D. (mm)*
1	I	Chromaton N-Super (0.125–0.16 mm)	7.5	3 × 3
2	II	Chromosorb W AW DMCS (0.15–0.18 mm)	10.0	3 × 2
3	III	Chromosorb W (0.18–0.25 mm)	10.0	3 × 3

The Kováts retention indices, I, column efficiency, N, selectivity, $r_{A/B}$, resolution, R_s, and capacity factors, k', of isomers were found from the following known relationships.

$$I_A = 100\,N + 100n \cdot \frac{\log t'_{R(A)} - \log t'_{R(N)}}{\log t'_{R(N+n)} - \log t'_{R(N)}}$$

where $t'_{R(A)}$, $t'_{R(N)}$ and $t'_{R(N+n)}$ are adjusted retention times of one of the isomers and alakanes with N and $N+n$ carbon atoms, respectively.

$$N = 5.54\left(\frac{t'_R}{w_{0.5}}\right)^2$$

where t'_R = adjusted retention time of (*E,Z*)-7,9-dodecadienyl acetate and $w_{0.5}$ is its peak width at half-height.

The selectivity of the stationary phases was expressed as the relative retention times of the isomers:

$$r_{A/B} = \frac{t'_{R(B)}}{t'_{R(A)}}$$

where $t'_{R(A)}$ and $t'_{R(B)}$ are adjusted retention times of (*E,Z*)- and (*Z,Z*)-7,9-dodecadienyl acetate, respectively.

$$R_s = \frac{t_{R(B)} - t_{R(A)}}{w_{0.5(A)} + w_{0.5(B)}}$$

where $w_{0.5(A)}$ and $w_{0.5(B)}$ are the peak width at half-height of the *E,Z*- and *Z,Z*- isomers, respectively.

$$k' = \frac{t'_R}{t_0}$$

where t_0 is the dead time. The dead time was determined according to the method of Peterson and Hirsch[7]:

$$t_0 = \frac{t_n t_{n+2} - t_{n+1}^2}{t_n + t_{n+2} - 2t_{n+1}}$$

where t_n, t_{n+1} and t_{n+2} are the retention times of members of the alkane homologous series.

RESULTS AND DISCUSSION

Stationary phases were tested in the range 100–200°C, so that the column was operated under supercooled mesophase conditions. At temperatures higher than 200°C liquid crystalline stationary phases showed bleeding.

The phase state of the liquid crystals changes with temperature. Table III shows measurements of the column efficiency, N, at column temperatures between 100 and 200°C and values of the difference between Kováts retention indices of (*Z,Z*)- and (*E,Z*)-7,9-dodecadienyl acetate. As the column temperature increases and the crystalline stationary phase tends towards the mesophase region, its solvation power increases and the column efficiency improves. After the column temperature rises above the mesophase range, the crystalline stationary phase melts and the column efficiency decreases.

The effect of column temperature and the state of the liquid crystalline stationary phase on the Kováts retention indices of geometric isomers is illustrated in Fig. 1. The Kováts retention indices measured on the cholesteryl stationary phases II and III increase linearly with increase in temperature. On phase I at temperatures

TABLE III

COLUMN EFFICIENCY (N) AND DIFFERENCE BETWEEN KOVATS RETENTION INDICES (ΔI) OF (*Z,Z*)- AND (*E,Z*)-7,9-DODECADIENYL ACETATE AT VARIOUS TEMPERATURES

Column temperature (°C)	*Column*					
	1		*2*		*3*	
	N	*ΔI*	*N*	*ΔI*	*N*	*ΔI*
100	986	6	–	−3	–	13
110	1758	19	23	−2	1391	14
120	4846	18	16	−2	1795	14
130	5016	18	27	−2	2004	14
140	4863	17	48	−1	2142	14
150	5160	16	94	−1	2210	15
160	4948	15	147	0	2211	16
170	4865	15	229	1	2237	16
180	1788	13	333	2	2075	16
190	2171	12	489	2	1996	17
200	2825	12	703	2	2039	17

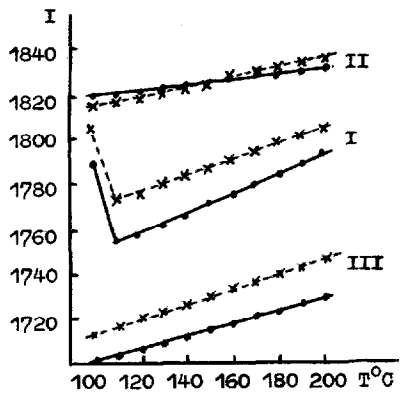


Fig. 1. Kováts retention indices of (○) (*E,Z*) and (×) (*Z,Z*)-7,9-dodecadienyl acetate on the liquid crystals I, II and III as stationary phases.

below melting point (120°C), the retention indices start to increase again. On phase II, (*Z,Z*)-7,9-dodecadienyl acetate eluates before the *E,Z*-isomer in the temperature range 100–150°C, but after it at temperatures above 160°C.

The difference between the retention indices of (*Z,Z*)- and (*E,Z*)-7,9-dodecadienyl acetate increases with increase in temperature on cholesteryl stationary phases II and III. The differences between those of geometric isomers increases with decreasing of temperature from 200 to 110°C and reaches 19 index units on the with nematic liquid crystal phase I, then it decreases sharply.

The selectivity of the stationary phases is demonstrated in Fig. 2. The values were directly dependent on temperature, and the highest selectivity towards isomers was exhibited by phase I.

The best resolving power was also exhibited by stationary phase I. The effect of its phase state on the resolution of (*E,Z*)- and (*Z,Z*)-7,9-dodecadienyl acetate is

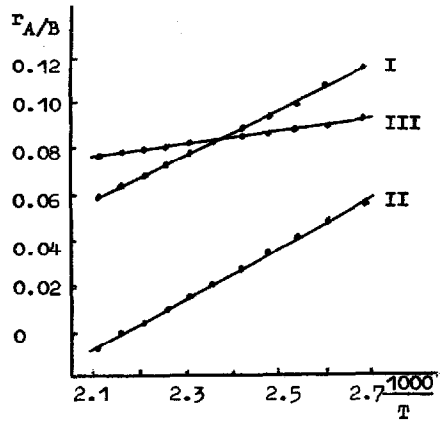


Fig. 2. Relative retentions of (*Z,Z*)- and (*E,Z*)-7,9-dodecadienyl acetate on the liquid crystals I, II and III as stationary phases.

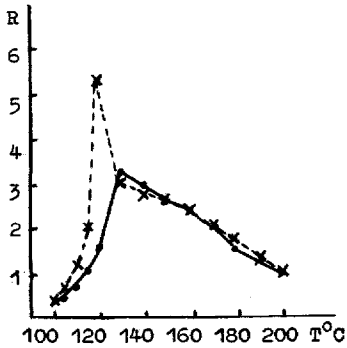


Fig. 3. Dependence of resolution, R_s, for (*E,Z*)- and (*Z,Z*)-7,9-dodecadienyl acetate on the column temperature with column (○) heating or (×) cooling. Colum 1.

illustrated in Fig. 3, which is a plot of the resolution R_s of the isomers against column temperature. Maximum resolution (R_s = 5) was achieved at the melting point (120°C) of 1,4-phenylene bis (4′-*p*-heptyloxybenzoate) when the column had been cooled.

Attempts to separate the isomers of monoolefinic insect pheromones on these packed columns failed. Fig. 4 illustrates the separation of the geometric isomers of European vine moth (A) and codling moth (B) pheromones on column 1 (3 m × 3 mm I.D.) with 10% nematic liquid crystal 1,4-phenylene bis(4-*p*-heptyloxybenzoate) on Chromaton N-Super, 0.125–0.16 mm. The carrier gas nitrogen flow-rate was 20 ml/min and the column temperature was 160°C.

Capillary columns were made in our laboratory. The efficiency of a column in separating isomers was expressed as the capacity factor, k', of isomers, the selectivity, r,

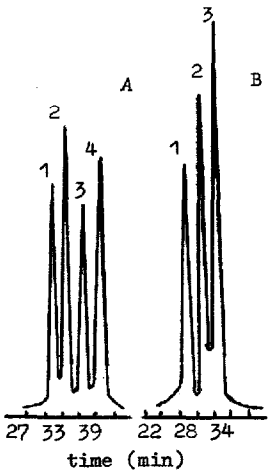


Fig. 4. Separation of geometric isomers of (A) European grape vine moth and (B) codling moth pheromones on column 1 (3 m × 3 mm I.D.) packed with Chromaton N-Super (0.125–0.16 mm) coated with 10% 1,4-phenylene bis(4′-*p*-heptyloxybenzoate). Nitrogen flow-rate, 20 ml/min; column temperature, 160°C. (A) 1 = (*Z,E*)-; 2 = (*E,Z*)-; 3 = (*Z,Z*)-; 4 = (*E,E*)-7,9-dodecadienyl acetates. (B) 1 = (*E,Z*)-; 2 = (*Z,Z*)-; 3 = (*E,E*)-8,10-dodecadienol.

TABLE IV

CAPACITY FACTORS (k') OF OLEFINIC ALCOHOLS AND ACETATES

Peak in Fig. 5	*Compound*	k'
1	(*E*)-7-Decenyl acetate	0.65
2	(*Z*)-7-Decenyl acetate	0.72
3	(*E*)-8-Decenyl acetate	0.73
4	(*Z*)-8-Decenyl acetate	0.83
5	(*E*)-9-Dodecenyl acetate	1.24
6	(*Z*)-8-Dodecenyl acetate	1.34
7	(*E*)-10-Dodecenyl acetate	1.35
8	(*Z*)-9-Dodecenyl acetate	1.37
9	(*Z*)-10-Dodecenyl acetate	1.52
10	(*E,Z*)-7,9-Dodecadienyl acetate	2.85
11	(*E,E*)-7,9-Dodecadienyl acetate	2.97
12	(*E,E*)-8,10-Dodecadienyl acetate	3.11
13	(*E,Z*)-7,9-Dodecadienol	3.63
14	(*E,E*)-7,9-Dodecadienol	3.81
15	(*E,E*)-8,10-Dodecadienol	4.04
16	(*Z*)-11-Tetradecenol	3.59
17	(*E*)-6-Hexadecenol	5.54
18	(*E*)-9-Hexadecenol	5.61
19	(*Z*)-6-Hexadecenol	5.87
20	(*Z*)-9-Hexadecenol	6.02
21	(*Z*)-11-Hexadecenol	6.09

and the resolution, R_s. The results obtained on a metal capillary column (50 m × 0.25 mm I.D.) with 1,2,3-tris(β-cyanethoxy)propane as stationary phase (N = 38 000 theoretical plates) are presented in Tables IV and V. The column is effective for the separation of geometric and positional isomers of olefinic pheromones. The value of

TABLE V

SELECTIVITY ($r_{A/B}$) AND RESOLUTION (R_s) OF GEOMETRIC AND POSITIONAL ISOMERS OF OLEFINIC ALCOHOLS AND ACETATES

Separated compounds (peaks Fig. 5)	$r_{A/B}$	R_s	*Separated compounds (peaks Fig. 5)*	$r_{A/B}$	R_s
1–2	1.14	2.74	6–7	1.00	0.00
3–4	1.16	3.25	8–7	1.01	0.00
1–3	1.13	2.24	5–9	1.23	4.58
2–4	1.15	2.80	10–11	1.05	1.10
2–3	1.01	0.00	11–12	1.04	1.26
5–8	1.10	2.00	13–14	1.05	1.15
7–9	1.13	2.75	14–15	1.06	1.42
6–8	1.02	0.59	17–19	1.06	0.81
6–9	1.19	1.74	18–20	1.07	0.91
8–9	1.11	2.17	17–18	1.01	0.00
5–7	1.09	1.87	19–20	1.02	0.00

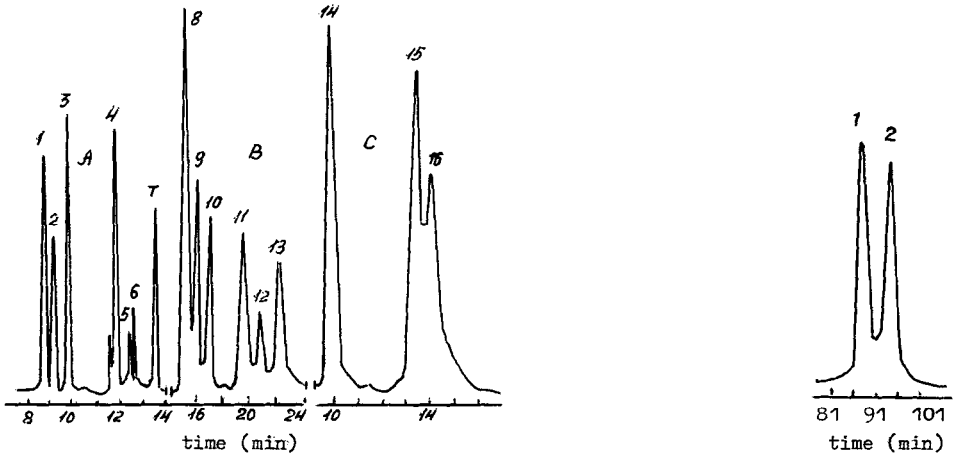


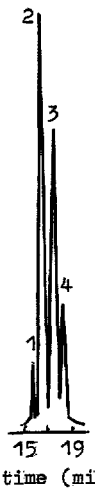
Fig. 5. Separation of insect pheromone isomers on a metal capillary column (50 m × 0.25 mm I.D.) with 1,2,3-tris(β-cyanetoxy)propane. (A) Temperature, 160°C; nitrogen flow-rate, 5.6 ml/min. (B) Temperature, 160°C; nitrogen flow-rate, 7.5 ml/min. (C) Temperature, 170°C; nitrogen flow-rate, 9.2 ml/min. For peak identification, see Table IV.

Fig. 6. Separation of (1) (*Z*)- and (2) (*E*)-7-eicosen-11-one on a glass capillary column (50 m × 0.25 mm I.D.) coated with CMB. Nitrogen flow-rate, 19 cm/s; column temperature, 178°C.

TABLE VI

KOVÁTS RETENTION INDICES (*I*), SELECTIVITY ($r_{A/B}$) AND RESOLUTION (R_s) OF GEOMETRICAL ISOMERS OF SOME INSECT PHEROMONES ON GLASS CAPILLARY COLUMNS

Compound	*Stationary phase*					
	CMB (178°C)			*DEGS (160°C)*		
	I	$r_{A/B}$	R_s	*I*	$r_{A/B}$	R_s
(*E*)-8-Decenyl acetate	–	1.25	1.54	1795	1.11	–
(*Z*)-8-Decenyl acetate	–			1818		
(*E*)-7-Dodecenyl acetate	1656	1.17	1.94	1943	1.04	0.99
(*Z*)-7-Dodecenyl acetate	1624			1956		
(*E*)-8-Dodecenyl acetate	1635	1.00	0.00	–	1.07	1.60
(*Z*)-8-Dodecenyl acetate	1636			–		
(*E*)-9-Dodecenyl acetate	1636	1.00	0.00	1943	1.05	0.97
(*Z*)-9-Dodecenyl acetate	1634			1955		
(*Z*,*E*)-5,7-Dodecadienyl acetate	1682	1.03	0.48	2102	1.03	0.62
(*E*,*E*)-5,7-Dodecadienyl acetate	1688	1.15	2.96	2120	1.05	1.07
(*Z*,*Z*)-5,7-Dodecadienyl acetate	1715	1.12	2.84	2129	1.09	2.03
(*E*,*Z*)-5,7-Dodecadienyl acetate	1739			2136		
(*Z*,*E*)-7,9-Dodecadienyl acetate	1697	1.10	1.86	2094	1.05	1.59
(*E*,*Z*)-7,9-Dodecadienyl acetate	1710	1.09	1.82	2105	1.03	1.92
(*Z*,*Z*)-7,9-Dodecadienyl acetate	1732	1.07	1.30	2113	1.04	1.57
(*E*,*E*)-7,9-Dodecadienyl acetate	1743			2119		
(*E*)-7-Eicosen-11-one	2179	1.07	1.62	–	–	–
(*Z*)-7-Eicosen-11-one	2165			–		



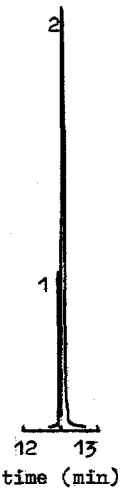


Fig. 7. Separation of geometric isomers of European grape vine moth pheromone on a capillary column (50 m × 0.25 mm I.D.) coated with CMB. Nitrogen flow-rate, 13.2 cm/s; column temperature, 178°C. 1 = (*Z*,*E*)-; 2 = (*E*,*Z*)-; 3 = (*Z*,*Z*)-; 4 = (*E*,*E*)-7,9-dodecadienyl acetate.

Fig. 8. Separation of (1) (*E*)- and (2) (*Z*)-8-dodecenyl acetate on a capillary column (50 m × 0.25 mm I.D.) coated with DEGS. Nitrogen flow-rate, 0.36 ml/min; column temperature, 160°C.

the capacity factor depends on variations in functional group, chain length and position and geometry of the double bond in the molecule. The alcohols elute after acetates and *cis* isomers after *trans* isomers on this stationary phase. The relative retention times of *trans* and *cis* isomers increase with reduction in chain length and increase in the distance between the double bond and the functional group. Chromatograms of the pheromones studied are shown in Fig. 5.

Glass capillary columns with DEGS (174 184 theoretical plates) and CMB (93 464 theoretical plates) were coated by the static method. The Kováts retention indices, selectivities and resolution of geometric isomers are summerized in Table VI. The separation properties of these columns towards geometric isomers of diunsaturated pheromones are better than those of the previous column. The temperature dependence of the efficiency and selectivity of CMB was studied. As the column temperature increased from a value corresponding to the crystalline state, the efficiency of the column and the separation of isomers continued to improve as the stationary phase approached the mesomorphic transition temperature (178°C). An increase in temperature above the transition point resulted in a decrease in column efficiency and separation. The selectivity of CMB showed no temperature dependence. Chromatograms of geometric isomers of some insect pheromones are shown in Figs. 6–8.

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