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GAS–LIQUID CHROMATOGRAPHIC ANALYSES

XXIV*. CAPILLARY COLUMN STUDIES OF THE CHLORINATED VERATROLES (1,2-DIMETHOXYBENZENES)

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SUMMARY

The gas chromatographic retention behaviour of veratrole and all nine chlorinated veratroles was studied on SE-30 and OV-351 capillary columns. Temperature programming from 100°C at 6°C min^{-1} and isothermal operation at 140, 160, 180 and 200°C were used. The complete separation of a mixture was obtained on SE-30, the isomers being eluted in order of their degree of chlorination. On OV-351, however, the 3,4,5-trichloro and tetrachloro isomers overlap with temperature programming, being separated at 140, 160 and 200°C. Retention indices and increments of retention indices for each position of substitution are examined and the effect of increasing temperature on retention is discussed.

INTRODUCTION

Previously, we studied the gas chromatographic (GC) separation of series of chlorinated aromatics, *i.e.*, acetate esters of chlorophenols[1,2], chloroguaiacols[2–4] and chlorocatechols[2], chlorinated 4-hydroxybenzaldehydes[5], chlorophenols[6,7], chlorocatechols[6] and chlorobenzenes[7]. Capillary columns have been used with suitable temperature programming. The retention indices of chlorinated phenyl acetates[8] and chlorobenzenes[9] have been reported on both non-polar and polar capillary columns, the retention index increments for each position of substitution together with the effect of increasing temperature on the values being discussed.

Chlorinated veratroles are important substances occurring, *e.g.*, as metabolites of chlorinated guaiacols[10] (2-methoxyphenols) and probably also of chlorinated catechols (1,2-dihydroxybenzenes). Chlorinated veratroles may also be responsible for taste and odour problems as previously reported with chlorinated anisoles[11,12]. In addition, to increase the mobility of chlorinated guaiacols and catechols in GC and GC–mass spectrometry (MS) experiments, they have been analysed as their methoxy derivatives, *e.g.*, veratroles from the products of the reaction of lignin model com-

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pounds with chlorine[13,14], the alkaline copper(II) oxide oxidation product of red pine chlorinated oxylignin[15] and effluents of kraft pulp mills[16,17]. Although some chloroveratrole isomers have previously been analysed by GC, a systematic GC study of all possible isomers on both polar and non-polar columns has not hitherto been reported.

This work extends the earlier studies by showing for the first time the retention behaviour of all chlorinated veratroles on SE-30 and OV-351 quartz capillary columns. Separations were carried out with temperature programming and isothermal operation. The retention data are given, with the retention indices and increments of retention indices for each position of substitution. The effect of increasing temperature on retention is discussed and the retention order of the isomers is compared with those of other series reported earlier[1–9].

EXPERIMENTAL

Samples

Chlorinated veratroles were prepared by using the generally known methylation procedure for chlorinated catechols[18] with dimethyl sulphate in sodium hydroxide solution. The same method was applied to the synthesis of 4,5-dibromoveratrole from the corresponding dibromocatechol. A mixture of the GC-pure components was used for GC analyses, the retention order being checked by GC–MS using non-polar and polar capillary columns with suitable operating conditions. The structure analyses of the isomers were based on their ^{1}H NMR, ^{13}C NMR and mass spectra. The spectroscopic behaviour of the isomers will be published later.

Commercial mixtures of *n*-alkanes were obtained from different sources.

Gas chromatography

Analyses were carried out on a Perkin-Elmer Sigma 3 gas chromatograph under the following operating conditions: injector and flame-ionization detector temperatures, 275°C; carrier gas (nitrogen) flow-rate, 1 ml min^{-1}; splitting ratio, 1:30; and chart speed, 10 mm min^{-1}. The columns used were a vitreous silica SE-30 wall-coated open-tubular (WCOT) column (25 m × 0.30 mm I.D.), supplied by SGE (North Melbourne, Australia), and a fused silica OV-351 WCOT column (25 m × 0.32 mm I.D.), supplied by Orion Analytica (Espoo, Finland). The column temperature was programmed from 100°C at 6°C min^{-1} until elution of peaks had ceased. The isothermal data were obtained at 140, 160, 180 and 200°C.

The retention times were measured from the time of sample injection and the retention indices were calculated off-line using a Univac 1100/60 computer. The dead volume was determined by regression analysis from a series of *n*-alkanes using the procedure given by Grobler and Balizs[19].

RESULTS AND DISCUSSION

Chromatograms of a mixture of veratroles separated on SE-30 and OV-351 with temperature programming are shown in Figs. 1 and 2. The corresponding retention data are presented in Table I, being tabulated relative to the parent compound, *i.e.*, veratrole, and to the 4,5-dibromo isomer. This isomer can be used as an

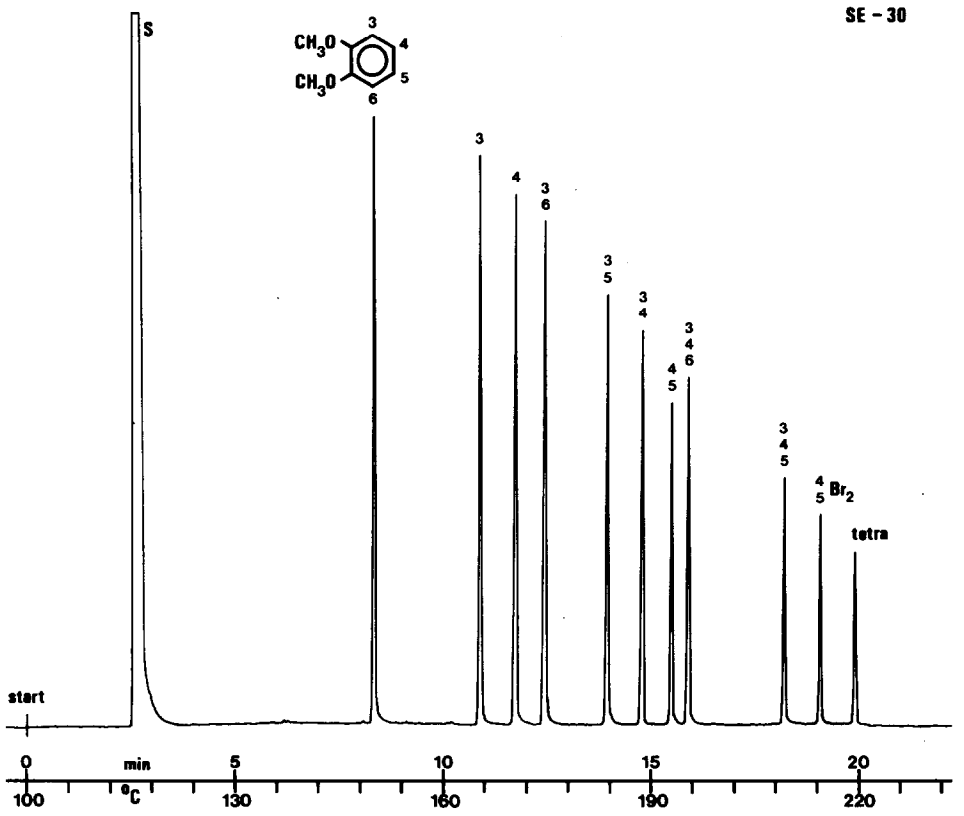

Fig. 1. Chromatogram of a mixture of veratrole and its halogenated derivatives, obtained on SE-30 with temperature programming. S = Solvent; the numbers indicate the chlorinated positions; $^{4}_{5}Br_2$ = 4,5-dibromoveratrole.

internal standard for analysing chlorinated veratroles in spent bleach liquors of pulp mills, for example. The retentions are also expressed as the ratios of the retention times of the compounds on OV-351 to those on SE-30.

The structure of the veratrole isomers is shown below, the numbers or symbols indicating the chlorinated positions relative to the methoxy groups.

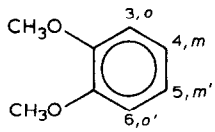

Fig. 1 shows that a non-polar column separated the mixture completely, whereas the use of a highly polar OV-351 column resulted in overlapping peaks of the 3,4,5-isomer (*o,m,m'*-) and tetrachloro isomer (Fig. 2). The 4,5-dibromo isomer on SE-30 has a lower retention than the tetrachloro isomer, whereas on OV-351, as expected, the bromo isomer is eluted last. This is due to the bulky bromine substituents and the relatively high retention time of the 4,5-dichloro isomer (*m,m'*-) on the

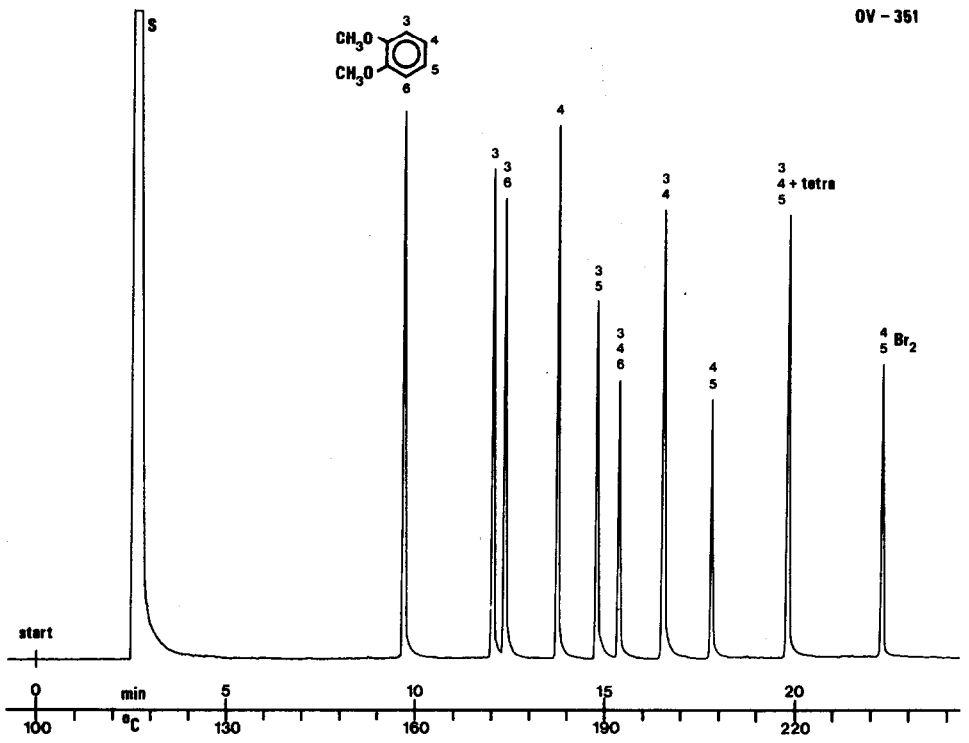

Fig. 2. Chromatogram of the same mixture as in Fig. 1, obtained on OV-351.

polar column. As shown, the 3,6-isomer (*o,o'*-) and the tetrachloro isomer have nearly the same retention time on the columns used, the greatest disparities being observed with the 4-chloro (*m*-) and 4,5-dichloro (*m,m'*-) isomers (Table I).

Retention indices determined on SE-30 and OV-351 at four isothermal temperatures and with temperature programming are presented in Tables II and III, and Tables IV and V show the incremental effects of chlorine substitution with temperature. The relationship between the relative adjusted retention time and the reciprocal of the absolute column temperature is illustrated in Fig. 3 and the isothermal retention orders of the compounds with the incremental effects are shown in Fig. 4.

The isomers are eluted on SE-30 in order of their degree of chlorination, the elution order remaining unchanged compared with the temperature programmed run (Figs. 1 and 4), and the retention generally increases with temperature (Table II). The 3-chloro isomer (*o*-) has a lower retention than the 4-chloro isomer (*m*-), as expected, owing to the *o*-substitution, as previously shown in other series[1–6,8]. With the dichloroveratroles, the 3,6-isomer (*o,o'*-), where the chlorine substituents in both positions are *ortho* to the methoxy groups, has the lowest retention. Enhanced retention occurs with the 3,5- (*o,m'*-) and 3,4-isomers (*o,m*-), the latter having a higher retention owing to the close proximity of the chlorine substituents. As a consequence of the relatively high retention time of the 4-chloro isomer (*m*-) and owing to the vicinal disubstitution, the 4,5-isomer (*m,m'*-) has the highest retention. The retention behaviour of the higher chlorinated veratroles follows that of the mono- and dichloro

TABLE I

RETENTION DATA FOR VERATROLE AND ITS HALOGENATED DERIVATIVES ON SE-30 AND OV-351 CAPILLARY COLUMNS WITH TEMPERATURE PROGRAMMING

Conditions as in Figs. 1 and 2.

Compound	*Column*						
	SE-30			*OV-351*			
	*ART**	*RRT***	*RRT****	*ART**	*RRT***	*RRT****	*RRT§*
Veratrole	8.39	1.00	0.44	9.74	1.00	0.44	1.16
3-Chloroveratrole	10.90	1.30	0.57	12.09	1.24	0.54	1.11
4-Chloroveratrole	11.76	1.40	0.62	13.79	1.42	0.62	1.17
3,4-Dichloroveratrole	14.79	1.76	0.77	16.59	1.70	0.74	1.12
3,5-Dichloroveratrole	13.96	1.66	0.73	14.81	1.52	0.66	1.06
3,6-Dichloroveratrole	12.45	1.48	0.65	12.39	1.27	0.55	1.00
4,5-Dichloroveratrole	15.50	1.85	0.81	17.82	1.83	0.80	1.15
3,4,5-Trichloroveratrole	18.20	2.17	0.95	19.88	2.04	0.89	1.09
3,4,6-Trichloroveratrole	15.89	1.89	0.83	15.39	1.58	0.69	0.97
Tetrachloroveratrole	19.90	2.37	1.04	19.88	2.04	0.89	1.00
4,5-Dibromoveratrole	19.09	2.28	1.00	22.33	2.29	1.00	1.17

* Absolute retention times (min) were measured from sample injection (Figs. 1 and 2).
** Relative retention time for veratrole taken as 1.00.
*** Relative retention time for 4,5-dibromoveratrole taken as 1.00.
§ Relative retention time for the corresponding compound on SE-30 taken as 1.00.

isomers, *i.e.*, the 3,4,6-isomer (*o,o',m*-) eluted earlier than the 3,4,5-isomer (*o,m,m'*-), the tetrachloro isomer having the highest retention.

The retention order observed for chlorinated veratroles on SE-30 is the same as previously reported for chlorinated catechol (1,2-dihydroxybenzene) diacetates[2]. The greatest disparity between the retention behaviour of these two series occurs with

TABLE II

RETENTION INDICES OF CHLORINATED VERATROLES ON SE-30 AT VARIOUS COLUMN TEMPERATURES

Compound	*Temperature*					
	Isothermal (°C)				*Programmed from 100°C at 6°C min^{-1}*	*Elution temperature (°C)*
	140	*160*	*180*	*200*		
Veratrole	1131	1114	1121	1128	1126	150
3-Chloroveratrole	1266	1262	1263	1281	1263	165
4-Chloroveratrole	1307	1301	1300	1318	1307	171
3,4-Dichloroveratrole	1448	1451	1455	1477	1461	189
3,5-Dichloroveratrole	1410	1412	1414	1436	1419	184
3,6-Dichloroveratrole	1340	1343	1348	1371	1342	175
4,5-Dichloroveratrole	1484	1483	1484	1503	1497	193
3,4,5-Trichloroveratrole	1606	1613	1622	1640	1636	209
3,4,6-Trichloroveratrole	1497	1503	1511	1536	1517	195
Tetrachloroveratrole	1677	1690	1705	1724	1726	219

TABLE III

RETENTION INDICES OF CHLORINATED VERATROLES ON OV-351 AT VARIOUS COLUMN TEMPERATURES

Compound	*Temperature*						$\frac{I_{OV\text{-}351}}{I_{SE\text{-}30}}$ *
	Isothermal (°C)				*Programmed from 100°C at 6°C min*$^{-1}$	*Elution temperature (°C)*	
	140	*160*	*180*	*200*			
Veratrole	1699	1703	1718	1752	1699	158	1.53
3-Chloroveratrole	1847	1863	1883	1915	1851	173	1.48
4-Chloroveratrole	1951	1959	1975	1994	1959	183	1.51
3,4-Dichloroveratrole	2108	2127	2150	2171	2134	200	1.47
3,5-Dichloroveratrole	2009	2025	2045	2067	2023	189	1.43
3,6-Dichloroveratrole	1864	1886	1916	1941	1870	174	1.40
4,5-Dichloroveratrole	2188	2203	2216	2228	2213	207	1.49
3,4,5-Trichloroveratrole	2287	2312	2332	2351	2342	219	1.43
3,4,6-Trichloroveratrole	2034	2060	2088	2119	2058	192	1.37
Tetrachloroveratrole	2265	2301	2331	2359	2342	219	1.36

* Ratio determined at 160°C; for values on SE-30, see Table II.

the 3,6- (*o,o'*-) and 3,4,6-isomers (*o,o',m*-), the veratroles being eluted earlier. Obviously this is due to the greatest effect of the chlorine substituent(s) adjacent to the small methoxy group(s) in veratroles compared with the bulky acetoxy group(s) in catechol diacetates. As shown previously[6], a different elution sequence for the free chlorocatechols on SE-30 is observed, the 3-chloro isomer being eluted earlier than catechol, for example.

Generally, an increased retention is observed on highly polar OV-351 stationary phase, the elution order of the compounds with different degrees of chlorination being unaltered (Fig. 4). However, owing to the decreased retention of the *o,o'*-isomers and to the enhanced retention of the *m*-isomers, three exceptions in the elution sequence compared with that on SE-30 are observed, *i.e.*, the 3,6-isomer (*o,o'*-) eluted between the monochloro isomers (*o*- and *m*-) and the 3,4,6-isomer (*o,o',m*-) left the column earlier than the 3,4- (*o,m*-) and 4,5-isomers (*m,m'*-). The tetrachloro isomer generally has a lower retention than the 3,4,5-isomer (*o,m,m'*-), but Fig. 3 shows a reversed elution sequence of the compounds at 200°C. As mentioned above, the isomers were unresolvable in the temperature-programmed run.

On comparing the retention indices determined at different column temperatures given in Tables II and III, it is evident that there is a relationship between the elution temperature and a temperature-programmed retention index[20,21], particularly on SE-30.

The incremental differences generally increase with increasing temperature on both stationary phases, as is evident in Tables IV and V, the effect being more apparent on SE-30. As with chlorinated phenyl acetates[8], the incremental increases tend to be relatively constant with different degrees of chlorine substitution and vary rather with the position of substitution. The differences between the isomers are greater with the chlorinated veratroles, however. As previously reported[9], these effects are opposite with the chlorobenzene isomers.

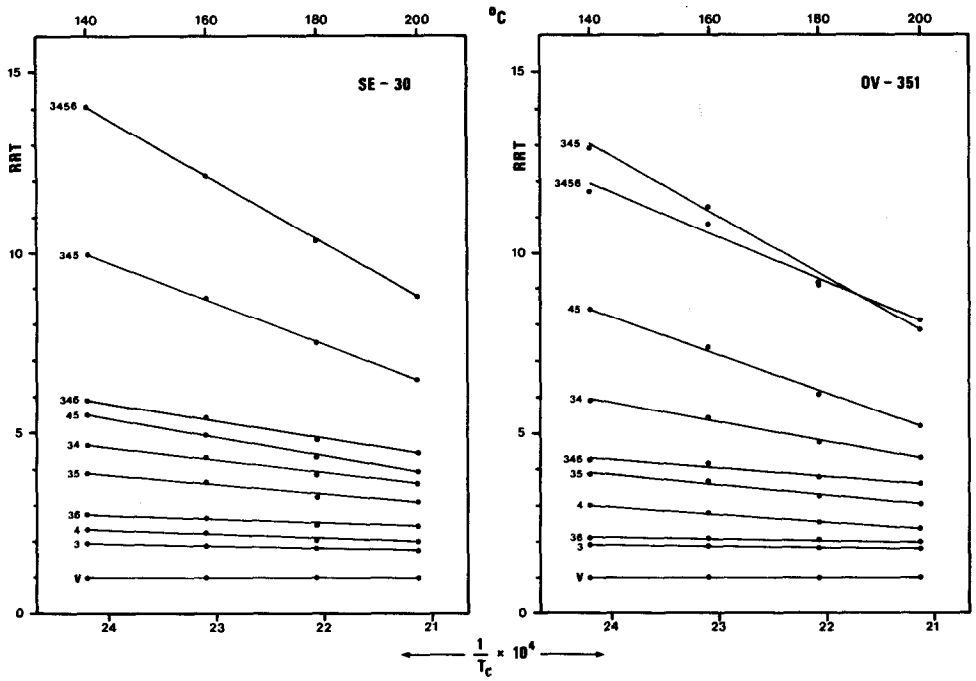

Fig. 3. Plots showing the relationship between the relative adjusted retention time and the reciprocal of the absolute column temperature on SE-30 and OV-351. Relative adjusted retention time (RRT) for veratrole taken as 1.00. V = Veratrole; the numbers indicate the chlorinated positions.

The highest retention increments on both columns are shown by the 4-chloro (*m*-) and 4,5-dichloro (*m,m'*-) isomers, *i.e.*, on SE-30 at 160°C from 187 to 185 index units (i.u.) and on OV-351 from 256 to 250 i.u., the ratios (1.37 and 1.36) of the increments constituting the greatest disparities between the columns used (Table V). The lowest incremental changes occur with the 3,6- (*o,o'*-), 3,4,6- (*o,o',m*-) and tetrachloro isomers, showing on SE-30 retention index increases for the chlorine atom from 115 to 130 to 144 i.u., respectively. The corresponding increases obtained on OV-351 are from 92 to 119 to 150 i.u., *i.e.*, generally lower than on SE-30, the minimum ratio (0.80) being observed for the 3,6-isomer (*o,o'*-) (Table V).

The retention data in Tables II–V and particularly the retention index and increments ratios on the two different columns indicate that the polar effects are maximized with the 4-chloro isomer (*m*-) and 4,5-dichloro isomer (*m,m'*-) and minimized with the 3,6-dichloro isomer (*o,o'*-), owing to the presence of the two chlorine groups adjacent to the methoxy groups. With increasing degree of chlorination, it is apparent that the steric effects are more dominant than the polar effects.

Related observations can be made for the thin-layer chromatographic data (R_F values) of other methoxy-substituted benzenes, *e.g.*, chlorinated guaiacols[22,23]. It is also interesting that on introducting of a chlorine atom into the *o*-position with respect to the methoxy group in guaiacol and chloroguaiacols, a remarkable increase in the methoxy carbon chemical shifts is observed (from *ca.* 56 to 60 ppm). This is due to the steric inhibition of resonance, which is greater with increasing *ortho* substitution[24]. The same effect has also been found with chlorinated veratroles[25] and it

TABLE IV

INCREMENTAL EFFECT OF CHLORINE SUBSTITUTION WITH ISOTHERMAL TEMPERATURE ON SE-30

Compound	*140°C*		*160°C*		*180°C*		*200°C*		*140–200°C*	
	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$***	ΔI_{1Cl}§
3-Chloroveratrole	135	135	148	148	142	142	153	153	145	145
4-Chloroveratrole	176	176	187	187	179	179	190	190	183	183
3,4-Dichloroveratrole	317	159	337	169	334	167	349	175	334	167
3,5-Dichloroveratrole	279	140	298	149	293	147	308	154	295	148
3,6-Dichloroveratrole	209	105	229	115	227	114	243	122	227	114
4,5-Dichloroveratrole	353	177	369	185	363	182	375	188	365	183
3,4,5-Trichloroveratrole	475	158	499	166	501	167	512	171	497	166
3,4,6-Trichloroveratrole	366	122	389	130	390	130	408	136	388	129
Tetrachloroveratrole	546	137	576	144	584	146	596	149	576	144

* Total retention index increase.

** Retention index increase per chlorine atom.

*** Mean total retention index increase at four temperatures.

§ Mean retention index increase per chlorine atom at four temperatures.

TABLE V

INCREMENTAL EFFECT OF CHLORINE SUBSTITUTION WITH ISOTHERMAL TEMPERATURE ON OV-351

Compound	*140°C*		*160°C*		*180°C*		*200°C*		*140–200°C*		$\frac{\Delta I_{OV\text{-}351}}{\Delta I_{SE\text{-}30}}$ §§
	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$*	ΔI_{1Cl}**	$\Sigma\Delta I_{nCl}$***	ΔI_{1Cl}§	
3-Chloro-veratrole	148	148	160	160	165	165	163	163	159	159	1.08
4-Chloro-veratrole	252	252	256	256	257	257	242	242	252	252	1.37
3,4-Dichloro-veratrole	409	205	424	212	432	216	419	210	421	211	1.26
3,5-Dichloro-veratrole	310	155	322	161	327	164	315	158	319	160	1.08
3,6-Dichloro-veratrole	165	83	183	92	198	99	189	95	184	92	0.80
4,5-Dichloro-veratrole	489	245	500	250	498	249	476	238	491	246	1.36
3,4,5-Trichloro-veratrole	588	196	609	203	614	205	599	200	603	201	1.22
3,4,6-Trichloro-veratrole	335	112	357	119	370	123	367	122	357	119	0.92
Tetrachloro-veratrole	566	142	598	150	613	153	607	152	596	149	1.04

* Total retention index increase.
** Retention index increase per chlorine atom.
*** Mean total retention index increase at four temperatures.
§ Mean retention index increase per chlorine atom at four temperatures.
§§ Ratio determined at 160°C; for values on SE-30, see Table IV.

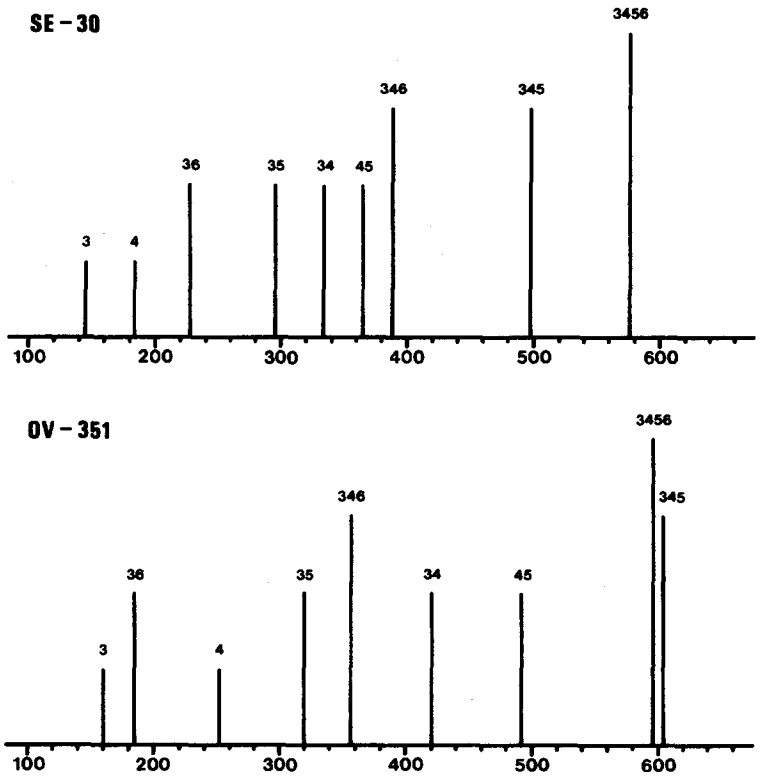

Fig. 4. Elution sequence and mean retention index increments of chlorinated veratroles on SE-30 and OV-351. The numbers indicate the chlorinated positions.

is expected that this phenomenon affects the GC retention behaviour of the veratrole isomers.

CONCLUSIONS

From a practical point of view, the present data are useful for prospective veratrole analysis, particularly as the GC behaviour of some isomers is presented here for the first time. Generally, the use of a non-polar column is recommended. However, for analysing trace amounts of compounds, *e.g.*, from fish and other environmental samples, the use of non-polar and polar columns with an electron-capture detector is to be preferred.

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