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GLASS CAPILLARY COLUMN GAS CHROMATOGRAPHY OF PHTHALATE ESTERS

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SUMMARY

Kováts retention indices of phthalate esters (*ortho*, *meta* and *para* isomers) were determined by glass capillary column gas chromatography for identification of the esters in biological fluids. The behaviour of homogeneous and heterogeneous diesters of the same molecular weight is discussed. Diastereoisomers of di-(3,3,5-trimethylcyclohexyl) phthalate were resolved on an SE-30 capillary column.

INTRODUCTION

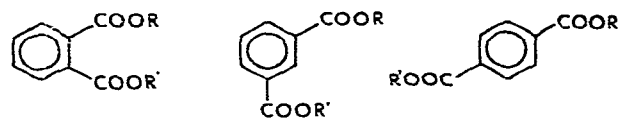
Various medical devices such as blood storage bags, blood administration assemblies and haemodialysis and cardiopulmonary by-pass units are made of poly(vinyl chloride) (PVC). As PVC is brittle, plasticizers such as phthalate esters are added to give the desired flexibility and softness, and may account for as much as 20-40% of the final weight of the material¹.

Plasticizers are not chemically bonded to the polymer but physically included throughout the plastic polymer matrix where they serve as intramolecular lubricants². Under certain conditions they can migrate from the plastic into the environment.

The most widely used plasticizers are phthalates (*ortho* esters) and particularly di-(2-ethylhexyl) phthalate (DEHP). This has been found as a contaminant in animal tissues such as rat liver³, mitochondria of the beef heart muscle⁴, lungs and abdominal fat⁵ and human blood and plasma stored in PVC bags⁵⁻¹¹, probably derived from air pollutants¹², foods such as milk^{13,14}, oils^{15,16} fish and water¹⁷⁻¹⁹ and other foodstuffs²⁰.

Terephthalates and isophthalates (*para* and *meta* isomers) are also found as contaminants as they are used in the manufacture of inks and paints²¹. It is generally asserted that DEHP appears not to be very toxic. However, subtle effects on various enzyme systems have been noted²². Reviews have been published on the much debated toxicity and teratogenicity of phthalate esters²³⁻²⁵.

Most of methods reported for the analysis and identification of phthalate esters involve the use of gas-liquid chromatography (GLC). In this paper, we report a systematic study carried out on glass capillary columns of the chromatographic behaviour of phthalate esters, according to the relative positions and nature of the



Phthalate

Isophthalate

Terephthalate

Fig. 1. Phthalate ester isomers.

R and R' groups (Fig. 1), where R and R' are *n*-alkyl, branched alkyl or cycloalkyl groups.

EXPERIMENTAL

GLC separations were carried out with Pye Unicam Series 104, Model 84 (Pye Unicam, Cambridge, Great Britain) and Carlo Erba 2300 (Carlo Erba, Milan, Italy) chromatographs, both fitted with flame-ionization detectors and glass capillary columns. They were equipped with an all-glass solid injection device as described by Ros²⁶, slightly modified in our laboratory²⁷. Glass capillary columns were prepared in our laboratory as described by Berthou and co-workers^{27,28}.

TABLE I

PARAMETERS OF THE COLUMNS AND CHROMATOGRAPHIC CONDITIONS

Parameter	Stationary phase	
	OV-101	SE-30
Concentration of phase (%) (static method of coating)	0.5	0.5
Length, <i>L</i> (m)	50	42
Inner diameter (mm)	0.3	0.3
Film thickness (μm)	0.35	0.39
Theoretical plates, n^*	105,000	98,000
Capacity ratio, k'^{***}	4.8	7.75
n_i/L (m^{-1})	2100	2300
Coating efficiency (%) ^{***}	53	56
Carrier gas	H ₂ or N ₂	He
Flow-rate (ml/min)	2	2
Temperature ($^{\circ}\text{C}$)	230	250
Separation No. [‡] :		
C ₂₀ -C ₂₂	—	28
C ₂₂ -C ₂₄	20	29
C ₂₄ -C ₂₆	24	32
C ₂₆ -C ₂₈	27	31
C ₂₈ -C ₃₀	—	31

* $n_{\text{exp.}} = 5.54 \left(\frac{t_r}{w_{1/2}} \right)^2$, where t_r is the retention time and $w_{1/2}$ is the peak width at half-height.

** The capacity ratio, k' , was measured on 5 α -androstande-3 α ,17 β -bis-trimethylsilyl ether.

*** Coating efficiency (%) = $\frac{\text{HEPT}_{\text{min.}}}{\text{HEPT}_{\text{exp.}}}$ (ref. 30); $\text{HEPT}_{\text{min.}} = r \sqrt{\frac{1 + 6k' + 11k'^2}{3(1+k')^2}}$ (r = column radius).

‡ Separation number (Trennzahl^{31,32}) (SN): $\text{SN} = \left[\frac{t_r(C_{n+1}) - t_r(C_n)}{w_{1/2}(C_{n+1}) + w_{1/2}(C_n)} - 1 \right]$, where C_{n+1} and C_n are two consecutive *n*-alkanes.

The parameters of the two columns and the chromatographic conditions used are given in Table I. The retention indices (I) according to Kováts²⁹ were measured in the isothermal mode using n -alkanes.

Each result expressed was calculated as the average of three measurements, with an error of 0.1%.

Preparation of standards

C_{14} - C_{34} n -alkanes were obtained from Fluka (Buchs, Switzerland). The following phthalates were used: diethyl, di-(3,3,5-trimethylcyclohexyl) (DTMCHP) and di-(n -butyl glycol) (DBGP) (Fluka); di-(2-ethylhexyl) (DEHP) (Aldrich-Europe, Beerse, Belgium); di-(n -butyl) (DBP) (Merck, Darmstadt, G.F.R.) and dicyclohexyl (DCHP) (Prolabo, Paris, France). The purity of each of these compounds was checked in our laboratory, and was found to be at least 98%.

For analytical purposes, diesters were prepared by esterification of 100 mg of phthaloyl chloride (Fluka) or isophthaloyl or terephthaloyl chloride (Merck) with 10 ml of alcohol (pure or a mixture of two). After addition of pyridine (0.5 ml) as catalyst, the reaction mixture was heated at 60° overnight. Methanol, ethanol, 1-propanol, 1-hexanol and 1-octanol were purchased from Prolabo, 1-heptanol, 1-nonanol, 1-decanol, 1-undecanol and 1-dodecanol from Fluka and 1-pentanol from UCB (Brussels, Belgium). The diesters remained stable for several months before gas chromatographic analysis, and were diluted with n -hexane.

RESULTS AND DISCUSSION

Phthalates (*ortho* isomers)

Retention indices of phthalates from dimethyl to di-(n -decyl) were measured on the two stationary phases (SE-30 and OV-101) at different column temperatures (Table II).

Plots of retention index against alkyl chain length (expressed as number of carbon atoms) appeared to be straight lines (Fig. 2), but a more accurate calculation (least-squares fitting) indicated non-linearity in the slope between di-(n -pentyl) and

TABLE II
RETENTION INDICES OF PHTHALATES ON CAPILLARY COLUMNS

Phthalate	SE-30, 250°, He	OV-101, 230°	
		H ₂	N ₂
Dimethyl	—	—	1453
Diethyl	1583	—	1581
Di-(n -propyl)	1758	1756	1756
Di-(n -butyl)	1940	1938	1937
Di-(n -pentyl)	2122	2121	2120
Di-(n -hexyl)	2308	2306	2305
Di-(n -heptyl)	2497	2495	2494
Di-(n -octyl)	2685	2682	—
Di-(n -nonyl)	2876	—	—
Di-(n -decyl)	3067	—	—

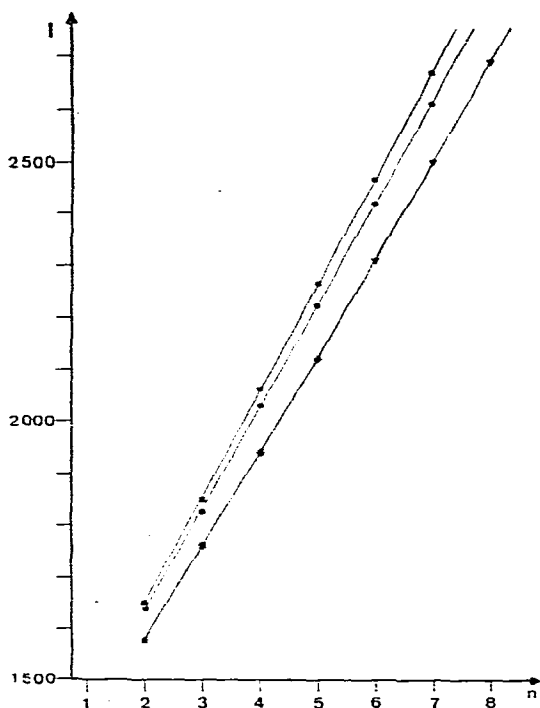


Fig. 2. Plot of retention indices of phthalate esters *versus* alkyl chain length. SE-30, 250°. 42 m \times 0.3 mm I.D. column. R = R' = C_nH_{2n+1}. *, ∇ , *ortho*; \odot , *meta*; \blacksquare , *para*.

di-(*n*-hexyl) phthalates. Zulaica and Guiochon³³ suggested that the retention indices (*I*) of phthalate esters could be expressed as

$$I = n\Delta\text{CH}_2 + \Delta\text{Bz}(\text{CO}_2)_2$$

where ΔCH_2 is the contribution of any methylene group of the linear alkyl side-chain, whatever its location, and $\Delta\text{Bz}(\text{CO}_2)_2$ is the contribution of dicarboxylic group. Hence $\Delta\text{Bz}(\text{CO}_2)_2$ is determined for $n = 0$ and ΔCH_2 as the slope of the curve (Table III).

On the OV-101 column at 230°, the plot of retention index against alkyl chain length exhibited a slight deviation from linearity for dimethyl and diethyl phthalates (Fig. 3). This effect, previously observed by Zulaica and Guiochon³³ and Bloom³⁴ on packed columns, is due to the influence of the ester group on the α -CH₂ group, which did not extend appreciably beyond the β -methylene group.

TABLE III

PARAMETERS OF THE EQUATION $I = n\Delta\text{CH}_2 + \Delta\text{Bz}(\text{CO}_2)_2$ DETERMINED BY LEAST-SQUARES FITTING FOR PHTHALATES (SE-30, 250°)

n in R = R' = C _n H _{2n+1}	$\Delta\text{Bz}(\text{CO}_2)_2$	ΔCH_2
3-10	1190	93.5
3-5	1212	91
6-10	1169	95

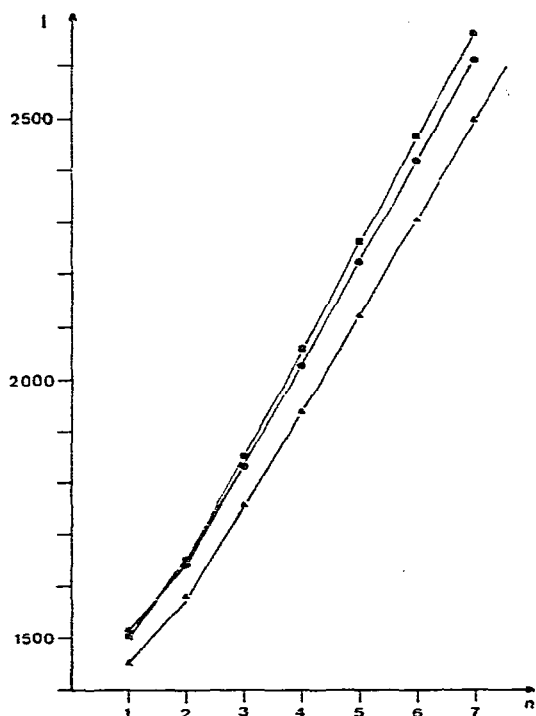


Fig. 3. Plot of retention indices of phthalate esters versus alkyl chain length. OV-101, 230°. 50 m \times 0.3 mm I.D. column. $R = R' = C_nH_{2n+1}$. \blacktriangle , ortho; \bullet , meta; \blacksquare , para.

From a comparison of the results in Table II, measured on the same capillary column, it can be seen that changing the carrier gas does not affect the results, but that the retention indices are temperature dependent.

Terephthalates and isophthalates

The analytical conditions were the same as above. Retention indices from dimethyl to di-(*n*-octyl) esters are listed in Table IV, and it can be seen that they are not affected by the carrier gas but are slightly dependent on column temperature.

TABLE IV
RETENTION INDICES OF TEREPHTHALATES AND ISOPHTHALATES

Ester	Terephthalates		Isophthalates		
	SE-30, 251°	OV-101, 230°	SE-30, 251°	OV-101, 230°	
				N ₂	H ₂
Dimethyl	—	1530	—	1512	—
Diethyl	1650	1649	1639	1638	1638
Di-(<i>n</i> -propyl)	1851	1850	1829	1828	1828
Di-(<i>n</i> -butyl)	2060	2058	2030	2025	2025
Di-(<i>n</i> -pentyl)	2261	2258	2222	2219	2219
Di-(<i>n</i> -hexyl)	2463	2460	2417	2414	2414
Di-(<i>n</i> -heptyl)	2665	2661	2608	2605	2605
Di-(<i>n</i> -octyl)	—	2860	—	—	—

TABLE V

PARAMETERS OF THE EQUATION DETERMINED BY LEAST-SQUARES FITTING FOR TEREPHTHALATES AND ISOPHTHALATES

$R = R' = C_nH_{2n+1}$	SE-30, 250°		OV-101, 230°	
	$\Delta Bz(CO_2)_2$	ΔCH_2	$\Delta Bz(CO_2)_2$	ΔCH_2
<i>Para</i> : propyl to heptyl	1244.5	101.5	1248	101
<i>Meta</i> : propyl to heptyl	1249	97	1247	97

The plots of Kováts retention indices against alkyl chain length are linear from di-(*n*-propyl) and exhibit a slight deviation for the two first members (Fig. 3). $\Delta Bz(CO_2)_2$ and ΔCH_2 were also calculated (Table V). With isophthalates, ΔCH_2 is lower and $\Delta Bz(CO_2)_2$ higher than the values for terephthalates and both are higher than those of *ortho*-phthalates, in which there are strong interactions between methylene groups of the side-chain.

Branched and cyclic phthalates

As previously observed on packed columns for branched dialkyl barbiturates³⁵ and phthalates^{34,36}, the retention indices of branched phthalates are lower than those of the linear homologues (Table VI). As the side-chain is shorter, as the difference ΔI is larger.

TABLE VI

RETENTION INDICES OF BRANCHED PHTHALATES AND LINEAR HOMOLOGUES
OV-101, 230°, carrier gas H₂.

Phthalate	<i>I</i>	ΔI
Diisopropyl	1641	117
Di-(<i>n</i> -propyl)	1758	
Diisobutyl	1847	91
Di-(<i>n</i> -butyl)	1938	
Diisopentyl	2045	77
Di-(<i>n</i> -pentyl)	2122	

Di-(2-ethylhexyl) phthalate and the corresponding linear homologue di-(*n*-octyl) phthalate have often been confused in literature. Their retention indices were therefore measured on SE-30 at 250° and compared with that of di-(*n*-heptyl) phthalate (Table VII). The value for the last compound is close to that for di-(2-ethylhexyl) phthalate. The surface interaction of a branched compound with the stationary phase is less strong than that of a linear compound and the Kováts retention index is therefore lower.

From a comparison of the retention indices of cyclic and homologous linear phthalates measured under the same conditions (Table VII), it is obvious that cyclization of a linear chain increases the retention index and this increase depends on the ring size. These observations are corroborated by literature data³⁷. If I_x is the

TABLE VII

RETENTION INDICES OF BRANCHED CYCLIC AND LINEAR PHTHALATES ON SE-30 AT 250°

<i>Phthalate</i>	<i>I</i>	<i>ΔI</i>
Di-(<i>n</i> -heptyl)	2497	
Di-(2-ethylhexyl)	2509	
Di-(<i>n</i> -octyl)	2685.5	
Di-(<i>n</i> -hexyl)	2308	167
Dicyclohexyl	2475	
Di-(<i>n</i> -heptyl)	2497	238
Dicycloheptyl	2735	

retention index for a cyclic compound and I_2 the retention index of the corresponding *n*-alkane, the plot of $(I_x - I_2)$ versus number of carbon atoms is a parabola with a maximum for 9 carbon atoms. In the particular instances of rings with 7, 8 or 9 carbon atoms, there is no strong interaction and the retention index is much higher than that of the homologous *n*-alkane. For cyclohexane and cyclopentane, intramolecular interactions are so strong that the difference (ΔI) between the cyclic and *n*-alkane is less important than for cycloheptane. Comparison between the Kováts retention indices of dicyclohexyl and dicycloheptyl phthalates and those of their linear homologues shows the same result.

Heterogeneous phthalates

Zulaica and Guiochon³³ considered that the Kováts retention indices of heterogeneous phthalates could be calculated as the half-sum of the retention indices of corresponding homogeneous diesters. In order to check this theory, heterogeneous phthalates ($\text{RO}_2\text{C}-\text{Bz}-\text{CO}_2\text{R}'$) were prepared by esterification of phthaloyl chloride with a mixture of two alcohols. The retention indices of esters from dimethyl to di-(*n*-decyl) phthalate were compared with those of heterogeneous phthalates of the same molecular weight, but with an R' alkyl chain length equal to $\text{R} + 2\text{CH}_2$ (Table VIII).

TABLE VIII

KOVÁTS RETENTION INDICES OF HOMOGENEOUS AND HETEROGENEOUS PHTHALATES ON SE-30 AT 250°

 $I_{\text{exp.}}$ = experimental; $I_{\text{calc.}}$ = calculated retention indices.

<i>Molecular weight</i>	$\text{R} + \text{R}' = n\text{C}$: <i>number of carbon atoms, n</i>	$I_{\text{exp.}}$		$\text{R} + \text{R}' = n\text{C}$:	
		$\text{R} = \text{R}'$	$\text{R}' = \text{R} + 2\text{CH}_2$	$I_{\text{calc.}}$	$I_{\text{exp.}} - I_{\text{calc.}}$
222	4	1583	—	—	—
250	6	1758	1768	1761.5	6.5
278	8	1940	1942	1940	2
306	10	2122	2125	2124	1
334	12	2308	2310	2309	1
362	14	2497	2497	2496	1
390	16	2685	2687	2685	2
418	18	2876	2876	2876	0
446	20	3069	—	—	—

TABLE IX

COMPARISONS OF EXPERIMENTAL AND CALCULATED RETENTION INDICES OF HETEROGENEOUS PHTHALATES

<i>Phthalate</i>	<i>Molecular weight</i>	$I_{exp.}$	$I_{cal.}$	$I_{exp.} - I_{calc.}$
<i>n</i> -Hexyl- <i>n</i> -heptyl	348	2404	2402.5	1.5
<i>n</i> -Butyl- <i>n</i> -nonyl	348	2415	2408	7.0
<i>n</i> -Pentyl- <i>n</i> -decyl	376	2602	2594.5	7.5

Our results agree with theory: the addition of any methylene group to a side-chain results in an increase in retention index equal to the ΔCH_2 value. For ethyl-*n*-butyl phthalate we cannot explain the difference between the experimental and calculated retention indices.

When the alkyl chains lengths are different one from another, the results are not in agreement with theory (Table IX). The experimental retention indices of *n*-butyl-*n*-nonyl and *n*-pentyl-*n*-decyl phthalates are not those expected from calculation.

In order to check this observation, heterogeneous phthalates of molecular weight 334 were compared with their linear homologue di-(*n*-hexyl) phthalate. The sum $R + R'$ was always equal to twelve carbon atoms (Table X).

TABLE X

KOVÁTS RETENTION INDICES OF HETEROGENEOUS PHTHALATES OF MOLECULAR WEIGHT 334 ($R + R' = 12$ CARBON ATOMS) (SE-30, 250°)

<i>Phthalate</i>	$I_{calc.}$	$I_{exp.}$	$I_{exp.} - I_{calc.}$
Methyl- <i>n</i> -undecyl		2389	
Ethyl- <i>n</i> -decyl	2325.5	2343	27.5
<i>n</i> -Propyl- <i>n</i> -nonyl	2317	2325	8
<i>n</i> -Butyl- <i>n</i> -octyl	2312.5	2317	4.5
<i>n</i> -Pentyl- <i>n</i> -heptyl	2309.5	2310	0.5
Di-(<i>n</i> -hexyl)	2308	2308	0

As the two chains become more asymmetrical, the discrepancy between the experimental and calculated results increases: it appears that some interactions other than those determined must exist.

The same derivatives for terephthalates and isophthalates were also prepared. From the results listed in Table XI, and plots of Kováts retention indices against alkyl chain length (Fig. 4), it is obvious that the methyl-*n*-undecyl ester has a particular behaviour which cannot be explained only by charge redistribution according to the relative positions of the alkyl chains R and R' , and that the three experimental curves are different; in the case of phthalates, it appears to be a hyperbola ($1/x$): esters with very different R and R' chain lengths are easily separated.

Separation of diastereoisomers

The separation of optical isomers by gas chromatography is difficult and the use of chiral stationary phases or the preparation of derivatives of the diastereoisomers is necessary³⁸.

TABLE XI

KOVÁTS RETENTION INDICES OF HETEROGENEOUS PHTHALATES OF MOLECULAR WEIGHT 334 ($R + R' = 12$ CARBON ATOMS) (SE-30, 250°)

$$\Delta I = [I_{exp. \text{ di-(n-hexyl)}} - I_{exp.}]$$

Ester	Phthalates (ortho)		Isophthalates (meta)		Terephthalates (para)	
	$I_{exp.}$	ΔI	$I_{exp.}$	ΔI	$I_{exp.}$	ΔI
Methyl- <i>n</i> -undecyl	2389	81	2459	42	2483	14
Ethyl- <i>n</i> -decyl	2343	35	2423	5	2455	-14
<i>n</i> -Propyl- <i>n</i> -nonyl	2325	15	2419	2	2459	10
<i>n</i> -Butyl- <i>n</i> -octyl	2317	9	2419	2	2465	4
<i>n</i> -Pentyl- <i>n</i> -heptyl	2310	2	2416	2	2466	3
Di-(<i>n</i> -hexyl)	2308	0	2417	0	2469	0

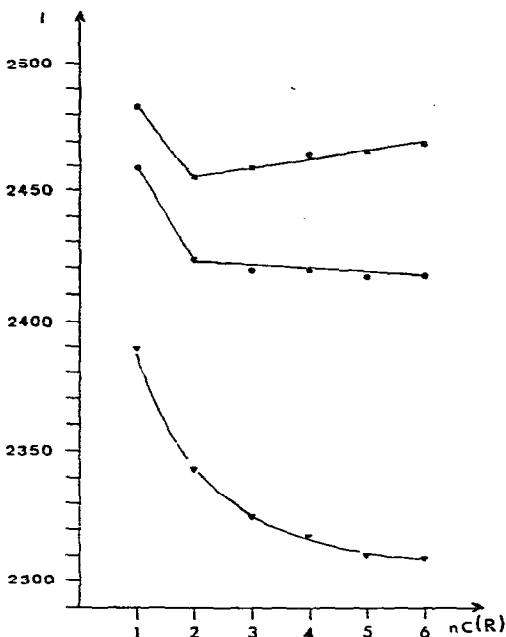


Fig. 4. Kováts retention indices of heterogeneous phthalate esters of same molecular weight (334) versus alkyl (R) chain length. SE-30, 250°. 42 m \times 0.3 mm I.D. column. $R + R' = 12$ C. ∇ , Ortho; \bullet , meta; \blacksquare , para.

By chromatography of commercial di-(3,3,5-trimethylcyclohexyl) phthalate (DTMCHP) (Fluka) on an SE-30 capillary column at 250°, two peaks are observed (Fig. 5). Saponification of this compound with sodium hydroxide gives only one product, identified by chromatography as *trans*-3,3,5-cyclohexanol. Commercial DTMCHP is prepared by esterification of phthalic acid or anhydride with the racemic *trans*-3,3,5-cyclohexanol. The separation number (SN) (see Table I) between the C_{26} and C_{28} *n*-alkanes is 31; the resolution of the column used for the separation of the DTMCHP diastereoisomers is 0.56 (Fig. 5). That is not sufficient for their determination, but it is adequate proof of their existence. Under these experimental conditions, a good resolution ($R = 1.5$) implies a ΔI between these two diastereoisomers of at least 8 units.

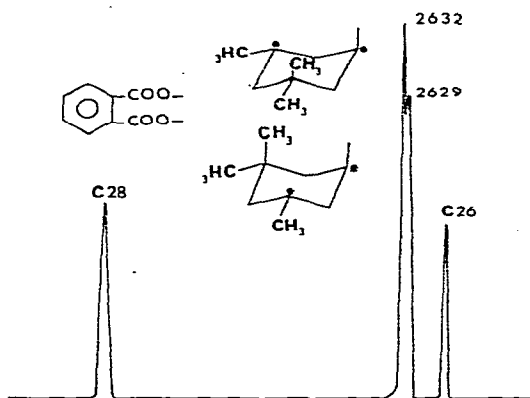


Fig. 5. Separation of di-(3,3,5-trimethylcyclohexyl) phthalate.

The DEHP molecule has also an asymmetrical carbon atom located on the 2-ethylhexyl side-chain. We could not resolve diastereoisomers of this compound by chromatography on capillary columns. The forces due to the cyclic side-chain in the DTMCHP molecule are so strong that the interactions between the solute and the solvent are altered and the diastereoisomers are resolved. In the particular instance of DEHP, free rotation around the bonds is easy and there is no change in the solute-solvent interactions.

CONCLUSION

Phthalates, isophthalates and terephthalates do not show the same chromatographic behaviour on capillary columns.

For homogeneous diesters ($R = R'$), with $R = n$ -alkyl, variations in the Kováts retention indices are in proportion to the side-chain length. With phthalates, a small correction must be used.

When R is a ring or a branched alkyl groups, calculation of Kováts retention indices is difficult: the retention time of a branched compound is lower than that of the homologous linear ester, whereas the corresponding cyclic compound is more retained.

When the lengths of R and R' are very different from one another, it is impossible to calculate the Kováts retention indices accurately.

It seems clear that the Kováts retention index is a satisfactory parameter for identifying phthalate esters (*ortho*, *meta* and *para*). In a few instances, such as when $R' = R + 2\text{CH}_2$, where the isomers [di-(*n*-heptyl) and (*n*-hexyl-*n*-octyl) phthalates, for example] have the same retention index, the use of mass spectrometry as a complementary method of identification is necessary. Some further results concerning on the mass fragmentation of phthalate esters will be published elsewhere.

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