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Journal of Chromatography A, 852 (1999) 525–534

JOURNAL OF CHROMATOGRAPHY A

Alkyl nitrates as achiral and chiral solute probes in gas chromatography
Novel properties of a β-cyclodextrin derivative and characterization of its enantioselective forces

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Received 9 February 1999; received in revised form 11 May 1999; accepted 12 May 1999

Abstract

Achiral and chiral interactions of alkyl nitrates ($R–O–NO_2$) with heptakis-(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (LIPODEX-D) in the gas phase were investigated chromatographically. Two major outcomes can be summarized. First, LIPODEX-D shows very fast temperature-dependent variations of the selectivity up to changes in the order of elution for $C_1–C_5$ alkyl nitrates. These changes in selectivity reveal that LIPODEX-D possesses different shape selectivities for small alkyl nitrates at different temperatures (40–80°C), i.e. with increasing isothermal separation temperature extended (chain-like) alkyl nitrates have increased retention relative to bulky alkyl nitrates. The observations are highly reproducible and might indicate conformational changes of the cyclodextrin, however, an ultimate proof would require further spectroscopic investigation. Secondly, the chiral separations of systematically varied sets of $C_4–C_{11}$ alkyl nitrates allowed the thermodynamic characterization of enantiodiscriminating interactions. Quantitative evidence is provided showing that the presence of an ethyl group at the asymmetric carbon atom of an n-alkyl nitrate gives a strong enthalpic contribution to the resulting enantioselectivity. The Gibbs free energy differences $-\Delta R_{R,S}(\Delta G)$ decrease systematically three to six times if the ethyl group is either shortened or enlarged by only a $–CH_2–$increment. The results are based on two separate thermodynamic approaches, i.e. the determination of thermodynamic quantities ($-\Delta_{R,S}(\Delta G)$, $-\Delta_{R,S}(\Delta H)$, $-\Delta_{R,S}(\Delta S)$, T_{iso}) and a theoretical concept of enthalpy–entropy compensation. The data from our laboratory experiments also indicate that van der Waals interactions are responsible for chiral discrimination.

Keywords: Enantiomer separation; Chiral recognition; Alkyl nitrates; Cyclodextrin

1. Introduction

During the last decade, alkyl nitrates (nitrooxy-alkanes; $R–O–NO_2$, R=alkyl) were found to be products of atmospheric photochemistry. In addition to ozone and peroxyacetyl nitrate (PAN;

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00635-4

$CH_3C(O)OONO_2$) they are important constituents of photochemical smog [1]. Until 1994 only a few single compounds from methyl nitrate up to some *iso*- and *n*-pentyl nitrates could be routinely analyzed in air samples [2]. More recently, we could show that alkyl nitrates up to *n*-heptadecyl nitrates are present in continental air of the lower troposphere, adding numerous branched and straight-chain homologues to the already known short chain alkyl nitrates [3–5]. Deriving from the $OH/O_2/NO$-reaction of all abundant alkanes [1] or the $h\nu/O_2/NO$-reaction of aldehydes [6] in the troposphere, they build an expansive complex mixture of single components including many chiral species. In the IUPAC nomenclature the $-O-NO_2$ group is called ''nitrooxy'' group. We have suggested a shorthand nomenclature structure-related to the hydrocarbon precursor of alkyl nitrates to characterize a single compound in complex mixtures [4,7], e.g. $2C_n$=2-nitrooxy-*n*-alkane, or $3C_6$=3-nitrooxy-*n*-hexane (for an example of alkyl nitrate structures see Fig. 1). The enantiomeric separations of several $\geq C_5$ alkyl nitrates using chiral high resolution gas chromatography (CHRGC) on heptakis-(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (LIPODEX-D) as the chiral selector have been reported by our group, as well as a discussion on the complexity of this class of compounds [7]. For example, we studied systematically for various numbers of carbon atoms (C_1–C_7) the numbers of possible positional isomers and what proportion of these have chiral centers. Remarkably, this was only feasible up to the heptyl nitrates. Beyond the heptyl nitrates the number of isomers increased exponentially. The C_1–C_7 alkyl (mono)nitrates alone add up to 72 different compounds if all the possible *iso*- and *n*-alkyl moieties are considered. This includes 41 chiral molecules, six of which have two asymmetric carbon atoms where diastereomers are also possible [7].

In our usual temperature-programmed chiral separations we observed an unexplainable effect in the enantioselectivity of $2C_n$ alkyl nitrate homologues (n=4–11) on LIPODEX-D, i.e. the inability to separate $2C_5$ nitrate into its enantiomers while all others ($2C_4$, $2C_{6-11}$) could be separated. This led us to study the separation behavior of all available alkyl nitrates (including small and achiral C_1–C_4 alkyl nitrates) particularly in the isothermal mode. In the

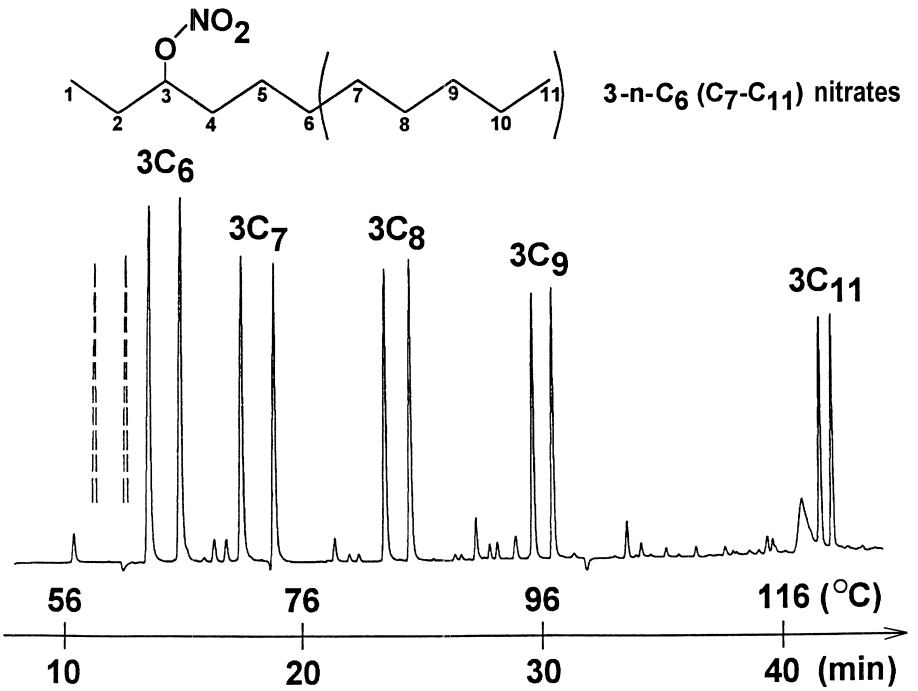

Fig. 1. HRGC/(LIPODEX-D)/ECD: Chiral separations of the homologous set of 3-*n*-alkyl nitrates ($3C_n$). Temp. progr.: 40°C (2 min), 2°C/min, 190°C. Dashed lines indicate where the enantiomers of $3C_6$ nitrate would be expected to elute.

first part of this publication we report a fast and reversible temperature-dependent selectivity change of the cyclodextrin stationary phase, a finding which is uncommon in gas chromatography. The effect is related to the shapes of the alkyl nitrate analytes and is independent from chirality.

Chiral alkyl nitrates up to eleven carbon atoms provided a stepwise investigation of the enantioselectivity of LIPODEX-D by varying the $-O-NO_2$-position and/or $-CH_2-$ increments. Cyclodextrins used in gas chromatography often have to be diluted in achiral polysiloxane stationary phases. It is important, and has been thoroughly discussed by Schurig et al., that non-enantioselective contributions (interactions of the solute probe molecules with the achiral stationary phase) have to be considered with a special approach in such diluted systems [8]. Our thermodynamic data are obtained from enantiomeric separations of the alkyl nitrates on the neat (undiluted) stationary phase. Two different ''approaches'' (thermodynamic calculations and comparisons) were carried out to discuss enantioselective interactions for chiral alkyl nitrates with the β-cyclodextrin derivative LIPODEX-D.

One approach is the calculation and comparison of enantioselective thermodynamic data, i.e. $-\Delta_{R,S}(\Delta G)$, $-\Delta_{R,S}(\Delta H)$, $-\Delta_{R,S}(\Delta S)$ and T_{iso} (isoentantioselective temperature). We have named it the ''enantioselective approach''. The second approach is an enthalpy–entropy compensation method applied in a similar way as described by Berthod et al. [9]. In this case we correlate retention data with thermodynamic data of the second eluting enantiomers of different chiral *n*-alkyl nitrates by varying the alkyl chain length without changing the position where the $-O-NO_2$ group is attached. We have named it the ''positionselective approach''. The second part of this publication deals with the interpretation of the results of these two thermodynamic approaches in consideration of the mechanistic aspects of chiral recognition in gas chromatography.

2. Experimental

2.1. Microsynthesis of alkyl nitrate references standards

Since only a few alkyl nitrates are commercially available, standard mixtures of the sets of *n*-alkyl nitrates with the nitrooxy group in the 2-, 3- and 4-position, respectively, were synthesized as recently described [7,10]. A microsynthetic two-phase esterification of the precursing alcohols in dichloromethane with a mixture of HNO_3 and H_2SO_4 was used. This procedure also allows the asymmetric synthesis of alkyl nitrates via enantiomerically pure alcohols [11,12] where the ''corresponding enantiomer'' of an alkyl nitrate with the same absolute configuration as the parent alcohol is obtained [7,13]. The reaction mechanism is known to be an electrophilic substitution of the proton with a nitryl cation NO_2^+ [14,15].

This synthesis was not applicable for the preparation of C_1–C_3 alkyl nitrates because dichloromethane and other halogenated hydrocarbons interfere the gas chromatographic separation with electron capture detection (ECD). These interferences are avoided if a mild nitration technique described by Olah et al. is used [16]. The parent alcohols are nitrated in acetonitrile with nitronium tetrafluoroborate (NO_2BF_4) and symmetric collidine (2,4,6-trimethylpyridine) as the NO_2^+-transferring reagents. The procedure was slightly modified to obtain alkyl nitrate solutions in pentane with concentrations in the μg/ml range [17]. The various alcohols used to synthesize the corresponding alkyl nitrates studied here were obtained from Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), Janssen Chimica (Geel, Belgium), Merck (Darmstadt, Germany), Merck–Schuchardt (Hohenbrunn, Germany) and Riedi de Haen (Seelze, Germany).

2.2. Gas chromatography

A Hewlett Packard (Palo Alto, CA) gas chromatograph 5890 Series II with on column injector and ^{63}Ni-ECD was used. Argon/methane (90/10%) was used as the ECD make up gas (35 ml/min) at a detector temperature of 240°C. Data acquisition was carried out on a Shimadzu C-R4A integrator (Kyoto, Japan). Hydrogen (grade 5.0) from Linde (Munich, Germany) was used as the carrier gas at a velocity of 55 cm/s at 100°C (70 kPa). The gas hold-up time t_0 was determined using the oxygen peak. The LIPODEX-D capillary column (length: 25 m; inner diameter: 0.25 mm; film thickness: not specified) with a neat heptakis-(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-

cyclodextrin stationary phase was purchased from Macherey Nagel (Düren, Germany).

2.3. Reproducibility

Thermodynamic calculations based on isothermally obtained data in gas chromatography depend on the accuracy and precision of the instrumentation parameters. The GC temperatures displayed by the instrument were controlled by an additional thermoelement which was attached to the GC oven. The oven temperatures were accurate within ±0.2 K. Thus, the data used for the calculations of the thermodynamic values were highly reproducible. The standard deviation (SD) of the enantioselectivity factors α for the separation of all chiral alkyl nitrates were in the range of ±0.001–0.006. Corresponding deviations for the $-\Delta_{R,S}(\Delta G)$ values calculated from the enantioselectivity factors α according to Eq. (1) (see below) were always smaller than 17 J/mol ($SD_{max}=\pm 17$ J/mol). The van't Hoff plots for the determination of $-\Delta_{R,S}(\Delta H)$ and $-\Delta_{R,S}(\Delta S)$ values for each individual alkyl nitrate consist of four to seven data points with resulting correlation coefficients of greater than 0.980. A total of ~300 individual isothermal enantiomeric separations were carried out to obtain the data sets.

3. Results and discussion

3.1. Temperature-dependent shape selectivity of LIPODEX-D

The temperature programmed achiral and chiral GC separations of the homologous series of long chain 2-, 3- and 4-*n*-alkyl nitrates (carbon chains> C_6) on a methyl-polysiloxane (DB1) and on the LIPODEX-D stationary phase, respectively, have been reported by our group [7]. No particular differences were observed for these long chain alkyl nitrates when we carried out the separations under isothermal conditions. The expected difference is the exponential increase (isothermal separation) of the retention times compared to a linear increase (temperature programmed separation) if the *n*-alkyl nitrates differ by $-CH_2-$ increments. Fig. 1 shows the temperature programmed (and therefore equidistant) separation of the homologous set of chiral 3-*n*-alkyl nitrates ($3C_n$, $n=6$–9 and 11). As a first qualitative result related to a temperature-dependent change in selectivity of the stationary phase note the smallest compound in Fig. 1, the 3-*n*-hexyl nitrate ($3C_6$). According to the thermodynamic rules of separation of homologous series (here: $3C_6$, $3C_7$, $3C_8$...), both $3C_6$-enantiomers should elute somewhat earlier (indicated with two sketched marks). In other words, the distance between $3C_6$, and $3C_7$ should be similar to the distance between $3C_7$ and $3C_8$, or $3C_8$ and $3C_9$. The actual GC temperatures according to the retention times are also depicted in Fig. 1. The temperature range of separation for the $3C_6$ nitrate enantiomers was 40°C (starting temperature) to 65°C (temperature at the retention time). The following results with C_1–C_5 alkyl nitrates will show that this is the temperature range where selectivity changes related to the shape of the different alkyl nitrate solute probes occur.

The separations of methyl nitrate up to the pentyl nitrates (C_1–C_5 nitrates) show a remarkable and reproducible temperature dependence. Fig. 2 depicts the isothermal separations of C_1–C_5 alkyl nitrates at 40°C, 60°C and 80°C. *n*-Alkyl nitrates with the $-O-NO_2$ group at the second carbon ($2C_n$ nitrates) are chiral with the exception of $2C_3$ nitrate. Only their chain length is increasing with the carbon number. Comparison of $2C_4$ nitrate and $2C_5$ nitrate, the two smallest chiral $2C_n$ nitrates, revealed rather dramatic differences in their chiral separation behavior on LIPODEX-D (Fig. 2), as well as if both compounds are compared to the available longer chain homologues, the $2C_{6-11}$ nitrates. The $2C_5$ nitrate turns out to be the only chiral $2C_n$ nitrate which cannot be separated on LIPODEX-D into its enantiomers (peak coalescence first kind [18]). Contrarily, the enantiomeric separation of $2C_4$ nitrate is superior (at all tested temperatures) to the chiral separations of the $2C_{6-11}$ nitrates (below discussed quantitatively). These findings would not be expected for this homologous set of compounds, especially the incomparable behavior of $2C_4$ nitrate and $2C_5$ nitrate which differ only in one methylene group.

The overall selectivity of LIPODEX-D for C_2–C_5 alkyl nitrates shows a steady and reproducible shift with temperature related to the molecular shape of

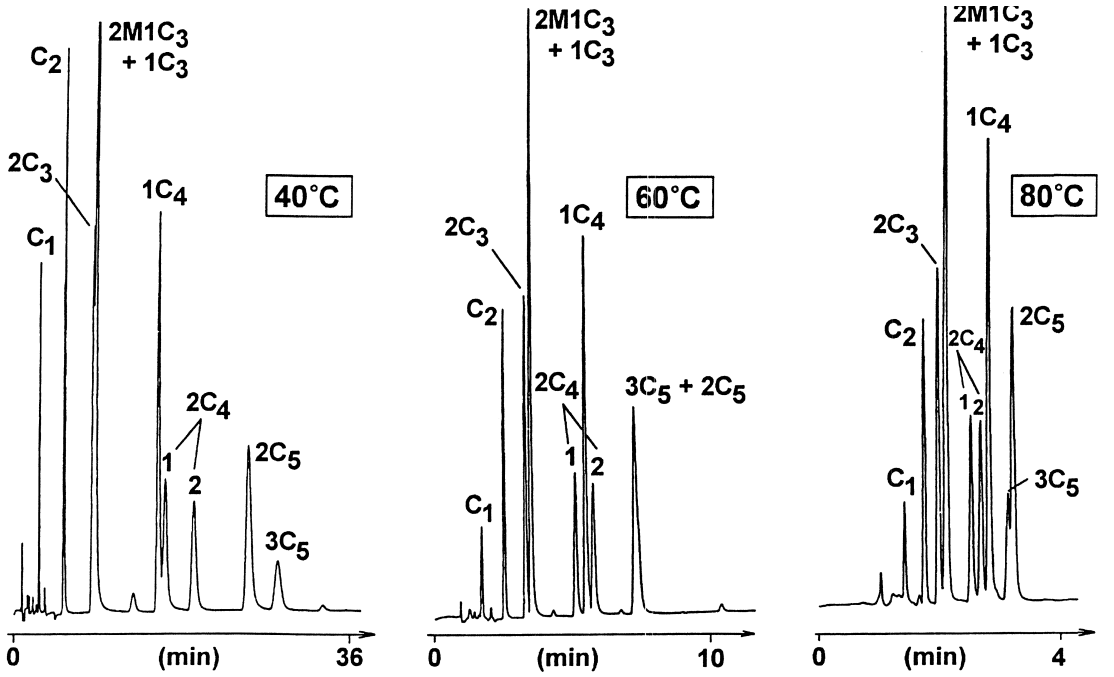

Fig. 2. HRGC/(LIPODEX-D)/ECD: Temperature-dependent changes in selectivity for short chain alkyl nitrates between 40 and 80°C.

the solute probe (for the following observations see also Fig. 2). With increasing temperature the $2C_4$ nitrate enantiomers are shifted to shorter retention times compared to the $1C_4$ nitrate. $1C_4$ nitrate changes the elution order with both $2C_4$ enantiomers. Simultaneously, the $2C_5$ nitrate is shifted to longer retention times compared to the $3C_5$ nitrate. Inversion of the retention order is observed in both cases, i.e. at higher temperatures (80°C) the $2C_4$ nitrate enantiomers and the $3C_5$ nitrate elute prior to $1C_4$ nitrate and $2C_5$ nitrate, respectively. 2-Methyl-1-nitrooxypropane ($2M1C_3$ nitrate) always coelutes with $1C_3$ nitrate although these molecules differ by a methyl group. The separation of the $1C_3$ nitrate and the $2C_3$ nitrate (the two smallest alkyl nitrate isomers) also shows this temperature-dependent selectivity change because these two rather small, similar molecules are obviously better separated at higher temperatures — an unusual observation in achiral gas chromatography. Thus, Fig. 2 is a noteworthy example for significant shape (structure) selectivity of a stationary phase towards small solute probes dependent on the separation temperature. Similar observations have been reported by Sander et al. described as 'the slot model' for the shape selectivity of smectic liquid christalline stationary phases [19], however the shape selectivity in this study did not change with temperature as in the case described here. With increasing temperature the cyclodextrin–solute interactions seem to change allowing extended solutes to have a better fit 'to' — not necessarily 'into' — the cyclodextrin structure compared to the more bulky solutes. This observation might indicate a change of the conformation of the cyclodextrin in this temperature range. However, further spectroscopic investigations will be needed to provide structural information of the cyclodextrin. Recently, O'Brien et al. reported a comparable effect in chiral liquid chromatography. The authors showed that modified cellulose stationary phases undergo a temperature-dependent change in conformation [20]. The temperature effect is reversible and very fast. As soon as the gas chromatograph has adjusted to a different temperature the specific isothermal separation can be carried cut. Thus, the three chromatograms depicted in Fig. 2 can be obtained within one hour. This result shows, that it is possible to develop useful applications for cyclodextrins in gas chroma-

tography which are not necessarily related to chiral separations.

3.2. Differentiation of the chiral recognition of alkyl nitrates via the enantioselective approach

We used undiluted heptakis-(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (LIPODEX-D) because Eq. (1) is only valid for the chiral separation on neat stationary phases [8,21].

$$-\Delta_{R,S}(\Delta G) = RT \ln(\alpha) \quad (1)$$

The theory for the calculation of the enantioselective thermodynamic parameters [$-\Delta_{R,S}(\Delta G)$, $-\Delta_{R,S}(\Delta H)$, $-\Delta_{R,S}(\Delta S)$, T_{iso}] reported here is commonly known [21–25]. Hence, we discuss only the results. Eq. (1) indicates that $-\Delta_{R,S}(\Delta G)$ is temperature dependent. Table 1 shows with three examples (out of 16 chiral *n*-alkyl nitrates) how the $-\Delta_{R,S}(\Delta G)$ values (and the α values) change with the separation temperature. As noted by Schurig et al. [8] (and references therein), data featuring very weak enantioselectivities with separation factors a smaller than 1.1 should not be used for mechanistic considerations. Our results in Table 1 provide a practical explanation to support this argument. The standard deviations (SD) of the obtained $-\Delta_{R,S}(\Delta G)$ values for all single chiral separations in the whole isothermal temperature range used in our experiments were very similar and can be expressed by the largest calculated value ($SD_{max} = \pm 17$ J/mol). In general, this deviation shows that it is not possible to clearly distinguish $-\Delta_{R,S}(\Delta G)$ values for enantiomeric separations with α values smaller than 1.1, i.e. the results in Table 1 for 4-nitrooxy-*n*-octane ($4C_8$ nitrate) at the different temperatures cannot be significantly distinguished and are not useful for discussion. Table 2 summarizes the obtained thermodynamic data. The $-\Delta_{R,S}(\Delta G)$ data are given at 80°C because values for all compounds could be determined at this temperature. It is remarkable that the $-\Delta_{R,S}(\Delta G)$ values of the single tested alkyl nitrates (let us assume that the structures would be unknown) split these compounds into three groups, i.e. the homologous series of 2-, 3- and $4C_n$ nitrates. However, the 2-*n*-butyl nitrate ($2C_4$) would fall into the $3C_n$ nitrate set. This behavior is no exception and can be clearly explained as follows. The $3C_n$ nitrate enantiomers exhibit a significant increase of 1.6–3.6 kJ/mol of enantioselective enthalpy differences $-\Delta_{R,S}(\Delta H)$ compared to the $2C_n$– and $4C_n$ nitrates. This confirms our hypothesis [7] that they undergo increased chiral discrimination due to the ethyl group at the asymmetric carbon atom C* because enantioselective interactions below the isoenantioselective temperature are enthalpy-controlled [26]. In addition to the $3C_n$ homologues, there is one other *n*-alkyl nitrate, the $2C_4$ nitrate, which also has an ethyl group attached at C* and which shows the same effect. The Gibbs free energy difference $-\Delta_{R,S}(\Delta G)$ of the $2C_4$ nitrate (293 J/mol) is increased by a factor of three and six compared to the other $2C_n$ nitrates (~100 J/mol) and $4C_n$ nitrates (~50 J/mol), respectively, and has the same value as those observed for the $3C_n$ nitrates (259–297 J/mol).

The enthalpy and entropy portions of the separations can be determined using the van't Hoff plots $\ln(\alpha)$ versus $1/T$ [21–25]. All van't Hoff plots obtained by the enantiomeric separation of each individual alkyl nitrate at different temperatures (40–120°C) resulted in straight lines (see the Experimental section) and are therefore not shown explicitly. Linear van't Hoff plots mean that the $-\Delta_{R,S}(\Delta H)$ values (obtained from the slope) are not temperature dependent and can be compared. The numerical values of $-\Delta_{R,S}(\Delta H)$ and $-\Delta_{R,S}(\Delta S)$ for the $3C_n$ nitrates and $2C_4$ nitrate do not indicate the similarity in enantioselective interaction as clearly as the $-\Delta_{R,S}(\Delta G)$ values do. Therefore, we also show a visual comparison by sketching all data points of the

Table 1
α-Values and Gibbs free energy differences (J/mol) for the three smallest chiral *n*-alkyl nitrates with the nitrooxy group at different positions (e.g. $2C_4$ = 2-nitrooxy-*n*-butane)

T (°C)	Enantioselectivity α[a]			$-\Delta_{R,S}(\Delta G)$[b]		
	$2C_4$	$3C_6$	$4C_8$	$2C_4$	$3C_6$	$4C_8$
40	1.208	1.342	–	479	766	–
50	1.176	1.253	1.040	436	606	105
60	1.150	1.184	1.032	387	469	87
70	1.126	1.131	1.025	338	351	70
80	1.105	1.092	1.019	293	259	55
90	1.083	–	–	241	–	–

[a] $SD_{max} = \pm 0.006$.
[b] $SD_{max} = \pm 17$ J/mol.

individual van't Hoff plots of the $2C_n$ nitrates and $3C_n$ nitrates in one graph (Fig. 3). The plots for the $2C_6$–$2C_{11}$ nitrates and $3C_6$–$3C_{11}$ nitrates are overlapping, respectively, indicating that there is no apparent difference in chiral recognition within the homologous series. Numbers for the Gibbs free energy difference $-\Delta_{R,S}(\Delta G)$ for chiral separations can have the same value by coincidence. However, overlapping van't Hoff plots can be regarded as a comparison of $-\Delta_{R,S}(\Delta G)$ including the partial contributions of the enthalpy and entropy involved. The plot for the $2C_4$ nitrate in Fig. 3 is shifted towards the overlapping $3C_n$ plots. This result indicates that the similarities of $-\Delta_{R,S}(\Delta G)$ occur not by coincidence but reflect to presence of an ethyl group in all $3C_n$ nitrates and in the $2C_4$ nitrate.

Table 2 further shows that the enthalpy- and entropy differences within the homologous series of $2C_n$ nitrates and $3C_n$ nitrates, respectively, correlate with the length of the solute probe molecules. The values of $-\Delta_{R,S}(\Delta H)$ and $-\Delta_{R,S}(\Delta S)$ continually increase with the loss of methylene increments. $4C_n$ nitrates cannot show this trend because chiral $4C_n$

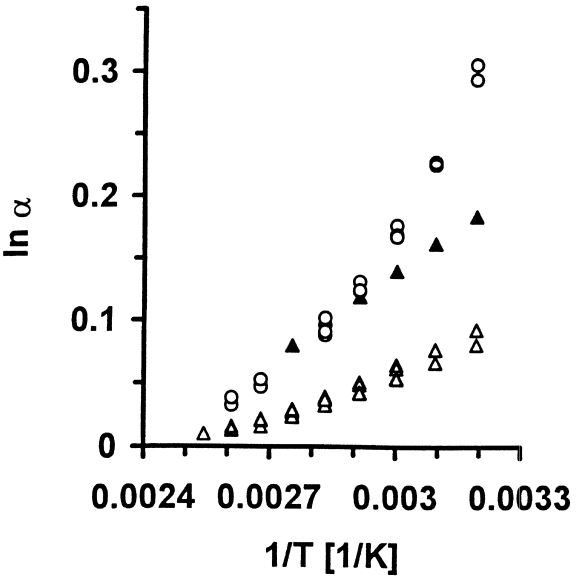

Fig. 3. Van't Hoff plots of chiral $2C_n$– and $3C_n$ alkyl nitrates. △, $2C_n$ nitrates, $n=6$–11; ○, $3C_n$ nitrates, $n=6$–9, 11; ▲, $2C_4$ nitrate. Individual plots are strongly overlapping. One symbol can represent several data points.

Table 2
Thermodynamic enantioselective quantities obtained from the isothermal chiral separations of the homologous series of *n*-alkyl nitrates on LIPODEX-D

n-Alkyl nitrates	*T*-Range[b] [°C]	$-\Delta_{R,S}(\Delta G)$ @ 80°C[b] [J/mol]	$-\Delta_{R,S}(\Delta H)$ [kJ/mol]	$-\Delta_{R,S}(\Delta S)$ [J/mol*K]	T_{iso}[c] [°C]
$2C_4$	40–90	293	1.96	4.76	140
$2C_5$	40–90	–[a]	–[a]	–[a]	–[a]
$2C_6$	40–90	109	1.21	3.18	110
$2C_7$	40–90	96	1.05	2.72	110
$2C_8$	60–100	96	0.96	2.47	120
$2C_9$	60–110	113	1.09	2.76	120
$2C_{10}$	80–110	109	0.88	2.17	130
$2C_{11}$	80–120	105	0.75	1.88	130
$3C_6$	40–80	259	4.77	12.8	100
$3C_7$	40–100	284	4.05	10.6	110
$3C_8$	50–100	268	3.51	9.15	110
$3C_9$	60–110	268	2.80	7.11	120
$3C_{11}$	80–120	297	2.42	6.02	130
$4C_8$	50–80	55	0.67	1.76	110
$4C_9$	50–80	55	0.67	1.76	110
$4C_{10}$	60–80	51	0.79	2.09	110

[a] Peak coalescence (first kind) in the whole temperature range.
[b] Retention and α-values were measured at 10°C intervals.
[c] Temperatures reported to the nearest 10°C (see also [9]).

alkyl nitrates exist only with longer alkyl chains ($n \geq$ carbon atoms) and the contribution of a single methylene increment is relatively small. The ratio of $[-\Delta_{R,S}(\Delta H)]/[-\Delta_{R,S}(\Delta S)]$ describes the isoenantioselective temperature (T_{iso}) where peak coalescence (third kind [18]) occurs. The T_{iso}'s decrease gradually towards shorter chain lengths within both sets of homologues indicating that the entropic part of the $[-\Delta_{R,S}(\Delta H)]/[-\Delta_{R,S}(\Delta S)]$-ratio rises faster than the enthalpic part and becomes more effective for smaller alkyl nitrate homologues. A model of enhanced inclusion into the cyclodextrin cavity with the loss of methylene increments of the solute probes might explain this observation although a mechanistic proof cannot be provided yet.

The $2C_4$ nitrate again shows an exceptional behavior and provides further explanation for the discussion above. Although the entropy value of the $2C_4$ nitrate fits perfectly into the increasing trend of $-\Delta_{R,S}(\Delta S)$ within the $2C_n$ homologues (a trend which lowers T_{iso}), the calculated T_{iso} of 140°C does not. An additional enthalpic component in the enantiodiscriminating interactions of the $2C_4$ enantiomers with LIPODEX-D increases the resulting T_{iso} to this number, revealing the quantitative proof of a relatively high enthalpic interaction due to an ethyl group. All $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ values were negative which means that an isoenantioselective temperature exists [9]. The disappearance of enantiomeric resolution at temperatures close to T_{iso} (and at T_{iso}) was observed for all compounds (data not shown), however, the isoenantioselective temperatures were too high to confirm peak inversion at temperatures above T_{iso}. This is a common problem in chiral GC because at increased temperatures enantiomeric separation is only possible if the α-values are large enough to compensate the decreasing capacity factors (retention times) [9,27].

In summary, the discussed data sets show that the addition of a single $-CH_2-$ increment to an alkyl chain can be highly significant for chiral recognition. In 1997, Lipkowitz et al. published an extensive computational study about how and where chiral recognition takes place in gas chromatography between small solute probes and permethylated β-cyclodextrin [28]. Some major outcomes were the following: (1) For host–guest complexation as well as for chiral recognition, the short range van der Waals dispersion forces are dominant over the electrostatic forces even if a hydroxyl group is present in the solute probe molecule. (2) The preferred binding site for small analytes appears to be the interior of the cyclodextrin. (3) A preferable binding to the secondary or the primary rim of the cavity is not observable (the secondary and primary rim is the more and less open side of the cavity, respectively). (4) Two of the solutes probes used by Lipkowitz et al. (acetic acid alkyl esters with a methyl- and ethyl group at C*) are comparable to the $2C_n$ nitrates and $3C_n$ nitrates (nitric acid alkyl esters) reported here. The data show that the ethyl derivative shows enantiodiscriminating forces twice as large as the methyl compound which is in agreement with our findings.

3.3. Differentiation of the chiral recognition of alkyl nitrates via the positionselective approach

Berthod et al. used an enthalpy–entropy compensation method to compare the chiral recognition mechanism of different cyclodextrin stationary phases [9]. We applied this method to compare the recognition mechanism of sets of *n*-alkyl nitrate enantiomers with the same position of the nitrooxy group, i.e. the homologous series of $2C_n$ nitrates and $3C_n$ nitrates. Data is taken from the later eluting enantiomer on LIPODEX-D. This theoretical approach can be summarized as follows. A solute is distributed between the two phases of a chromatographic system according to the difference in the interactions with these two phases, i.e. the difference in free energy of partition ΔG^0. It is important to recall that deviating from above, where we have discussed $-\Delta_{R,S}(\Delta G)$, now ΔG^0 is considered. ΔG^0 expresses the free energy of partition of a compound (here: one enantiomer) between an active phase (here: cyclodextrin) and an inert gas phase. The basis of the calculation is that a group of molecules (here: different enantiomers) undergoes similar physicochemical interactions with an active phase if they have the same Gibbs free energy change ΔG^0_β at a virtual temperature β where enthalpy–entropy compensation occurs. The origin of Eq. (2) and further literature is described in [9]. Plots of ln k versus $\Delta H^0/R$ can lead to a linear correlation if the

compensation temperature β is equal for different solute probes. Values for ΔH^0 were determined from the slope of ln k versus $1/T$ plots.

$$\ln k = -(\Delta H^0/\mathrm{R})(1/T - 1/\beta) + \Delta G^0_\beta/\mathrm{R}\beta + \text{const.} \quad (2)$$

For the resulting enthalpy–entropy compensation plots we have used k-values from the second eluting enantiomer of the $2C_n$ nitrates and $3C_n$ nitrates, respectively, at 80°C. Fig. 4 depicts the enthalpy–entropy compensation plot of the $3C_n$ homologues. It is clearly indicated that the ΔH^0 values correspond to the ln k-values (the regression coefficient is 0.970). Each point represents a different enantiomer. The $2C_n$ plot gave the same results (therefore not shown) with $2C_4$ nitrate and $2C_5$ nitrate off the regression line (regression coefficient: 0.959). Thus, we can also show with this positionselective approach that different n-alkyl nitrate enantiomers with the nitrooxy group at the same position have the same enantioselective interaction with LIPODEX-D. However, the most important result is the fact that the plot also visualizes the influence of the ethyl group. $2C_4$ nitrate lies almost exactly on the extension of the trendline for the $3C_n$ nitrates. We cannot explain the deviating behavior of the $3C_6$ nitrate. It could be due to an increased entropic component because it is the smallest chiral $3C_n$ nitrate. It definitely is not a result of the change in conformation of the cyclodextrin, as described for the C_1–C_5 alkyl nitrates above, because the data here are obtained isothermally at 80°C.

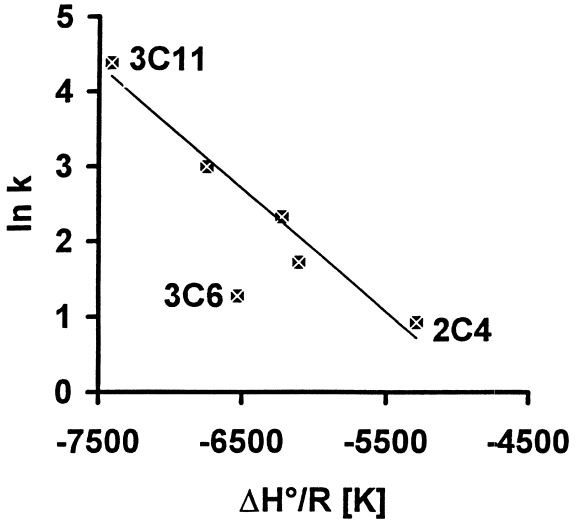

Fig. 4. Enthalpy–entropy compensation plot (ln k versus $\Delta H^0/\mathrm{R}$) for the later eluting enantiomers of $3C_n$ alkyl nitrates ($n = 11$, 9–6) and $2C_4$ nitrate). k-Values are obtained from isothermal separations at 80°C.

The calculation of the compensation temperatures β via the slope m with $\beta = T/(1 - mT)$ led to approximately 970 K and 1070 K for the $2C_n$ nitrate- and the $3C_n$ nitrate plots, respectively. Similarity of β would suggest essentially identical retention mechanism for the $2C_n$ nitrates and $3C_n$ nitrates [9], however, a closer discussion of this result can be misleading due to cumulative errors associated with the determination of enthalpy [9]. These temperatures represent theoretical quantities and they are too high to carry out any experiments under these conditions.

4. Conclusions

The neat stationary phase heptakis-(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (LIPODEX-D) changes its interaction properties for small alkyl nitrates ($\leq C_5$) systematically and fast with temperature. These changes are linked to a significant shape selectivity which discriminates extended and bulky alkyl nitrates. Changes in the conformation of the cyclodextrin stationary phase might explain these findings, however, further spectroscopic investigations are needed to confirm this.

In addition, the results of two independent thermodynamic approaches allow the discussion of some mechanistic aspects of enantiomeric separations. It was possible to differentiate enantioselective interactions within structurally similar compounds (here alkyl nitrates). The similar molecular properties of a large number of chiral alkyl nitrates, i.e. an unchanging polar group (–O–NO_2) in combination with a varying non-polar skeleton, enabled us to demonstrate that addition or removal of –CH_2– increments in a chiral solute probe molecule can lead to significant changes in the enantioselective interaction with cyclodextrin LIPODEX-D.

Acknowledgements

The financial support by the Deutsche Forschungsgemeinschaft (Project Number Ba 371/14-2) is gratefully acknowledged.

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