# Separation of Diastereomeric and Enantiomeric Alkyl Nitrates—Systematic Approach to Chiral Discrimination on Cyclodextrin LIPODEX-D

**Manfred Schneider and Karlheinz Ballschmiter***

**Abstract**: High-resolution gas chromatographic separation of all diastereomeric monomethyl-substituted cyclohexyl nitrates is shown on a nonpolar methylpolysiloxane stationary phase, and the first application of this procedure to the environmental diastereomeric analysis of alkyl nitrates is presented. Two characteristic signals in the achiral analysis of atmospheric samples could be assigned to the smallest alkyl nitrate containing two asymmetric carbon atoms, 3-methyl-2-pentyl nitrate. Retention indices in the temperature-programmed separation based on the *n*-alkanes were determined. The homologous series of 1-alkyl nitrates were found to be useful as ECD-visible *n*-alkanes. Enantiomeric separation of alkyl nitrates was achieved on heptakis(3-*O*-acetyl,-2,6-di-*O*-pentyl)-β-cyclodextrin (LIPODEX-D). The influence of the nitrooxy group and the alkyl chain length on the chiral discrimination on LIPODEX-D is discussed for 25 chiral alkyl nitrates. The absolute configurations of some alkyl nitrates were assigned by asymmetric synthesis of enantiomerically pure references. The complexity of the alkyl nitrate mixtures present in air samples does not allow a direct chiral separation as the alkyl nitrates partly coelute on the LIPODEX-D column. Column coupling of LIPODEX-D with a polar achiral stationary phase like polyalkylenglycol (PAG) was successfully applied to solve this problem, and the chiral alkyl nitrates present in a typical air sample were separated. A systematic nomenclature for alkyl nitrates is introduced to handle the steadily growing number of branched and long-chain alkyl nitrates detected in environmental analysis.

**Keywords**
analytical methods · alkyl nitrates · chiral resolution · cyclodextrins · gas chromatography

## Introduction

Nitric acid alkyl esters ($R-ONO_2$), conventionally named as alkyl nitrates, are one of the components of the photochemical smog (Los Angeles smog) formed by the reaction of alkylperoxy radicals with NO. They are also produced commercially and are used as diesel additives, propellants, explosives and pharmaceuticals.[1, 2] Atmospheric alkyl nitrates are produced by light-induced oxidation of anthropogenic and biogenic hydrocarbons by the OH radical/O2 system and $NO_x$ emissions. The photochemical formation of alkyl nitrates has been intensively investigated in the last decade.[3, 4] $C_1$ to $C_{14}$ alkyl nitrates are detectable as trace compounds in the troposphere.[5–8] Even in clean air of remote areas, alkyl nitrates could be detected, and evidence for their long-range transport has been reported.[9–11]

Alkyl nitrates build a complex but defined class of chemical components only differing in the alkyl moiety. Most of them are chiral compounds. The hydrocarbon skeleton allows structure-related enantioselective studies by varying the chain length and the branching of the alkyl nitrates. Branched alkyl nitrates also exist as diastereomers if more than one asymmetric carbon atom is present.

Due to the unique properties of cyclodextrin (CD) derivatives chiral high-resolution gas chromatography (CHRGC) has been significantly improved in the last few years. Improvements in stereochemical discrimination of chiral environmental xenobiotics have been achieved, and their enantioselective biotransformation demonstrated.[12, 13] Discussions on the selector–selectand interaction mechanism in the case of cyclodextrins as chiral selectors are difficult, because CDs are able to separate chiral molecules with entirely different stereochemical properties. This is due to the manifold of potential combinations of interactions with cyclodextrins (inclusion, dipole–dipole, hydrogen bonding, van der Waals). Multiple enantioselective retention mechanisms for one cyclodextrin stationary phase have been described.[14] The state of the art in CHRGC has recently been reviewed.[15] The difficulties in characterising the interaction with CD derivatives due to multimodal recognition processes have also been pointed out.[15] Interaction mechanisms can therefore only be applied to predict the suitability of a cyclodextrin stationary phase (CSP) for the separation of chiral compounds within a class of structurally similar compounds or homologues. Knowledge of structure-related enantioselectivity of a CSP can help reduce trial-and-error testing. For example, heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (LIPODEX-D) is described as a chiral selector for γ-lactones[16] and was recently successfully tested in the enantiomeric separation of a γ-lactone metabolite of the insecticide endosulfan.[17]

Enantiomeric and diastereomeric separation of alkyl nitrates is not only of academic interest in the study of chiral recogni-

[*] Prof. Dr. K. Ballschmiter, Dipl.-Chem. M. Schneider
Department of Analytical and Environmental Chemistry
University of Ulm, 89069 Ulm (Germany)
Fax: Int. code +(731)502-2763
e-mail: karlheinz.ballschmiter@chemie.uni-ulm.de

  0947-6539/96/0205-0539 $ 15.00+.25/0 

tion, but is also of substantial interest in environmental chemistry. Hydrocarbons, which are precursors for alkyl nitrates, originate from anthropogenic and biogenic sources. Biogenic chiral alkanes may be produced as nonracemic mixtures. The corresponding mixtures of enantiomeric alkyl nitrates formed directly from these precursors can therefore also be nonracemic.[17] Hence, biogenic and anthropogenic hydrocarbon sources can be distinguished by the enantiomeric ratios of distinct alkyl nitrates. Moreover, a biotic degradation of alkyl nitrates, which are only slowly hydrolysed in an aqueous medium,[18] can be detected by determing the enantiomeric ratios of environmental samples.

In this paper we describe the first example of stereoisomeric analysis of an environmental sample of alkyl nitrates. We show that two characteristic peaks almost always detected in environmental samples can be assigned to diastereomers of 3-methyl-2-pentyl nitrate, present in different proportions.

## Results and Discussions

**Nomenclature of alkyl nitrates based on the hydrocarbon precursor:** In the IUPAC nomenclature the -$ONO_2$ group is called nitrooxy group. For characterising complex alkyl nitrate mixtures we used a simple nomenclature correlated to the hydrocarbon precursors. The longest nonbranched alkyl chain is taken as the skeleton of the molecule. In the case of branched alkyl nitrates the alkyl side chains are given a higher priority than the nitrooxy group. As a general example the abbreviation, 2,4M 5 $C_7$ represents 2,4-dimethyl-5-heptyl nitrate or, more generally, a methyl-branched isononyl nitrate (Fig. 1).

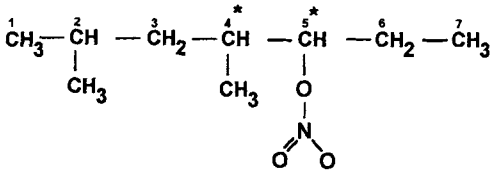

Fig. 1. 2,4M 5 $C_7$ nitrate.

We summarise the advantages of this new nomenclature as follows:

1) With increasing length and branching of the alkyl chain, the number of possible alkyl nitrates expands rapidly, and a simplified labelling system for the resulting complex mixtures is required.
2) Mistakes and double labelling can be avoided (e.g., 2M 3 $C_4$ = 3M 2 $C_4$, but 2M 3 $C_5$ ≠ 3M 2 $C_5$).
3) The alkane precursor in the photochemical formation of alkyl nitrates is implicit.

**Stereochemistry of alkyl nitrates:** The determination of the number of enantiomeric and diastereomeric alkyl nitrates is straightforward, since only central chirality with one or more asymmetric carbon atoms (C*) is possible. Alkyl nitrates possessing at least two C* also form pairs of diastereomers, and the method for calculating the number of enantiomeric and diastereomeric pairs has long been known.[19, 20]

We studied systematically, for various numbers of carbon atoms ($C_1$–$C_7$), the number of possible positional isomers and what proportion of these have chiral centres (Table 1). Remarkably, this was only feasible up to the heptyl nitrates. Beyond the heptyl isomers, the number of isomers increases exponentially, and we could not model this behaviour mathematically. Altogether there are 72 positional isomers possible for $C_1$ to $C_7$. This includes 41 chiral compounds, six of which have two C* where diastereomers are also possible.

Table 1. Number of positional isomers of alkyl nitrates up to $C_7$ and proportion of these with one and two chiral centres (C*).

| | $\Sigma RONO_2$ | 1 C* | 2 C* | $\Sigma$chiral |
|---|---|---|---|---|
| $C_3$ | 2 | 0 | 0 | 0 |
| $C_4$ | 4 | 1 | 0 | 1 |
| $C_5$ | 8 | 5 | 0 | 5 |
| $C_6$ | 17 | 8 | 1 | 9 |
| $C_7$ | 39 | 21 | 5 | 26 |
| $\Sigma C_1$–$C_7$ | 72 | 35 | 6 | 41 |

Alkyl nitrates were prepared by esterification of the corresponding alcohols (see Experimental Procedure). We synthesised alkyl nitrates from every commercially available alcohol. Alkyl nitrates up to $C_{15}$ and some cycloaliphatic compounds were synthesised. Unfortunately tertiary alkyl nitrates give insufficient yields and could not be prepared in this way. Generally, nonbranched alkyl nitrates are chiral, when the nitrooxy group is not at the 1-position and, for odd C numbers, not in the middle position (e.g., 1 $C_7$ and 4 $C_7$ nitrates are achiral). The chirality of every branched alkyl nitrate had to be assigned separately. In total we synthesised more than 80 alkyl nitrates.

**Achiral separation of alkyl nitrates:** Extending recent results in alkyl nitrate separation,[5, 7] Figure 2 depicts the separation of more than 60 alkyl nitrates including some dinitrates. We determined the retention indices (RI) based on the *n*-alkanes accord-

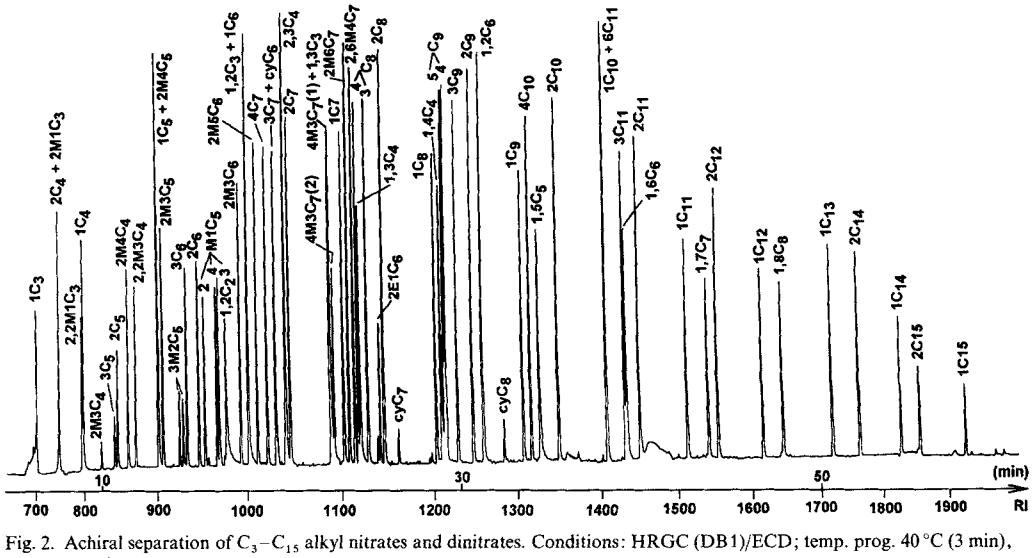


Fig. 2. Achiral separation of $C_3$–$C_{15}$ alkyl nitrates and dinitrates. Conditions: HRGC (DB1)/ECD; temp. prog. 40 °C (3 min), rate 3 °min$^{-1}$, 250 °C.



0947-6539/96/0205-0540 $ 15.00+.25/0 Chem. Eur. J. 1996, 2, No. 5

ing to Kovats' method, but in the temperature-programmed mode proposed by Van den Dool[21] on a methylpolysiloxane capillary. The homologous series of the 1-nitrates showed a unique behaviour. With increasing carbon number ΔRI has a constant value of 101.5 ± 0.5 RI units. The index increment on introducing the nitrooxy group at the 1-position is almost exactly 397 + 1.5 $C_n$ RI units; thus, a 1 $C_n$ alkyl nitrate has about the same retention index as an $n+4$ *n*-alkane. This RI increment decreases for secondary alkyl nitrates as the nitrooxy group moves to the middle of the alkyl chain (e.g., 350 RI units for 2-alkyl nitrates and 335 RI units for 3-alkyl nitrates). For practical purposes the homologous series of 1-alkyl nitrates can be used as retention-index markers for the electron-capture detector, that is, as ECD-visible *n*-alkanes.

Besides retention indices, relative retention times (RRT) are also a good measure for assigning retention, defined in chromatography as the strength of interaction with the stationary phase. We therefore calculated RRT of the alkyl nitrates. Considering the fact that secondary nitrates are the most abundant alkyl nitrates formed in the atmosphere,[22] we chose the 2$C_5$ and the 2$C_{10}$ nitrates as the reference compounds for short- and long-chain alkyl nitrates, respectively. These two reference compounds are always abundant in atmospheric samples and easy to assign in the chromatogram. RRT of some alkyl nitrates have already been determined with tetrachloroethylene as the reference,[23] but this is only useful in assigning short-chain alkyl nitrates within roughly the same boiling range as tetrachloroethene. Moreover, the reference marker should always be present in the sample. This is also a main reason for us choosing two alkyl nitrates as the marker molecules for the assignment of complex alkyl nitrate mixtures by their relative retentions. Table 2 summarises all the calculated retention indices and relative retention times in the order of elution on the methylpolysiloxane stationary phase (DB1).

**Enantioselective separation of alkyl nitrates:** In general the LIPODEX-D capillary column displayed very good enantioselectivity towards alkyl nitrates. In order to study characteristics of the retention mechanism, different mixtures of alkyl nitrates were synthesised. For the nonbranched alkyl nitrates, homologous series of chiral 2-, 3- and 4-nitrates were prepared. The available branched alkyl nitrates were divided into compounds with the nitrooxy group directly attached to the asymmetric centre C* and those where this was not the case.

For the *n*-alkyl homologous series, the enantioselective separation of the 3-alkyl nitrates was remarkably straightforward (Fig. 3). Enantioselectivity factors α were calculated to be up to 1.11. In general, α values are low for HRGC with cyclodextrin stationary phases.[15] Owing to the wide boiling range of the analytes, we had to use temperature-programmed gas chromatography. The decrease in the α values within a homologous

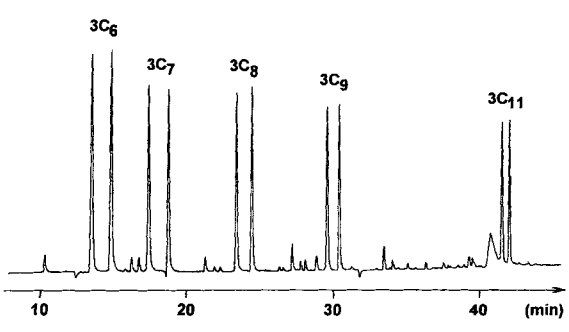


Fig. 3. Chiral separation of 3-alkyl nitrates. Conditions: HRGC (LIPODEX-D)/ECD; temp. prog. 40 °C (2 min), rate 2 °min$^{-1}$, 190 °C.

Table 2. RI and RRT (relative to 2$C_5$ or 2$C_{10}$) of alkyl nitrates on nonpolar DB1.

| Nitrate | RI | RRT(2$C_5$) | Nitrate | RI | RRT(2$C_{10}$) |
|---|---|---|---|---|---|
| 1$C_3$ | 700 | 0.512 | 4M3$C_7$ (1) | 1093 | 0.619 |
| 2M1$C_3$ | 739 | 0.643 | 1,3$C_3$ | 1093 | 0.619 |
| 2$C_4$ | 739 | 0.643 | 4M3$C_7$ (2) | 1096 | 0.624 |
| 2,2M1$C_3$ | 798 | 0.782 | 1$C_7$ | 1106 | 0.640 |
| 1$C_4$ | 801 | 0.788 | 2M6$C_7$ | 1112 | 0.649 |
| 2M3$C_4$ | 819 | 0.901 | 2,6M4$C_7$ | 1119 | 0.658 |
| 3$C_5$ | 844 | 0.982 | 4$C_8$ | 1122 | 0.664 |
| 2$C_5$ | 847 | 1.000 | 1,3$C_4$ | 1123 | 0.668 |
| 2M4$C_4$ | 862 | 1.064 | 3$C_8$ | 1133 | 0.681 |
| 2,2M3$C_4$ | 873 | 1.113 | 2E1$C_6$ | 1145 | 0.700 |
| 1$C_5$ | 903 | 1.257 | 2$C_8$ | 1150 | 0.707 |
| 2M4$C_5$ | 903 | 1.257 | cy$C_7$ | 1173 | 0.731 |
| 2M3$C_5$ | 908 | 1.284 | 1$C_8$ | 1208 | 0.794 |
| 3M2$C_5$ (1) | 927 | 1.395 | 1,4$C_4$ | 1213 | 0.803 |
| 3M2$C_5$ (2) | 931 | 1.416 | 5$C_9$ | 1217 | 0.808 |
| 3$C_6$ | 935 | 1.435 | 4$C_9$ | 1219 | 0.811 |
| 2$C_6$ | 949 | 1.511 | 3$C_9$ | 1233 | 0.833 |
| 2M1$C_5$ | 956 | 1.549 | 2$C_9$ | 1251 | 0.857 |
| 4M1$C_5$ | 969 | 1.621 | 1,2$C_6$ | 1261 | 0.874 |
| 3M1$C_5$ | 972 | 1.636 | cy$C_8$ | 1294 | 0.909 |
| 1,2$C_2$ | 975 | 1.678 | 1$C_9$ | 1310 | 0.941 |
| 2M3$C_6$ | 996 | 1.769 | 4$C_{10}$ | 1319 | 0.954 |
| 1,2$C_3$ | 1004 | 1.818 | 1,5$C_5$ | 1329 | 0.969 |
| 1$C_6$ | 1004 | 1.818 | 2$C_{10}$ | 1352 | 1.000 |
| 2M5$C_6$ | 1014 | 1.875 | 1$C_{10}$ | 1411 | 1.081 |
| 4$C_7$ | 1025 | 1.939 | 6$C_{11}$ | 1411 | 1.081 |
| 3$C_7$ | 1034 | 1.992 | 3$C_{11}$ | 1434 | 1.111 |
| cy$C_6$ | 1034 | 1.992 | 1,6$C_6$ | 1438 | 1.114 |
| 2,3$C_4$ | 1043 | 2.053 | 2$C_{11}$ | 1453 | 1.135 |
| 2$C_7$ | 1049 | 2.080 | 1$C_{11}$ | 1513 | 1.213 |
| 4M3$C_7$ (1) | 1093 | 2.329 | 1,7$C_7$ | 1543 | 1.248 |
| 1,3$C_3$ | 1093 | 2.329 | 2$C_{12}$ | 1554 | 1.264 |
| 4M3$C_7$ (2) | 1096 | 2.349 | 1$C_{12}$ | 1615 | 1.338 |
| 1$C_7$ | 1106 | 2.409 | 1,8$C_8$ | 1647 | 1.373 |
| 2M6$C_7$ | 1112 | 2.442 | 1$C_{13}$ | 1717 | 1.457 |
| 2,6M4$C_7$ | 1119 | 2.477 | 2$C_{14}$ | 1756 | 1.502 |
| 4$C_8$ | 1122 | 2.499 | 1$C_{14}$ | 1819 | 1.570 |
| 1,3$C_4$ | 1123 | 2.513 | 2$C_{15}$ | 1857 | 1.602 |
| 3$C_8$ | 1133 | 2.562 | 1$C_{15}$ | 1920 | 1.678 |
| 2E1$C_6$ | 1145 | 2.634 | | | |
| 2$C_8$ | 1150 | 2.661 | | | |
| cy$C_7$ | 1173 | 2.751 | | | |
| 1$C_8$ | 1208 | 2.987 | | | |

Table 3. Comparison of enantioselectivity factors α for *n*-alkyl nitrates of a given chain length for different positions of the $NO_2$ group.

| position | 3 | 2 | 4 |
|---|---|---|---|
| $C_6$ | 1.106 | 1.032 | – |
| $C_7$ | 1.083 | 1.019 | 1.012 |
| $C_8$ | 1.048 | 1.012 | 1.010 |
| $C_9$ | 1.029 | 1.009 | 1.007 |
| $C_{10}$ | [a] | 1.007 | 1.005 |
| $C_{11}$ | 1.013 | 1.004 | [a] |

[a] Not available.

series results mostly from the temperature-dependence of α. However, the results from different homologous series can be compared for the same chain length (Table 3). There is some indication of a collective retention mechanism for the chiral discrimination of alkyl nitrates at least within the homologous series. Without exception the enantioselectivity α of a 3-alkyl nitrate is higher than the corresponding values for 2- and 4-alkyl nitrates, and the order is always the same, $3C_n > 2C_n > 4C_n$. Therefore, properties common to the 3-nitrates must cause the higher enantioselectivity. All 3-nitrates have an ethyl group at the chiral centre (Fig. 4). It seems that

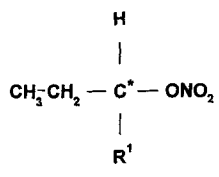


Fig. 4. 3-*n*-Alkyl nitrates.

the ethyl group contributes very strongly to the total enantiomeric discrimination. If the chain length $R^1$ is increased (homologous series of 3-nitrates) the enantioselective discrimination remains fairly constant. Regardless of whether the ethyl chain is shortened (2-nitrates) or lengthened (4-nitrates) chiral discrimination decreases.

The influence of the nitrooxy group on chiral separation is also significant. Alkyl nitrates in which the nitrooxy group is not directly attached to the asymmetric carbon atom (group 1 in Fig. 5) showed low enantioselective discrimination on LIPODEX-D. Branched alkyl nitrates with the nitrooxy group

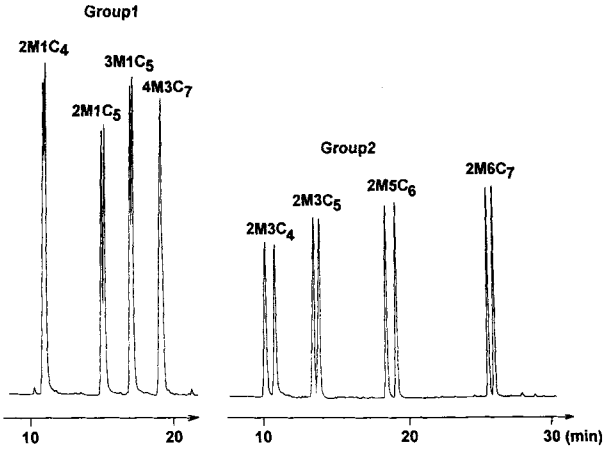


Fig. 5. Separation of chiral branched alkyl nitrates. In group 1 the nitrooxy group is not directly attached to the asymmetric carbon atom. Conditions: HRGC(LIPODEX-D)/ECD; temp. prog. 40 °C (2 min), rate 2 °min$^{-1}$, 190 °C.

directly fixed at the C* (group 2) showed better enantioselectivities, similar to those of the nonbranched alkyl nitrates, where the nitrooxy group is always fixed at C*. Direct comparison of alkyl nitrates with the same number of carbons invariably reveals a better separation for group 2 nitrates (2M3$C_4$ > 2M1$C_4$ and 2M3$C_5$ > 2M1$C_5$).

Since the asymmetric synthesis of 2$C_6$, 2$C_7$ and 2$C_8$ nitrates was possible (see Experimental Procedure), we were able to assign the absolute configuration of these alkyl nitrates (Fig. 6). The (*S*) enantiomer elutes prior to the (*R*) enantiomer. The absolute configurations of the other nitrates could not be determined, owing to the lack of enantiomerically pure reference alcohols, but we assume that the (*S*) enantiomer always elutes first for the homologous 2$C_n$ nitrates. There are several examples of stationary phases (including LIPODEX-D) where the elution order within a homologous series remains constant,[24–26] but there are also examples that show that the

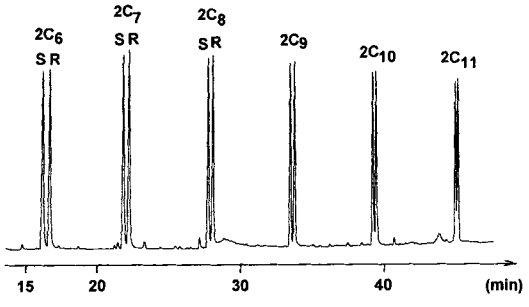


Fig. 6. Chiral separation of 2-alkyl nitrates. The (*S*) enantiomers elute before the (*R*) enantiomers. Conditions: HRGC(LIPODEX-D)/ECD; temp. prog. 40 °C (2 min), rate 2 °min$^{-1}$, 190 °C.

elution orders cannot be predicted definitively without having the pure references.[27]

Considering the results presented above, there are at least two factors responsible for chiral recognition of alkyl nitrates on LIPODEX-D: a polar component, the nitrooxy group directly fixed at C*, combined with nonpolar dispersion interactions of the alkyl chains, in particular with the ethyl group at C*.

In addition, it should be noted that at low temperatures (< 60 °C) separation behaviour on LIPODEX-D showed some curiosities. The homologous series of the 2-alkyl nitrates starts with 2$C_3$. All alkyl nitrates $C_n$ with $n < 6$ show a strong temperature dependence in chiral and achiral separation. Recent results in the area of thermodynamics of separation indicate that a cyclodextrin stationary phase can exhibit enthalpy–entropy compensation for some, but not all, compound types.[14]

**Separation of diastereomeric alkyl nitrates:** Diastereomers of aliphatic and alicyclic alkyl nitrates can be separated on methylpolysiloxane (DB1). Each monomethylcyclohexyl nitrate has *cis* (*c*) and *trans* (*t*) isomers. Hence, there are six diastereomers in total. In the *t*-1,2, *c*-1,3 and *t*-1,4 compounds the two substituents can either both be equatorial (e,e) or both be axial (a,a); the latter conformation is less stable. In the *c*-1,2, *t*-1,3 and *c*-1,4 compounds the substituents are a,e or e,a, and both conformations are present in dynamic equilibrium, but not separable by gas chromatography. The structures of *t*- and *c*-2M cy$C_6$ are given in Figure 7. The conformation of *t*-2M cy$C_6$ (e,e) is

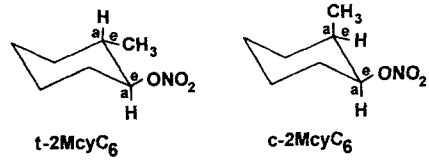


Fig. 7. Conformations of *trans*- and *cis*-2-methylcyclohexyl nitrate.

more stable than that of *c*-2M cy$C_6$. The reactivity of an equatorial substituent is known to be higher when it is involved in electrophilic substitutions.[28] This is the case here because, in our synthesis via the alcohol, the proton is replaced by a nitryl cation. We synthesised all three monomethylcyclohexyl nitrates individually and could separate each of them into their *cis* and *trans* diastereomers. It was even possible to separate all six diastereomers without coelution from a mixture of all three (Fig. 8). One peak is always more abundant, which obviously corresponds to the all-equatorial diastereomers. The *trans* diastereomers of 2M cy$C_6$ and 3M cy$C_6$ are also optically active. However, the separation of the enantiomers could not be achieved on LIPODEX-D.

Two available branched aliphatic alkyl nitrates, the 3M2$C_5$ and the 4M3$C_7$, have two asymmetric carbon atoms. Both alkyl nitrates could be separated into their dia-

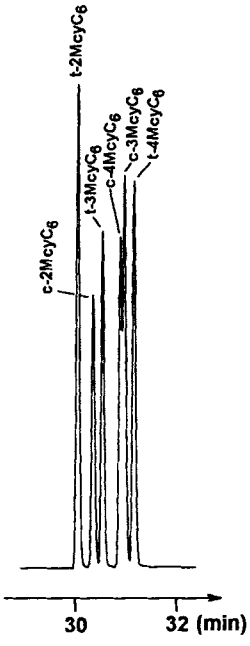


Fig. 8. Diastereomeric separation of all monomethylcyclohexyl nitrates. Conditions: HRGC (DB1)/ECD; temp. prog. 40 °C (3 min), rate 3 °min$^{-1}$, 250 °C.



0947-6539/96/0205-0542 $ 15.00+.25/0 Chem. Eur. J. 1996, 2, No. 5

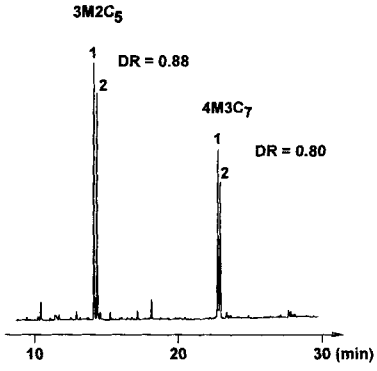


Fig. 9. Diastereomeric separation of $3M2C_5$ and $4M3C_7$. Conditions: HRGC(DB1)/ECD; temp. prog. 40 °C(3 min), rate 3 °min$^{-1}$, 250 °C.

stereomers. The diastereomeric ratios (DR = peak 2/peak 1) are 0.88 and 0.80, respectively (Fig. 9). There is no symmetry plane in both nitrates (i.e., no *meso* compound) and so each peak corresponds to a racemic mixture.

**Diastereomeric and chiral alkyl nitrates in air samples**: We were able to demonstrate the first application of stereoisomeric separation to the analysis of alkyl nitrates in environmental samples. The separated diastereomers of $3M2C_5$ eluted just before the "$3C_6$/$2C_6$" doublet (Fig. 2) on nonpolar and slightly polar stationary phases (DB1, CPSil8). Until now, two peaks in this characteristic four-peak pattern were always assigned to be hexyl nitrate isomers.[5, 7, 9, 23, 29] Actually, they are the two diastereomers of the $3M2C_5$ nitrate. The diastereomeric ratios in the air samples are the same as the DR value of 0.88 for the synthesised $3M2C_5$ (first peak more abundant than second). The synthesis used differs entirely from the atmospheric reaction pathway. Hence, it is likely that in both cases the same cause must be responsible for the fact that the diastereomeric ratio is not equal to 1. One explanation could be that the DR value represents the stability of the diastereomers and does not reflect the rates of formation.

For the environmental analysis of chiral alkyl nitrates a substantial difficulty has to be overcome. The complexity of mixtures present in air samples does not allow a direct chiral separation, because alkyl nitrates partly coelute on the LIPODEX-D column. Moreover, initial separation from other interfering molecules is necessary. We successfully preseparated alkyl nitrates from the bulk of compounds also present in air samples and detectable by the electron-capture detector, in order to improve the detection limits, especially for the long-chain alkyl nitrates.[11] The coelution of different alkyl nitrates up to chiral $C_{12}$ could be avoided by column coupling of LIPODEX-D with a polar achiral stationary phase like polyalkylenglycol (PAG). Figure 10 depicts the first chiral separation of $C_9$–$C_{12}$ alkyl nitrates present in a sample of rural air (from the vicinity of Ulm, Germany). The ratio of the separated enantiomers of alkyl nitrates show that they are present as a racemic mixture. This might have been expected, but has never been proven before. Further samples of alkyl nitrates exposed to higher bioactivity (e.g., from the microlayer of oceans or lakes) will be analysed in this way. It should be pointed out that the resulting chromatogram was only possible by preseparation and chiral separation, combined with column coupling to an achiral stationary phase.

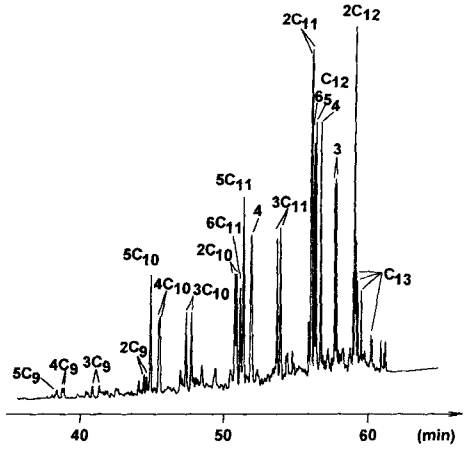


Fig. 10. Chiral separation of $C_9$–$C_{12}$ alkyl nitrates present in a sample of rural air (from the vicinity of Ulm). Conditions: CHRGC(LIPODEX-D + PAG)/ECD; temp. prog. 40 °C (2 min), rate A 2 °min$^{-1}$, 140 °C (1 min), rate B 10 °C min$^{-1}$, 220 °C.

## Conclusions

The lack of a consolidated nomenclature for abundant alkyl nitrates was not a critical issue until now, because the number of alkyl nitrates identified in environmental analysis was limited. The recent rapid increase in the number of alkyl nitrates detected in environmental samples, up to $C_{15}$ and possibly higher and including the branched compounds, makes a general and systematic labelling necessary. The suggested system provides a simple procedure for labelling complex mixtures (Fig. 2) and allows the hydrocarbon precursor to be identified directly.

In electron-capture detection the homologous series of 1-alkyl nitrates can be used directly as retention-index markers for the estimation of the Kovats Index in gas chromatography. A similar approach has been used in liquid chromatography based on the UV absorption of the alkyl nitrates.[30]

We have been able to separate diastereomers of alkyl nitrates, including aliphatic and alicyclic components, and in one case this method was applied to environmental samples.

We were able to show that a systematic approach to the study of structure-related chiral recognition in cyclodextrin stationary phases is possible. The chiral separation of alkyl nitrates has great potential for future work in atmospheric chemistry. It adds a large number of specific anthropogenic and geogenic markers to the well-established α-hexachlorocyclohexane enantiomers.[12, 13, 31] Hydrocarbon precursors can be indentified as being of biogenic or anthropogenic origin, and the biotic degradation of alkyl nitrates can be investigated. Further potential applications include the pharmaceutical investigation of chiral alkyl nitrates.

The complexity of the alkyl nitrate mixtures present in air samples does not allow a direct chiral separation, since alkyl nitrates partly coelute on the LIPODEX-D column. Coupling of columns with LIPODEX-D and a polar achiral stationary phase like polyalkylenglycol (PAG) has been successfully applied to solve this problem. Two-dimensional gas chromatography would be a more demanding alternative in terms of instrumentation.

## Experimental Procedure

**Gas chromatography:** A Hewlett Packard (Palo Alto, USA) gas chromatograph 5890 series II with an $^{63}$Ni electron capture detector (ECD) was used for analysis. Argon/methane (9:1) was used as the ECD carrier gas (35 mL min$^{-1}$) at a detector temperature of 240 °C for the LIPODEX-D and 260 °C for the DB1 and the LIPODEX-D/PAG-coupled capillary column. The lower temperature for the LIPODEX-D stationary phase was used to avoid phase destruction in the detector. Data acquisition was carried out with a Shimadzu C-R4A integrator (Kyoto, Japan). Hydrogen (purified 5.0) from Linde (Munich, Germany) was used as carrier gas.

**Capillary columns and conditions:** For stereoselective high-resolution gas chromatography three capillary columns were applied with on-column injection:
A) LIPODEX-D capillary (Macherey Nagel, Düren, Germany); stationary phase: heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin; 2 m retention gap + 25 m,

i.d.: 0.25 mm, $d_f$: not given by supplier; temp. program: 40 °C (2 min), rate 2 °C min$^{-1}$, 190 °C; hydrogen: 48 cm s$^{-1}$ (150 °C); 70 kPa.
B) DB1 capillary (J & W Scientific, Rancho Cordova, USA); stationary phase: 100% dimethylpolysilaxane; 2 m retention gap + 60 m, i.d.: 0.25 mm, $d_f$: 0.25 µm; temp. program: 40 °C (3 min), rate 3 °C min$^{-1}$, 250 °C; hydrogen: 38 cm s$^{-1}$ (150 °C), 95 kPa.
C) LIPODEX-D/PAG-coupled capillary (PAG: Supelco, Bellefonte, USA); LIPODEX-D was the same as capillary A; stationary phase: LIPODEX-D/polyalkylenglycol (PAG); 2 m retention gap + 25 m LIPODEX-D + 30 m PAG, i.d.: 0.25 mm, $d_f$: 0.25 µm); temp. program: 40 °C (2 min), rate A: 2 °C min$^{-1}$, 140 °C (1 min), rate B: 10 °C min$^{-1}$, 220 °C; hydrogen: 40 cm s$^{-1}$ (150 °C), 100 kPa.

**Preparation of racemic and optically pure alkyl nitrates:** Standard mixtures of alkyl nitrates of any desired composition can readily be derived by using a recently described method based on microsynthetic esterification of the alcohol precursors [29] with a mixture of $HNO_3$ and $H_2SO_4$. The alcohols were obtained from Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), Janssen Chimica (Geel, Belgium), Merck (Darmstadt, Germany), Merck-Schuchardt (Hohenbrunn, Germany) and Riedl de Haen (Seelze, Germany). The reaction mechanism is known to be an electrophilic substitution of the proton for the nitryl cation $NO_2^+$ [32,33]. The advantage of this reaction is that the oxygen atom remains attached to the alcohol, and the configuration of the asymmetric carbon atom is thus preserved. Thus, when the absolute configuration of an optically pure alcohol precursor is known, the absolute configuration of the resulting alkyl nitrate can be inferred. The configuration retains the same label, because the nitrooxy group has the same priority as the hydroxyl group in relation to alkyl moieties. This correlation was recently described as "corresponding enantiomers" [17]. Hence, asymmetric synthesis of alkyl nitrates is possible starting from the optically pure alcohols [34,35]. Optically pure (*S*)-(+)-2-hexanol and (*S*)-(+)-2-heptanol were obtained from Aldrich (Steinheim, Germany), and (*S*)-(+)-2-octanol from Merck-Schuchardt (Hohenbrunn, Germany).

**Acknowledgements**: The support of the Deutsche Forschungsgemeinschaft (Ba 371/14-2) is gratefully acknowledged.

Received: June 16, 1995 [F152]

[1] P. Q. E. Clothier, B. D. Aguda, A. Moise, H. O. Pritchard, *Chem. Soc. Rev.* **1993**, *22*, 101–108.
[2] R. Meyer, *Explosivstoffe* **1973**, VCH, Weinheim.
[3] J. M. Roberts, *Atmos. Environ.* **1990**, *24A*, 243–287.
[4] R. Atkinson, S. M. Aschmann, W. P. L. Carter, A. M. Winer, J. N. Pitts, *J. Phys. Chem.* **1982**, *86*, 4563–4569.
[5] O. Luxenhofer, K. Ballschmiter, *Fresenius J. Anal. Chem.* **1994**, *350*, 395–402.
[6] F. Flocke, A. Volz-Thomas, D. Kley, *Atmos. Environ.* **1991**, *25A*, 1951–1960.
[7] O. Luxenhofer, *Dr. rer. nat. Dissertation* **1995**, University of Ulm, Germany.
[8] A. C. de Kock, C. R. Anderson, *Chemosphere* **1994**, *29*, 299–310.
[9] E. Atlas, *Nature* **1988**, *331*, 426–428.
[10] C. S. Atherton, *Geophys. Res. Letters* **1989**, *16*, 1289–1292.
[11] R. Rieger, M. Schneider, K. Ballschmiter, *Meteor Berichte 95–1* **1995**, Universität Hamburg, 118–130.
[12] J. Faller, H. Hühnerfuss, W. A. König, R. Krebber, P. Ludwig, *Environ. Sci. Technol.* **1991**, *25*, 676–678.
[13] S. Mössner, T. R. Sparker, P. R. Becker, K. Ballschmiter, *Chemosphere* **1992**, *24*, 1171–1180.
[14] A. Berthod, W. Li, D. W. Armstrong, *Anal. Chem.* **1992**, *64*, 873–879.
[15] V. Schurig, *J. Chromatogr.* **1994**, *666*, 111–129.
[16] W. A. König, *Nachr. Chem. Tech. Lab.* **1989**, *37*, 471–476 .
[17] M. Schneider, K. Ballschmiter, *Fresenius J. Anal. Chem.* **1995**, *352*, 756–762.
[18] J. Kames, U. Schurath, *J. Atmos. Chem.* **1992**, *15*, 79–92.
[19] Y. Izumi, A. Tai, *Stereodifferentiating Reactions* **1977**, Kodansha Scientific Books, Tokio,.
[20] H. Kamp, *Einführung in die Stereochemie* **1981**, VCH, Weinheim.
[21] a) E. Kovats, *Helv. Chim. Acta* **1958**, *41*, 1915–1932; b) H. Van den Dool, P. D. Kratz, *J. Chromatogr.* **1963**, *11*, 463–471.
[22] R. Atkinson, S. M. Aschmann, W. P. L. Carter, A. M. Winer, J. N. Pitts, *J. Phys. Chem.* **1982**, *86*, 4563–4569.
[23] E. Atlas, S. Schauffler, *Environ. Sci. Technol.* **1991**, *25*, 61–67.
[24] K. Grob, H. Neukom, H. Schmarr, A. Mosandl, *HRGC & CC* **1990**, *13*, 433–436.
[25] W. A. König, S. Lutz, C. Colberg, N. Schmidt, G. Wenz, E. von der Bey, A. Mosandl, C. Günther, A. Kustermann, *HRGC & CC* **1988**, *11*, 621–625.
[26] W. A. König, S. Lutz, G. Wenz, E. von der Bey, *HRGC & CC* **1988**, *11*, 506–509.
[27] A. Mosandl, K. Rettinger, K. Fischer, V. Schubert, J. Schmarr, B. Maas, *HRGC & CC* **1990**, *13*, 382–385.
[28] H. Bayer, *Lehrbuch der organischen Chemie* **1973**, 17th ed., Hirzel, Stuttgart.
[29] O. Luxenhofer, E. Schneider, K. Ballschmiter, *Fresenius J. Anal. Chem.* **1994**, *350*, 384–394.
[30] M. Wachsmann, *Diploma Thesis* **1991**, University of Ulm, Germany.
[31] R. L. Falconer, T. F. Bidleman, D. J. Gregor, R. Semkin, C. Teixeira, *Environ. Sci. Technol.* **1995**, *29*, 1297–1302.
[32] E. L. Blackall, E. D. Hughes, *Nature* **1952**, *170*, 972–973.
[33] E. L. Blackall, E. D. Hughes, C. Ingold, R. B. Pearson, *J. Chem. Soc.* **1958**, 4366–4374.
[34] G. A. Olah, R. Malhotra, S. C. Narang, *Nitration: Methods and Mechanisms* **1989**, VCH, New York.
[35] R. L. Shriner, E. A. Parker, *J. Am. Chem. Soc.* **1933**, *55*, 766–771.