Journal of Chromatography, 457 (1988) 366–371
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 957

Note

Retention behaviour of diastereomeric truxillic and truxinic diamides and separation of an enantiomeric pair in high-performance liquid chromatography

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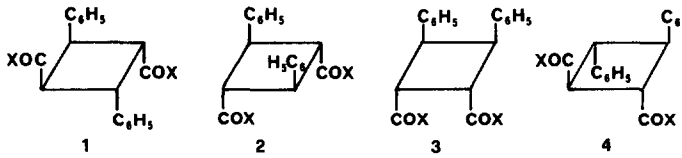
(First received March 21st, 1988; revised manuscript received August 18th, 1988)

The retention behaviour of a solute reflects all interactions that occur between the solute and the solvent and between the solute and the bonded phase in reversed-phase liquid chromatography (RPLC). These factors include the polarity of the solutes, the polarity and the dielectric constant of the mobile phase, the length of the hydrocarbonaceous bonded chain in the stationary phase and the stereochemistry and size of the solute molecules.

We are interested in the contribution to the retention caused by the interaction of the area of the non-polar segment of the diastereomeric α- and ε-truxillic (1 and 2, respectively) and β- and δ-truxinic (3 and 4, respectively) derivatives, where X = $N(CH_3)_2$, with the alkyl chains of the bonded phase (C_{18} and C_8).



Also we describe the use of a chiral stationary phase (CSP Pirkle 1A) for the separation of these diastereomers. The enantiomers of δ-truxinic N,N-dimethylamide (4) were separated using a more efficient and recently reported chiral stationary phase.

The superiority of octadecyl-bonded RPLC over the use of the normal silica gel phase for separating diastereomers is also shown.

EXPERIMENTAL

Chemicals and solvents

N,N-Dimethylamides 1–4 were prepared by interfacial condensation of the corresponding acid chloride dissolved in dry diethyl ether with dimethylamine hydrochloride dissolved in water in the presence of dilute sodium hydroxide with stirring. Preparative details for the diamides[1] and for the corresponding acid precursors obtained by photodimerization of cinnamic acid[2] have been reported previously. We should point out that the reported[1] m.p. of compound 2 was erroneous,

0021-9673/88/$03.50

being actually the m.p. of the corresponding acid chloride. The m.p. of compound 2 is in fact 187–188°C. High-performance liquid chromatographic (HPLC)-grade water, acetonitrile, 2-propanol and hexane were obtained from Fluka (Buchs, Switzerland).

HPLC apparatus and conditions

The HPLC apparatus consisted of the following components: a Varian Model 5060 liquid chromatograph with a 10-μl Valco sample loop, a Jasco Model Uvidec-100 III UV spectrophotometric detector and a Varian CDS 401 Data System. The chromatographic columns were MOS-Hypersil C_8 and C_{18} Hypersil silica (all 5 μm) (150 mm × 3.9 mm I.D.) from Shandon (Runcorn, U.K.) and LiChrosorb RP-18 (10 μm) (250 mm × 4 mm I.D.) from Merck (Darmstadt, F.R.G.), a Pirkle Type 1A chiral column, packed with (*R*)-N-3,5-dinitrobenzoylphenylglycine ionically bonded to γ-aminopropylsilanized silica from Regis Chemical (Morton Grove, IL, U.S.A.) and a Pirkle chiral column packed with *cis*-3-(1,1-dimethylethyl)-4-phenyl-2-azetidinone bonded silica gel[3].

Capacity factors (k') were calculated according to the equation $k' = (t - t_0)/t_0$, where t_0 is the retention time of an unretained solute. The void volume of the column was determined by injecting water (for the reversed phase) or hexane (for normal and chiral phases) when pumping a mixed mobile phase. Retention times were mean values of two replicate determinations.

RESULTS AND DISCUSSION

Reversed-phase HPLC

The solvophobic theory assumes that the solute interactions with bonded phases are weak, whereas the contribution of the solute–solvent interaction is fairly large. An increase in the concentration of the organic modifier in the eluent leads to a decrease in retention. According to this model, the variation of the logarithm of the capacity factors of the four diamides with the water–acetonitrile mixture is as shown in Fig. 1, the slope being very sensitive to minor changes in the composition of the mobile phase.

The retention mechanism of RPLC is very complex. However, considering that in compounds 1–4 the large molecular size and the functional groups are identical, the intermolecular solute–solvent interaction can be related mainly to the single parameter of the polarity of the solutes. The polarity sequence of these compounds is $\alpha \ll \delta \ll \varepsilon < \beta$, as from their measured solution dipole moments[4] (α = 1.90 D, δ = 3.04 D, ε = 5.95 D and β = 6.22 D). Hence in the reversed-phase mode we expect the elution order to be $\beta < \varepsilon \ll \delta < \alpha$. However, as shown in Fig. 1, this is not so.

Remarkably, minor stereochemical effects due to the relative configuration of the ring substituents strongly affect the retention behaviour. The layered alkyl "skin" of the bonded phase interacts with the non-polar region of the solutes and according to this partition mechanism[5] the bonded phase interacts better with large solute molecules, such as compounds 1–4, if its solvophobic surface area relative to the alkyl chain length is large enough to overlap with the hydrocarbonaceous segment area of the molecules.

These interactions are depicted in Fig. 2, constructed using Dreiding models for compounds 1–4. The plane shows the surface of the alkyl-bonded phase as normal to

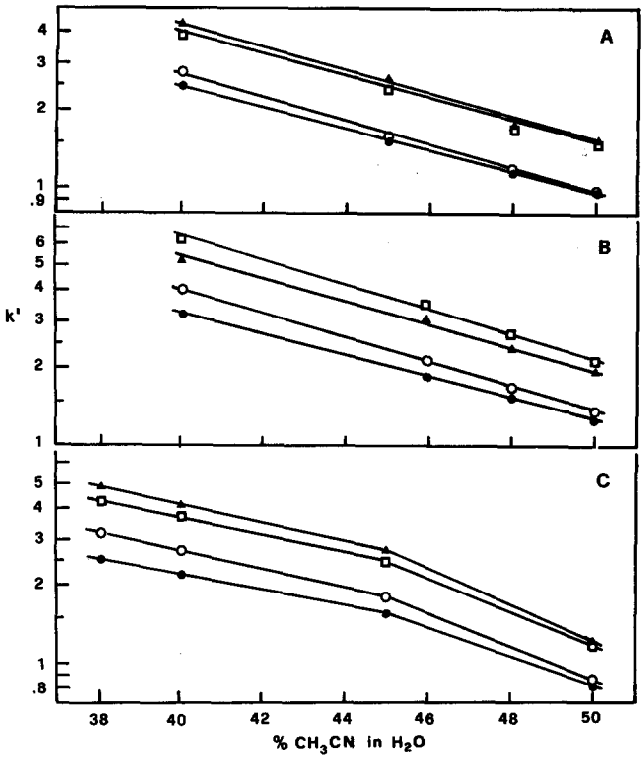


Fig. 1. Effect of acetonitrile concentration in water on the capacity factors (k') of (●) compound 1; (▲) compound 2; (○) compound 3; (□) compound 4. (A) On Shandon C_{18} phase; (B) on Merck C_{18} phase; (C) on C_8 phase.

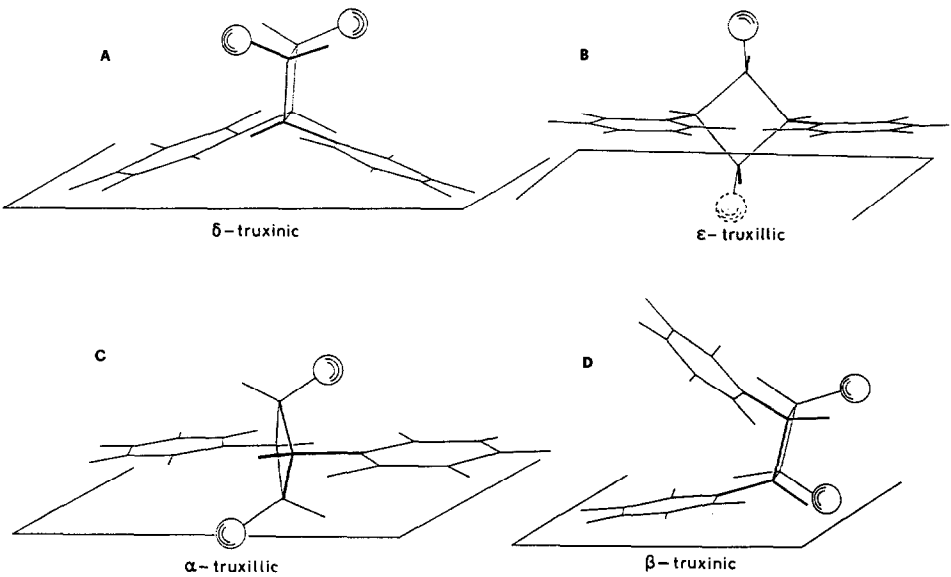


Fig. 2. Interaction of compounds 1–4 with the bonded alkyl chain of the reversed-phase: (A) compound 4; (B) compound 2; (C) compound 1; (D) compound 3. Spheres illustrate the bulky amide groups.

the cyclobutane ring and the spheres illustrate the bulky amide groups. In particular, the hydrocarbonaceous surface in Fig. 2B intersects the ring (which lies on the page) and both amide groups are disected behind the page.

In these compounds the preferred conformations of the phenyl rings and of the amide groups shown, arising from the rotation around the single bond connecting the cyclobutane carbon to the aromatic or carbonyl carbon, respectively, were previously found by contour energy maps[2] and can be easily obtained by a careful examination of their Dreiding models. Moreover, the interatomic distances between the *para* position of the phenyl groups in the four diastereomers are very different and only in the ε-truxillic (2) and δ-truxinic (4) configurations can the phenyl rings remain, in the preferred conformation, almost coplanar *without* hindrance resulting from the bulky polar amide groups both pointing in opposite directions from the phenyl rings plane. This non-polar region of the molecules can therefore perfectly overlap with the alkyl surface of the bonded phase, thus explaining their higher chromatographic retention. In contrast, in the α-truxillic configuration 1 a polar amide group is intercalated in the plane containing the phenyl rings, thus strongly disturbing the overlap of the non-polar region of the solute with the hydrocarbonaceous sheath of the stationary phase. In the β-truxinic configuration 3, the dihedral angle between the phenyl rings in the preferred conformation is very small (about 60°) and therefore again large area interactions of the non-polar region of the molecule with the bonded phase are not possible. Hence the retentions of the α and β compounds are lower than those of the ε and δ compounds. This behaviour was not expected from the strong polarity difference between α and β compounds. A typical separation of the four diastereomers is shown in Fig. 3.

The marked difference between the retention times of the α and β and those of the ε and δ compounds is therefore related to the interaction mechanism depicted above and it is confirmed when using C_{18} phases from different manufacturers, as shown in Fig. 1A and B. Fig. 1A and B also show that the resolution between the ε and δ isomers

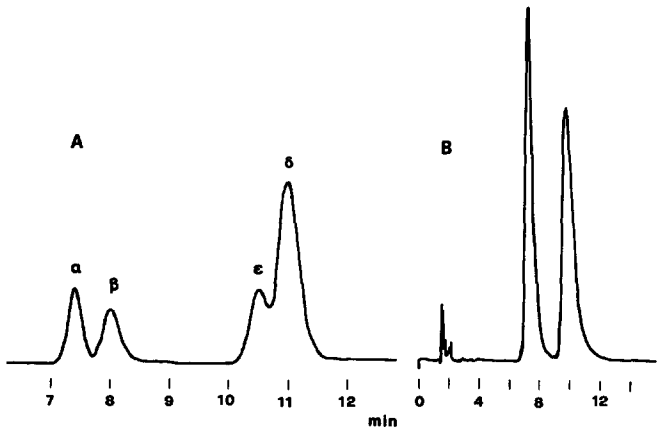


Fig. 3. Typical chromatograms showing (A) separation of the four diastereomers on a C_{18} reversed-phase column, mobile phase acetonitrile–water (46:54), flow-rate 1 ml/min, detection at 225 nm; (B) separation of the enantiomers of compound 4 on a CSP 2 column, mobile phase 2-propanol–hexane (10:90), flow-rate 2 ml/min, detection at 254 nm.

and between the α and β isomers is poor on the Shandon C_{18} column whereas it is much better on the Merck C_{18} column. This result is probably due to a different carbon content bonded to the silica or to a different history. In fact, the k' values of all compounds are larger on the Merck than on the Shandon phase, evidently owing to a larger solute–stationary phase interaction, which leads to a higher selectivity in the isomeric pair.

Remarkably, a change in the retention order of the ε/δ pair occurs between the C_{18} columns from the two manufacturers.

Shortening of the alkyl chain length of the bonded reversed-phase leads, as expected, to a decreased retention of a given solute, as shown by comparing the k' values obtained with C_{18} phases with those obtained with C_8 phase (Fig. 1). In addition, the decrease in log k' in Fig. 1C with a 50% acetonitrile mobile phase, resulting in incomplete separation of the compounds, is related to the weaker interaction of these large solute molecules with the shorter alkyl C_8 chains compared with the stronger solute–solvent interaction. Similar deviations from the theoretical linear relationship of log k' *vs.* phase composition have been reported for compounds exhibiting hydrogen bonding with the aqueous phase.

Chiral stationary phase

As δ-truxinic diamide (4) possesses only a simple two-fold axis of symmetry, the racemic compound synthesized by us is in principle resolvable into an enantiomeric pair.

In view of the considerable success demonstrated by the Pirkle amino acid-derived stationary phase CSP 1A in the enantiomeric resolution of compounds of forty structural types[6,7], we tried this phase. However, no enantiomeric resolution of the δ-truxinic compound 4 was obtained. Using 2–20% of 2-propanol in hexane as the mobile phase at a flow-rate of 1 ml/min and detection at 254 nm, only a single peak appeared. The configuration of the four large substituent groups inhibits the separability of enantiomers, presumably owing to steric congestion in a region important to chiral recognition. The CSP 1A was instead extremely selective in the separation of diastereomeric α-truxillic and δ-truxinic diamides. The k' values were 2.86 and 2.28, respectively, in 2-propanol–hexane (10:90). Remarkably, the ε and β isomers (both possessing the strongly polar amide groups *cis* oriented and not disturbed by the phenyl groups extending in the opposite direction) were retained in the column. In this instance, the retention behaviour is presumably controlled by dipole interactions of the amide groups with the acyl group of the CSP.

A new Pirkle chiral stationary phase (CSP 2) derived from *cis*-3-(1,1-dimethylethyl)-4-phenyl-2-azetidinone[3] was effective for the separation of the δ-isomer into its enantiomers. Fig. 3 illustrates the facility with which the enantiomers of compound 4 were resolved on this new phase, with a separation factor $\alpha = 1.30$ and $k' = 3.44$.

The improved chiral recognition of CSP 2 with respect to that of CSP 1A may be due to the presence in the former of an additional stereogenic centre and to the better ability of the enantiomers to "conform" to the shape of CSP 2[3].

Normal-phase HPLC

With the exception of inverted k' values for the δ and ε isomers, the

chromatographic behaviour of compounds 1–4 is related to their dipole moments, as expected. At a flow-rate of 1.5 ml/min and using 2-propanol–hexane (10:90) as the mobile phase, the capacity factors k' were 14.5 for the δ, 9.6 for the ε and 1.35 for the α isomers. The extremely polar β isomer (μ = 6.22 D) is irreversibly retained in the column. This behaviour, in addition to the broadening of the peaks for the δ and ε isomers, is due to the strong interaction of the *cis* amide dipoles with the silanol groups of the adsorbent. Hence the general performance of this phase for the separation of this class of diastereomers is not as high as that of the reversed-phase.

ACKNOWLEDGEMENTS

We thank Mr. J. E. McCune and Mr. J. Burke of the University of Illinois at Urbana-Champaign for the use of the CSP 2.

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