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Quinine versus carbamoylated quinine-based chiral anion exchangers A comparison regarding enantioselectivity for N-protected amino acids and other chiral acids

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Abstract

First, a chiral stationary phase was prepared by immobilization of the naturally occurring alkaloid quinine onto a 3-mercaptopropyl-modified silica gel via a radical addition reaction and it was evaluated for direct HPLC enantioseparation of acidic chiral compounds under buffered hydro-organic mobile phase conditions. Second, its enantioselectivity and retention characteristics for a representative set of N-derivatized α-amino acids and other chiral acids were compared with those of a similar weak chiral anion exchanger based on *tert*.-butyl carbamoylated quinine derivative as chiral selector. The results clearly indicate that the introduction of the carbamoyl functionality at the secondary hydroxyl group at C_9 of quinine provides new and additional sites for intermolecular interactions with chiral analytes and this can profoundly change and improve chiral recognition ability, especially for amide, carbamate and sulfonamide derivatives of amino acids including DNB, Bz, Ac, For, Z, Fmoc, Boc and DNS-protected amino acids. The impact of this new and rigid hydrogen donor–acceptor group – directing stereoselective selector–selectand complexation by intermolecular hydrogen bonding – in comparison to the plain quinine selector is further evaluated by alkylation of the nitrogen atom of either the selector carbamate and/or of the amino function of leucine (*N*-methyl leucine) derivatized either by DNB or DNP groups.

Keywords: Chiral stationary phases, LC; Anion exchangers; Enantiomer separation; Chiral selectors; Amino acids, DNB derivatives; Amino acids, DNP derivatives; Quinine

1. Introduction

Chiral stationary phases (CSPs) with quinine (QN) carbamate derivatives (see Fig. 1, depicting *tert*.-butyl carbamoylated quinine as chiral template and named CSP II in this contribution) have proved to successfully facilitate the direct high-performance liquid chromatographic enantioseparation of chiral acids (selectands, SAs). Advantageously, these CSPs are operated with buffered hydro-organic mobile phases in the anion-exchange mode where the tertiary amine moiety in the quinuclidine ring is

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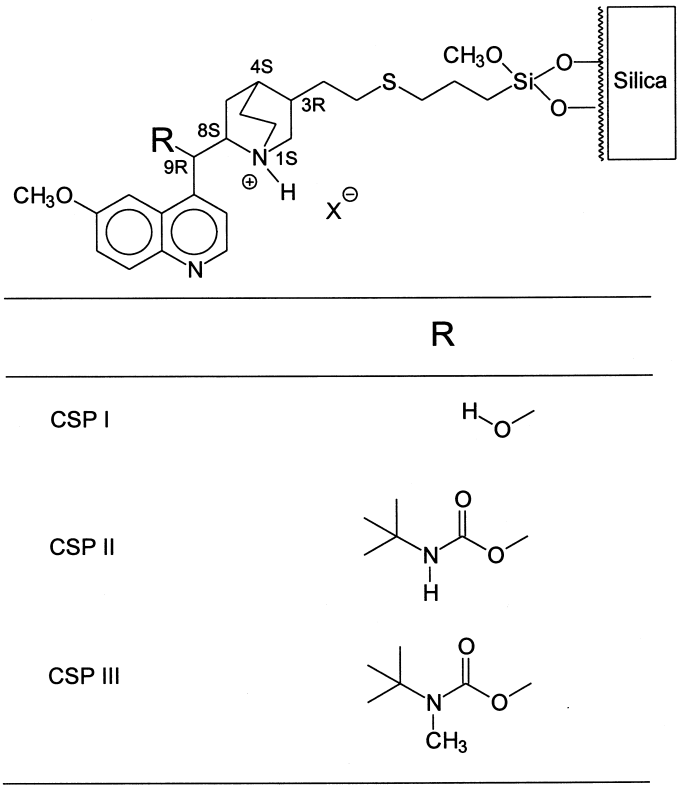


Fig. 1. Structures of chiral stationary phases: CSP I with quinine as selector, CSP II with *tert*.-butyl carbamoylated quinine as selector, CSP III with *N*-methyl-*tert*.-butyl carbamoylated quinine as selector.

positively charged. As shown in earlier publications, these CSPs exhibit high enantioselectivity for the resolution of a broad range of chiral acidic SAs, such as, e.g., N-derivatized amino acids, α-aryloxyalkyl carboxylic acids and α-arylalkyl carboxylic acids like Profens [1–8]. Generally, various CSPs derived from the carbamoylated quinine lead structure have been prepared. It appeared that CSPs based on chiral selectors (SOs) with a bulky substituent at the carbamate function yielded higher enantioselectivity values, in particular for N-derivatized amino acids, while other quinine-derived CSPs, e.g., those with aromatic carbamate substituents, show better enantioseparation capability for other SAs, such as profens and α-arylalkyl carboxylic acids, and have a broader spectrum of applicability.

For an anion-exchange type separation mode the acidic functional group of the SA molecules (carboxylic, sulfonic, phosphonic group, etc.) need to be ionized (negatively charged) to be able to interact with the positively charged quinuclidine moiety of the SO by intermolecular ionic interaction. Accordingly, these CSPs can be classified as weak chiral anion exchangers. These intermolecular electrostatic interactions are accompanied by additional attractive and/or repulsive forces, such as hydrogen bonding, π–π interactions, dipole–dipole, van der Waals and steric interactions, resulting in enantioseparation of different magnitude for racemic anionic SAs.

Exceptionally high enantioselectivity was found for the resolution of *N*-3,5-dinitrobenzoyl (DNB) α-amino acid derivatives. This is a result of cooperatively acting ionic forces, attractive π–π interaction between the electron poor π-acidic DNB group of the SA and the electron rich π-basic quinoline ring of the SO, and hydrogen bonding between the amide group of the DNB amino acid and the carbamate function of the SO (see Fig. 2A) [2].

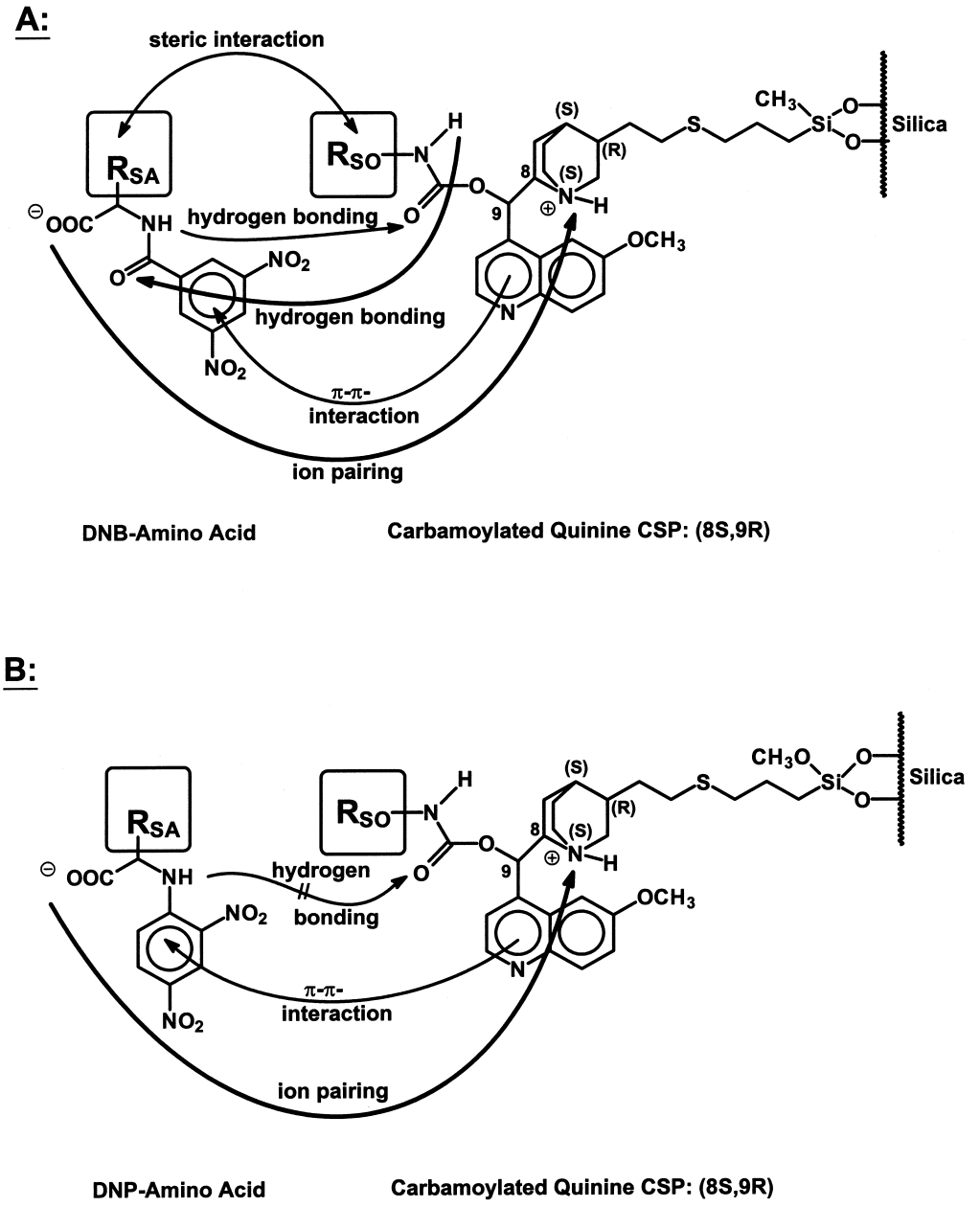


Fig. 2. Tentative chiral host–guest complexation model. Intermolecular interactions of (A) DNB amino acids and (B) DNP amino acids with the carbamoylated quinine selector (R_{SO}: carbamate substituent, e.g., *tert.*-butyl, R_{SA}: amino acid side chain), according to Ref. [2].

These additional and directing intermolecular hydrogen bonds turned out to be crucial and influenced the overall enantioselectivity (α values) significantly. Thus the structurally similar *N*-2,4-dinitrophenyl (DNP) amino acid derivatives lacking the amide group, i.e., the hydrogen donor–acceptor system, were resolved with distinctively lower α values and in reversed elution order (see Fig. 2B). Further evidence for the tentative binding models depicted in Fig. 2 was gained by Fourier transform infrared

spectroscopy (FT-IR), nuclear magnetic resonance (NMR) and X-ray studies. The X-ray structure [8] underlines the importance of a hydrogen bonding effect of the SA's amide function on the postulated chiral SO–SA recognition mechanism. However, the aim of this work is to evaluate the contribution of the amide group on the enantiorecognition capability of the CSPs for specific analytes by methylating either on the SA side (3,5-DNB-*N*-methyl leucine) or on the SO side (*N*-methyl-*tert*.-butyl-CQN). For chiral SA molecules which are enantioseparated according to a different binding model (e.g., DNP amino acids) these structure modifications (N-methylation of amino acid nitrogen and SO's carbamate, respectively) may have little or no effect.

Earlier, Salvadori and co-workers [9–11] had introduced QN as a chiral SO (CSP I, see Fig. 1). Leaving the hydroxyl group on position C_9 underivatized, they operated this CSP under normal-phase conditions. Thus, following the Pirkle concept, a different retention mechanism may take place. In this mode, various chiral arylalkylcarbinols and binaphthyl derivatives could be resolved reasonably well. The potential of the QN-CSP originally synthesized by Salvadori and co-workers and lacking the carbamate function at position C_9 had not yet been tested in the anion-exchange mode. Therefore CSP I was evaluated under buffered hydro-organic mobile phase conditions, in order to draw conclusions on the important role of the SO's carbamate function in the chiral recognition mechanism. As chiral solutes various N-protected α-amino acids, including DNB and DNP derivatives, as well as other chiral acids were selected.

2. Experimental

2.1. Materials

QN for pharmaceutical purpose was obtained from Apoka (Vienna, Austria). Porous silica material Kromasil 100 Å, 5 μm (~340 $m^2\ g^{-1}$) from EKA-Nobel (Bohus, Sweden) was used for the preparation of 3-mercaptopropyl-modified silica gel that was synthesized according to the literature [12]. A calculated coverage of ca. 0.95 mmol thiol groups per gram modified silica resulted by this functionalization step. α,α′-Azo-bis-isobutyronitrile (AIBN), 3-mercaptopropyl-trimethoxy-silane, and 1-hexene were purchased from Aldrich. Analytical grade chloroform was used as solvent for the syntheses of the modified silica materials.

Mobile phases for chromatography were prepared from analytical-reagent grade ammonium acetate (Loba Feinchemie, Fischamend, Austria), HPLC-grade water (purified by a Milli-Q-Plus filtration unit from Millipore, Bedford, MA, USA) and methanol of HPLC-grade (Baker). Chiral test compounds and amino acids were provided by different sources, mainly Aldrich, Sigma and Bachem (Bubendorf, Switzerland), derivatives thereof were prepared in the laboratory according to standard derivatisation procedures [13–15].

2.2. Synthesis of CSPs

2.2.1. Synthesis of tert.-butyl carbamoylated quinine

Quinine base (9 mmol) was dissolved in toluene, and three drops of dibutyl tin dilaurate and *tert*.-butyl isocyanate (9.9 mmol) were added. The reaction mixture was refluxed for 4 h. The solvent was evaporated and the crude product stirred with *n*-hexane. Re-crystallization from cyclohexane gave pure white product in 70% yield (m.p.: 122°C).

$[\alpha]_{589} = -10.9$; $[\alpha]_{546} = -15.8$; ($c = 1.01$; methanol).

IR (KBr): 1718, 1622, 1593, 1532, 1508, 1267, 1035 cm^{-1}.

^{1}H-NMR (200 MHz, CD_3OD): δ 1.2–1.4 (s, 9H); 1.5–1.7 (m, 2H); 1.7–2.0 (m, 3H); 2.25–2.45 (m, 1H); 2.55–2.8 (m, 2H); 3.0–3.2 (m, 1H); 3.2–3.4 (m, 3H) 4.01 (s, 3H); 4.9–5.1 (m, 2H); 5.7–5.9 (m, 1H); 6.5 (d, 1H); 7.4–7.5 (m, 1H); 7.5–7.65 (m, 2H); 7.9–8.0 (d, 1H); 8.7 (d, 1H) ppm.

2.2.2. Synthesis of N-methyl tert.-butyl carbamoylated quinine

Quinine base (9 mmol) was dissolved in toluene. An equimolar amount of 4-nitrophenyl chloroformate was added to the solution at ambient temperature. After a few minutes the active intermediate, *O*-(4-nitrophenyloxycarbonyl) quinine hydrochloride, precipitated as a pure white compound. It was filtered off, thoroughly washed with toluene and immedi-

ately used for the subsequent reaction step. Thus, the active intermediate was dissolved in chloroform and a two-fold molar excess of triethylamine as well as a 1.5 molar excess of *N*-methyl *tert*.-butylamine were added. The reaction mixture was stirred overnight at ambient temperature. After evaporation of the solvent the crude product was purified by flash chromatography (acetonitrile). The product was obtained in 31% yield (m.p.: 90–91°C).

$[\alpha]_{589} = -17.3$; $[\alpha]_{546} = -23.1$; ($c = 0.865$; chloroform).

IR (KBr): 1742, 1623, 1596, 1509, 1264 cm^{-1}.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.2 (m, 3H); 1.5–1.8 (m, 9H); 2.2 (s, 2H); 2.6 (m, 3H); 3.0 (m, 3H); 3.3 (m, 1H); 3.9 (s, 3H), 4.1 (m, 2H); 5.0 (t, 2H); 5.8 (m, 1H); 6.3 (d, 1H); 7.4 (m, 3H); 8.0 (d, 1H); 8.7 (d, 1H) ppm.

2.2.3. Immobilization of the chiral selectors

3.0 g of 3-mercaptopropyl-modified silica (4.58% C, 1.12% H corresponding to 0.95 mmol SH per gram modified silica) was suspended in 100 ml chloroform, 2.0 g of the corresponding chiral selector (QN, *tert*.-butyl carbamoylated QN, or *N*-methyl *tert*.-butyl carbamoylated QN) and 200 mg of AIBN as radical initiator were added to the suspension which was refluxed for 12 h under a nitrogen atmosphere. After sedimentation and removal of the solvent, the modified silica was washed with chloroform, methanol and petroleum benzene. The dried modified silica were subjected to elemental analysis (CHN).

CHN analysis: CSP I: 11.75% C, 1.73% H and 1.04% N, corresponding to a calculated selector density of: 299 μmol g^{-1} CSP (based on carbon) or 371 μmol g^{-1} CSP (based on nitrogen).

CSP II: 12.7% C, 1.89% H, 1.25% N, corresponding to a calculated selector density of: 271 μmol g^{-1} CSP (based on carbon) or 298 μmol g^{-1} CSP (based on nitrogen).

CSP III: 12.23% C, 1.64% H, 0.89% N, corresponding to a calculated selector density of: 245 μmol g^{-1} CSP (based on carbon) or 212 μmol g^{-1} CSP (based on nitrogen).

As an additional step ''end-capping'' of the remaining unmodified thiol groups was performed. Thus, the respective modified silica material suspended in 100 ml chloroform together with 2 ml 1-hexene and 200 mg AIBN was refluxed for 12 h as described above, washed, dried and sieved. Elemental analysis of the dried modified silica materials gave the following results:

CSP I: 12.33% C, 1.78% H, 1.07% N; CSP II: 13.27% C, 1.97% H, 1.25% N; CSP III: 13.23% C, 1.81% H, 0.90% N.

It was calculated that between 75 and 80 μmol of the remaining thiol groups per g CSP have been modified with hexyl groups in the ''end-capping'' procedure.

More details about the syntheses of the chiral selectors and CSPs are given elsewhere [6].

CSPs I–III were packed into stainless steel columns (150×4.0 mm I.D.) by a conventional slurry packing procedure at the Forschungszentrum Seibersdorf, Austria.

2.3. Instrumentation

Chromatography was performed using an HP 1050 liquid chromatograph from Hewlett-Packard (Vienna, Austria), consisting of a low-pressure quaternary gradient pump, an autosampler, a variable-wavelength detector and an HP Chemstation (with Windows 3.11) for data evaluation. The pH of the mobile phase was measured with an Orion pH meter Model 520A (from Orion, Vienna, Austria) and represents the apparent pH (pH_a) values measured in the hydro-organic mobile phase mixture.

2.4. Chromatographic conditions

A mixture of methanol–0.1 *M* ammonium acetate buffer (80:20) was used as mobile phase. The pH_a of this mixture was adjusted to 6.0 by adding analytical-reagent grade glacial acetic acid. Mobile phases were filtered through a Nalgene nylon membrane filter (0.2 μm) from Nalge (New York, NY, USA) and degassed. Flow-rate was 1 ml min^{-1} at ambient temperature and a UV detection wavelength of 254 nm was chosen.

3. Results and discussion

Like previously tested CSPs derived from QN and/or QN carbamates operated under aqueous buffered mobile phase conditions (working pH range between 4 and 7) CSPs I–III can also be classified as

weak anion exchangers but showing different enantioselectivities for N-acyl and N-aryl derivatives of α- and β-amino acids and other acidic compounds depending on the conditions and more so on the SO–SA structure variations.

First the influence of the buffer concentration in the mobile phase on retention and enantioselectivity of the SAs was investigated for CSP I (Fig. 3). Generally, solute retention decreases with increasing buffer concentrations (Fig. 3a) while enantioselectivity remained more or less unaffected (Fig. 3b). These results are in agreement with the behavior previously found for QN carbamate type anion exchangers [7] and clearly establish anion-exchange as primary retention mechanism.

Representative and conclusive enantioseparations of SAs with various structural features on CSPs I to III are listed in Table 1 and depicted in Figs. 4 to 6.

Although the free secondary hydroxyl group of the QN selector (CSP I) and the amide function of the QN carbamate selector (CSP II) represent a hydrogen donor site, these potential intermolecular interaction sites differ in directionality and/or rigidity, in spatial arrangement and environment, in solvation/desolvation energy as well as in hydrogen-bond acidity/basicity. Thus, the favorable influence of the unique carbamate function compared to the hydroxyl interaction site leads to an unexpected switch of the stability of the SO–SA diastereomers accompanied with an extraordinary increase of α values of N-acyl amino acid SAs (amide group attached to the stereogenic center). This effect is demonstrated by

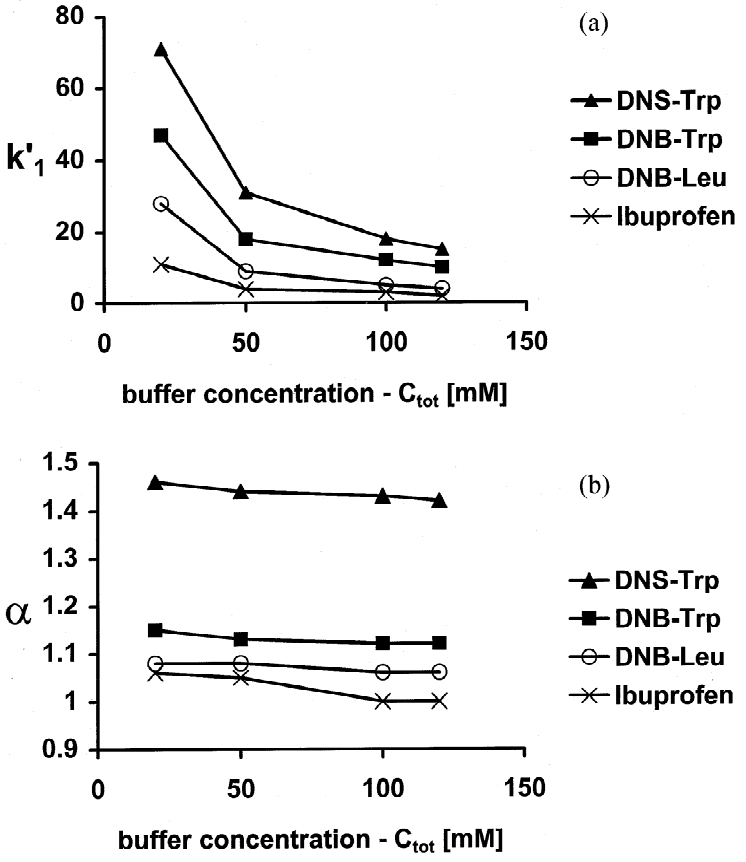
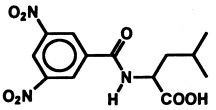
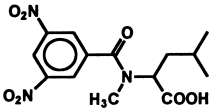
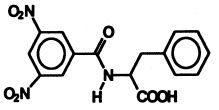
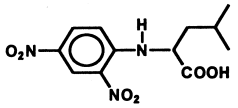
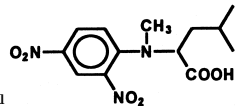
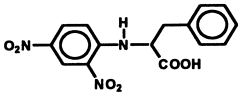
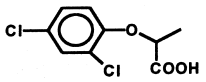
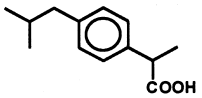


Fig. 3. Influence of buffer concentration on (a) retention and (b) enantioselectivity of selected analytes on CSP I. Chromatographic conditions: mobile phase: methanol–ammonium acetate (80:20), pH_a 6.00, flow-rate 1 ml min^{-1}, ambient temperature; indicated values of buffer concentration refer to the total buffer concentration in the mobile phase mixture.

Table 1
Chromatographic retention and enantioselectivity data of diverse acidic compounds on CSP I, CSP II and CSP III[a]

Compound	CSP I			CSP II			CSP III		
	k_1'	α	e.o.[b]	k_1'	α	e.o.[b]	k_1'	α	e.o.[b]
DNB-Leu 	15.66	1.15	*S*	11.73	15.87	*R*	9.54	1.59	*R*
DNB-N-Me-Leu 	12.31	1.04	n.d.[c]	10.90	1.07	*R*	8.24	1.02	n.d.
DNB-Phe 	17.80	1.05	*S*	16.19	10.78	*R*	12.48	1.58	*R*
DNP-Leu 	26.39	1.35	*S*	24.01	1.31	*S*	16.37	1.54	*S*
DNP-N-Me-Leu 	19.18	1.15	*S*	23.30	1.38	*S*	15.62	1.43	*S*
DNP-Phe 	41.25	1.30	*S*	39.94	1.28	*S*	18.27	1.67	*S*
Dichlorprop 	12.84	1.15	*S*	10.94	1.19	*S*	8.25	1.33	*S*
Ibuprofen 	5.79	1.06	*S*	5.78	1.00	–	4.46	1.08	n.d.

[a] For chromatographic conditions see Section 2.
[b] e.o.=Elution order: configuration of the first eluted enantiomer.
[c] n.d.=Not determined.

the chromatograms in Fig. 4 exemplifying the separation of DNB-Leu enantiomers on CSPs I and II. In contrast to QN carbamate type CSPs represented by CSP II, CSP I showed much lower enantioselectivity for DNB derivatives of amino acids (1.15 versus 15.87 on CSP II for DNB-Leu). Clearly, the carbamate gains stereodirecting character and provokes a change of the chiral recognition mechanism indicated by the reversal of elution order on QN carbamate type CSPs (higher affinity of *S*-enantiomer of DNB-Leu on QN carbamate CSP II whereas on QN CSP I the *R*-enantiomer has higher intrinsic affinity to the chiral SO).

Obviously, hydrogen bonding is of minor impor-

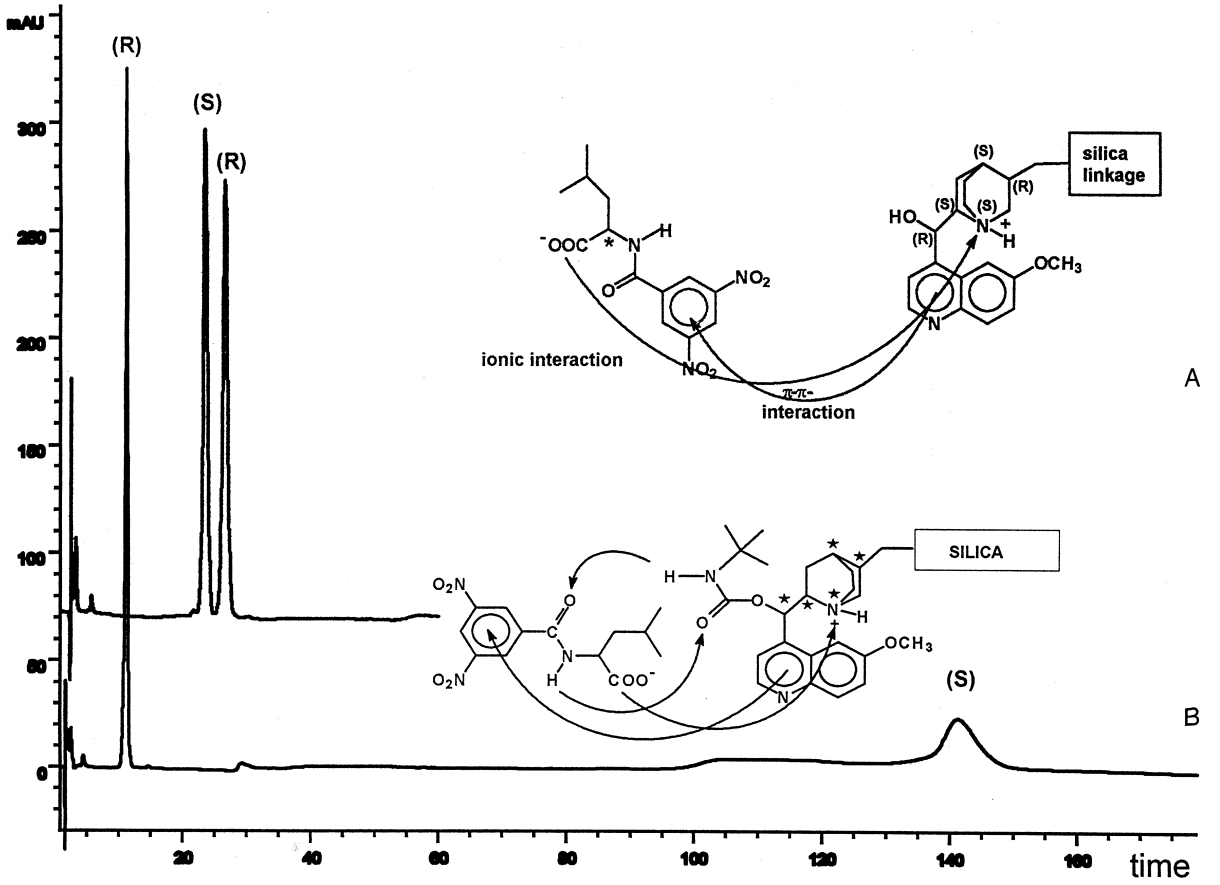


Fig. 4. Chromatograms of the enantioseparation of DNB-Leu on (A) CSP I and (B) CSP II with proposed sites of intermolecular interaction between selector and selectand, for chromatographic conditions see Section 2.

tance in the case of DNP amino acids, like DNP-Leu, and aryloxy carboxylic acids, like dichlorprop, etc. as there is no change in elution order and the α values remain relatively similar for all three CSPs (see Table 1). On CSP I, besides the strong but non-stereoselective ionic interaction, only the charge-transfer interaction site between the quinoline moiety of the SO and the DNB, DNP or aryl(oxy) substituent of the SA seems to dominate the overall binding mechanism.

The ambivalent character (hydrogen donor and acceptor properties) of the rigid amide and carbamate groups give rise to the outstanding α values of DNB-Leu on CSP II in combination with other intermolecular SO–SA interactions as ionic interaction and co-operatively acting $\pi-\pi$ interaction (see Fig. 5A). Upon methylation of the SO's carbamate group the hydrogen donor properties are lost and consequently enantiorecognition ability of CSP III for DNB-Leu decreases dramatically from $\alpha=15.87$ to $\alpha=1.57$ (compare chromatograms A and B in Fig. 5). A similar effect has been observed when the SA's amide function is alkylated. The enantioselectivity of e.g., DNB-*N*-methyl leucine is nearly lost both on CSP II ($\alpha=1.07$) and on CSP III ($\alpha=1.03$) (compare chromatograms A and C as well as C and D in Fig. 5). From these experiments it can be conclusively deducted that hydrogen bond formation between the SA's amide group and the SO's carbamoyl group is strikingly important. It seems that one hydrogen

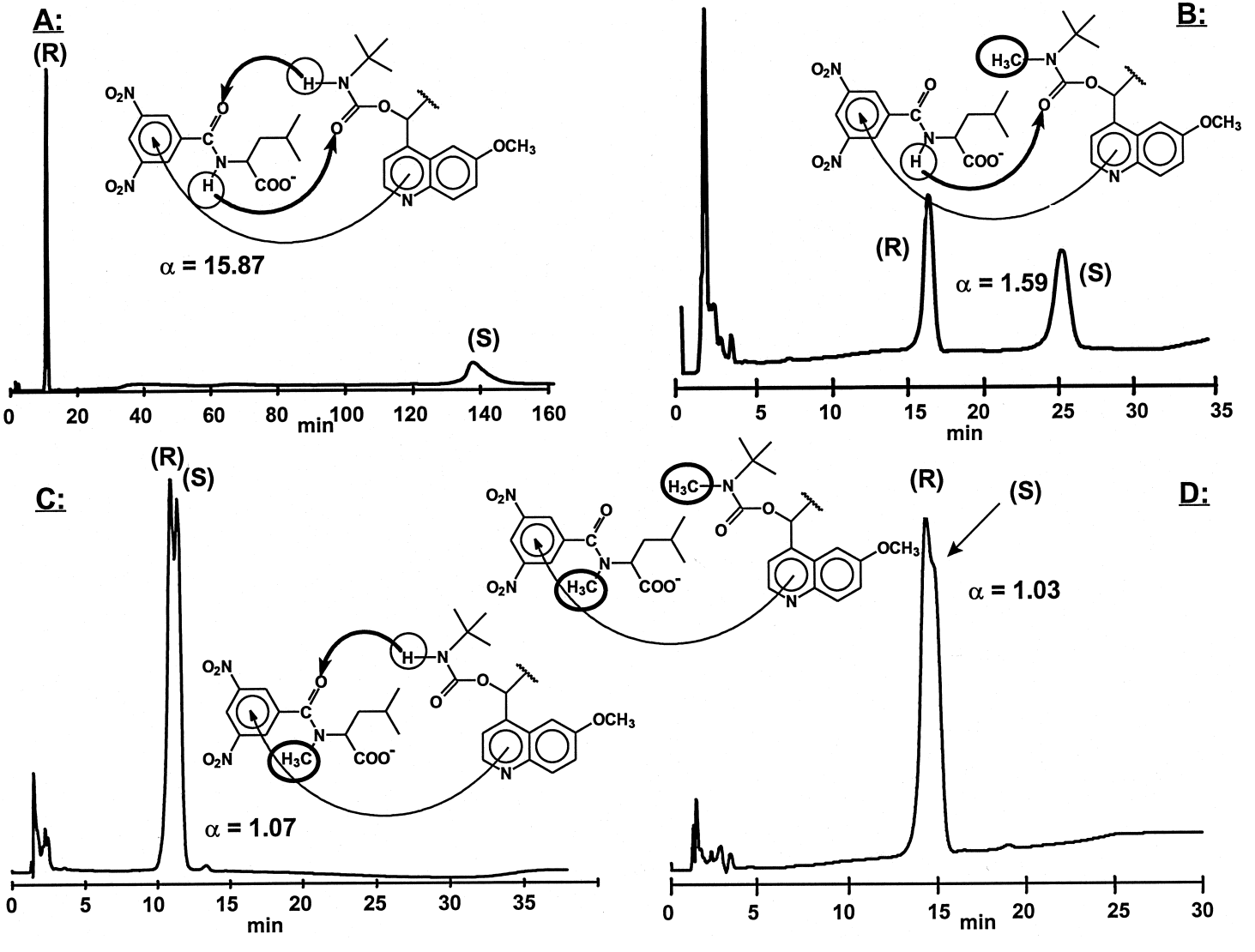


Fig. 5. Chromatograms and tentative SO–SA interactions of the analytes *N*-3,5-dinitrobenzoylated leucine (A, B) and *N*-methyl leucine (C, D) on CSP II (A, C) and CSP III (B, D), for chromatographic conditions see Section 2.

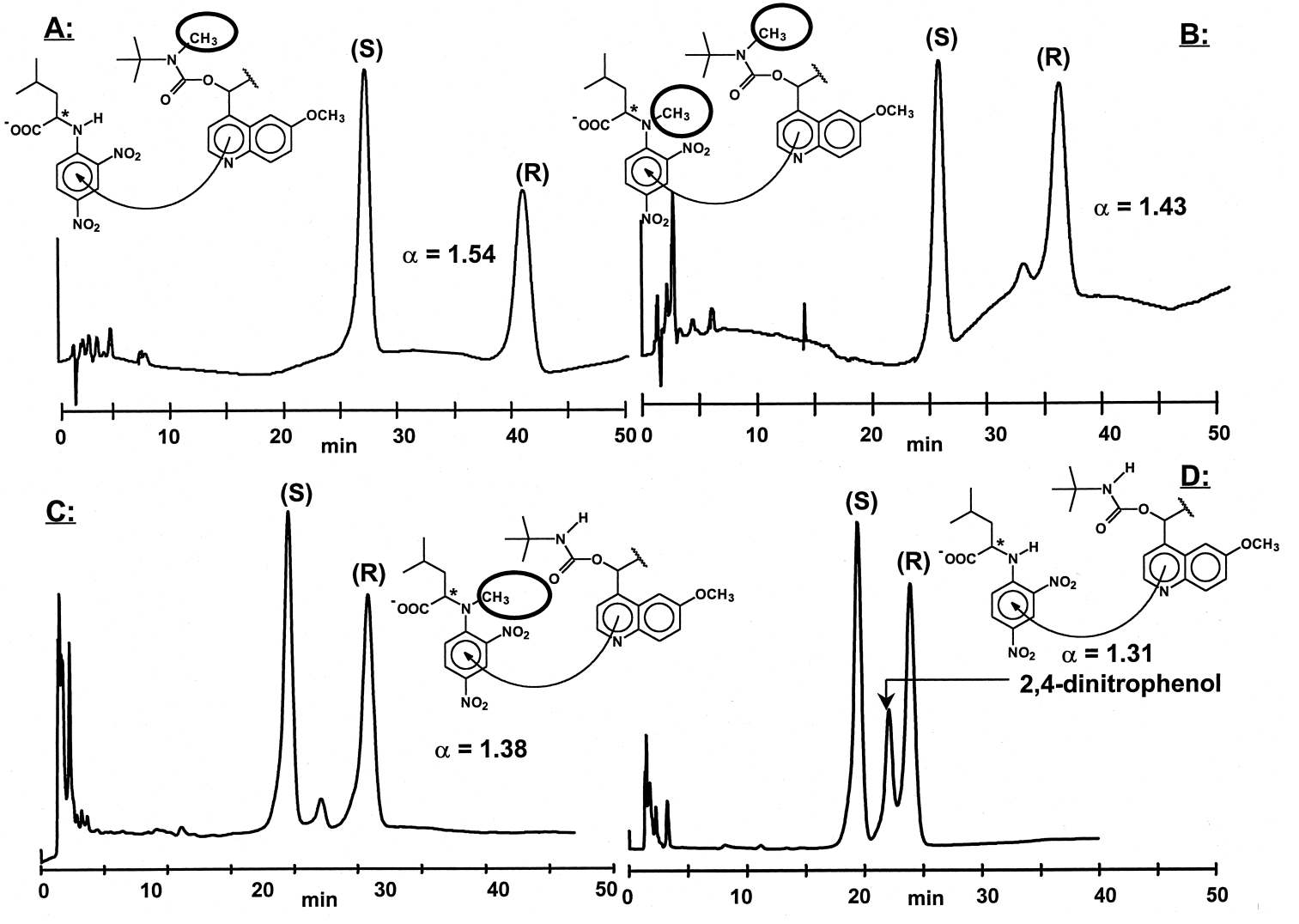


Fig. 6. Chromatograms and tentative SO–SA interactions of the analytes *N*-2,4-dinitrophenyl leucine (A, D) and *N*-methyl leucine (B, C) on CSP II (C, D) and CSP III (A, B), for chromatographic conditions see Section 2.

donor–acceptor conformation of the SO–SA molecule is more favorable with respect to enantioselectivity.

This is convincingly supported by the experiments depicted in Fig. 6: if no or only very weak intermolecular hydrogen bonds between the –NH– of the SA and the SO's carbamate are involved in the overall chiral recognition process for SO–SA complex stabilization, then the N-methylation approach should have no significant effect on α values. Hence, enantioselectivity values for both DNP-Leu and DNP-N-Me-Leu are comparable on both QN carbamate CSPs (CSP II and CSP III). Steric effects might be responsible for the slight differences in α values.

4. Conclusions

Both QN carbamate type CSPs and the CSP derived from unmodified native QN with its free secondary hydroxyl moiety at C_9 operated as anion exchanger with hydro-buffered mobile phases possess stereodiscriminating potential for DNB, DNP and other N-acyl and N-aryl amino acid derivatives including 9-fluorenylmethoxycarbonyl (Fmoc), benzyloxycarbonyl (Z), acetyl (Ac), benzoyl (Bz), dansyl (DNS), 3,5-dinitrobenzoyl (DNB) and 2,4-dinitrophenyl (DNP) beside a broad spectrum of other acidic compounds. Ionic interaction between the negatively charged carboxylate function of the SAs and the positively charged basic nitrogen in the quinuclidine moiety of the SO is the primary driving force for intermolecular SO–SA association. The rigid hydrogen donor acceptor sites of the carbamate group and of the N-acylated amino acid favorably increases the overall stereorecognition capability of QN carbamate type anion exchangers for a wide variety of SAs. Thus both the alkylation of these sites of interaction of the CSPs but also of the amino acid SAs dramatically reduce enantioselectivity. On the other hand, if intermolecular hydrogen bonding is of minor importance for the stereodiscrimination process, N-alkylation has only little effects on stereoselectivity. Overall, the proposed SO–SA binding models are in agreement with data obtained by FT-IR [16], X-ray structure analysis [8] and molecular modeling studies [17]. In any case, the introduction of a carbamate group as substituent to the quinine moiety definitively enlarged the scope of applicability of quinine derived chiral SOs to be used in chromatography. In contrast to the normal-phase operation mode of CSP I presented earlier [9–11], its operation in anion-exchange mode allows the injection of aqueous samples and has the advantage that numerous mobile phase variables (type and concentration of buffer, type and concentration of organic modifier, pH of mobile phase, temperature, etc.) can be used to control overall retention and enantioselectivity.

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