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6,6′-Dinitrobiphenyl-2,2′-dicarboxylic acid ionically bonded to aminopropyl silica: new axially chiral phase of C_2 symmetry for high-performance liquid chromatographic separation of enantiomeric amino alcohol derivatives

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Abstract

(*R*)-(+)-6,6′-Dinitrobiphenyl-2,2′-dicarboxylic acid, ionically bonded to 3-aminopropylsilanized silica (CSP 1), is the first ionically bonded axially chiral phase of C_2 symmetry. The phase is specifically efficient for the separation of a wide range of enantiomeric vicinal benzamido alcohols, whether open-chain, cyclic or polycyclic. To assess the steric and polar effects of the 6,6′-substituents, the performance of CSP 1 is compared with that of the analogous (but less effective) phase CSP 2, based on (*S*)-(+)-6,6′-dimethylbiphenyl-2,2′-dicarboxylic acid. Some conclusions about the relation between the separation effectivity and the analyte structure, as well as about the factors involved in the chiral recognition process, are given.

1. Introduction

During the last decade the preparation and use of chiral stationary phases has represented a very progressive trend in analytical as well as preparative organic chemistry, thanks particularly to the pioneering work of Pirkle and Pochapsky [1] who, using a simple rationale, succeeded in making more or less "tailor-made" chiral stationary phases of wide applicability and good accessibility.

Most chiral stationary phases (CSPs) described so far are based on systems whose asymmetry is derived from one or more *centres* of chirality (*i.e.* asymmetric atoms). Interestingly, only very few chiral phases based on other kinds of chirality (*e.g.* helical or axial) have been examined, in spite of their considerable separation potential. Systems possessing a C_2 symmetry axis offer a special promise for chiral recognition (and separation of the enantiomers) because in this particular case the number of possible competing diastereoisomeric situations in the interaction of the analyte with the phase is strongly reduced (for a discussion and review see ref. 2). To our knowledge, only three C_2 symmetrical chiral stationary phases have been described [3–5] so

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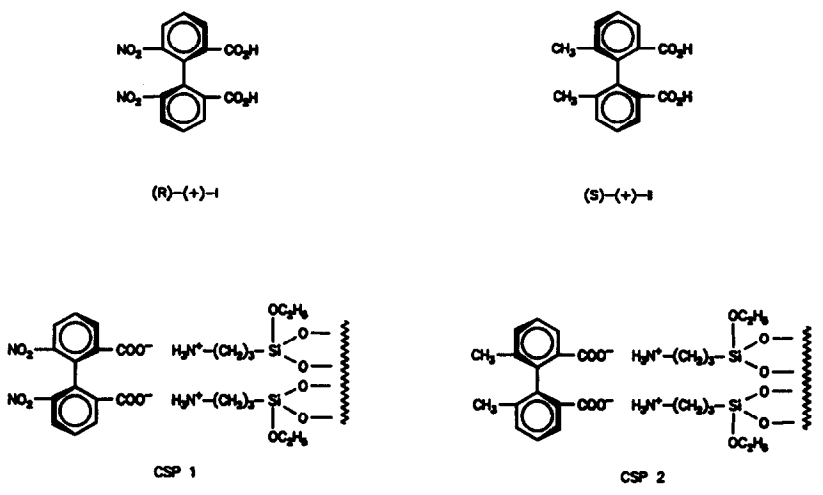

Fig. 1. Structure of (*R*)-(+)-**I**, (*S*)-(+)-**II**, CSP 1 and CSP 2.

far (separating helicenes [3], heterocyclic systems [4] and amines [5])[a].

In this communication we present results obtained with CSPs based on simple biphenyl compounds of C_2 symmetry, (*R*)-(+)-6,6′-dinitrobiphenyl-2,2′-dicarboxylic acid [(*R*)-(+)-I] and (*S*)-(+)-6,6′-dimethylbiphenyl-2,2′-dicarboxylic acid [(*S*)-(+)-II] (Fig. 1), in the separation of benzoyl derivatives of vicinal amino alcohols.

2. Experimental

2.1. General

Liquid chromatography was carried out using a Varian 2510 pump, a Varian 2550 UV detector (detection at 254 nm) and a polarimetric detector (Chiralizer, Knauer).

Analyses were performed on 250 × 4 mm stainless-steel columns (slurry-packed in the usual manner) with 10% 2-propanol in heptane as the mobile phase (flow-rate 0.5 ml/min). Sample solutions (about 1 mg/ml) were introduced via a 20-μl loop. Hold-up times were determined using 1,3,5-tri-*tert.*-butylbenzene.

2.2. Materials

(*R*)-(+)-6′6′-Dinitrobiphenyl-2,2-dicarboxylic acid [(*R*)-**I**] and (*S*)-(+)-6′,6′-dimethylbiphenyl-2,2-dicarboxylic acid [(*S*)-**II**] were prepared and resolved as described previously [12–14]; (*R*)-**I**: m.p. 231–233°C, $[\alpha]_D^{20}$ + 130.1° (*c* 0.5, methanol) reported [13]: m.p. 229–230.5°C and $[\alpha]_D^{20}$ + 127° (*c* 2.4, methanol); (*S*)-**II**: m.p. 212–214°C, $[\alpha]_D^{20}$ + 21.1° (*c* 1.0, methanol) reported [14] m.p. 213–214°C and $[\alpha]_D$ + 22.1° (*c* 1.0 methanol). 3-Aminopropylsilanized silica was a Tessek product (Separon SGX-NH_2, 7 μm, 1.48 mmol NH_2/g, based on nitrogen analysis). The benzamido and *p*-nitrobenzamido alcohols were mostly available from our previous studies; the pertinent references concerning their preparation and/or physicochemical properties are as follows: compounds **1a**, **1b**, **2b** [15], **2a** [16], **3a–7a**, **10a**, **11a** [17], **8a**, **9a** [18], **12a**, *cis*-**28a** [19], **13a**, **14a** [20], **15b**, **16b**, **16c**, **17a** [21], **18a**, **18g** [22], **19a**, **21a** [23], **19b**, **20b**, **21b**, **22b**, **29a**, **30b**, **32a** [24], **23a**, **24b** [25], **25a**, **26a**, **27a** [26], *trans*-**28a** [27], **30a** [28].

The benzamido acetates were prepared by treating the corresponding benzamido alcohols

[a] Few other CSPs [6–11], derived from axially chiral 2,2′-disubstituted 1,1′-binaphthyls but attached to the support by only one of the naphthalene rings, have been described. These, however, do not possess C_2 symmetry and thus do not offer the advantage of reducing the number of competing diastereoisomeric situations.

with acetic anhydride in pyridine at room temperature overnight.

2.3. Preparation of stationary phases

A solution of the appropriate acid (2.3 mmol) in methanol (15 ml) was mixed with a slurry of Separon SGX-NH_2 (3.0 g, 4.44 mmol) in methanol (10 ml). The mixture was shaken intermittently for 1 day at room temperature, filtered, and the adsorbent was washed with methanol, acetone, ether and pentane (70 ml each) and dried at 50–60°C *in vacuo*. The washings were evaporated and the recovered unreacted acid was accurately weighed. The amount of acid retained on the adsorbent was obtained by subtracting the weight of the recovered acid from that originally applied. For CSP 1 the content of (*R*)-**I** was 0.46 mmol/g; for CSP 2 the content of (*S*)-**II** was 0.47 mmol/g.

3. Results and discussion

The ionic stationary phases CSP 1 and CSP 2 (Fig. 1) were prepared by simple treatment of 3-aminopropylsilanized silica with solutions of acids (*R*)-**I** and (*S*)-**II**, respectively, packing the columns and washing with 10% 2-propanol in heptane until the baseline was steady. No leaching, substantial change in retention times or loss of effectivity of the columns was observed during 8 months' daily performance using mostly 10% 2-propanol in heptane as the mobile phase[a] (see Table 1).

Since the sorbent retained less than 0.5 equivalents of the acids per equivalent of the NH_2 groups originally present, we suppose that both the carboxyl groups are ionically bonded to the neighbouring amino groups as depicted in Fig. 1 and that the whole system therefore meets the requirements of C_2 symmetry.

The acids (*R*)-**I** and (*S*)-**II** represent a pair of compounds in which the groups in positions 6 and 6′ differ much in polarity: this should give some information about the polar and steric factors involved in the chiral recognition in the studied biphenyl system. The fact that the acids I and II differ in their absolute configuration (different arrangement about the chiral axis) is of course immaterial. We have found that CSP 1 specifically resolves benzamido derivatives of vicinal amino alcohols.

There are only scattered reports on chiral separation of amino alcohols [30–39], mostly with emphasis on β-blockers. No systematic study has been described so far concerning this important group of compounds.

Having a large collection of model vicinal amino alcohols and their derivatives, we were able to investigate in more detail the relation between structure of the analytes and their separation behaviour. Compounds analysed on the phases CSP 1 and CSP 2 are listed in Fig. 2 and the HPLC analytical results are given in Table 1.

The phase CSP 1 resolves benzoyl derivatives of vicinal amino alcohols, irrespective of whether they are acyclic (*threo* or *erythro*) or cyclic (*trans* or *cis*, common or large rings), the resolution being invariably much better than on CSP 2. Under the same conditions, *p*-nitrobenzamido derivatives have a longer retention time and are somewhat better separated than the benzamido derivatives. In the cyclic series, *trans*-isomers of compounds with smaller rings have greater α-values than the *cis*-isomers; this difference decreases with increasing ring size. For many of the cyclic benzamido alcohols investigated baseline separation was achieved. As expected, the elution time becomes shorter with increasing ring size.

In the case of a mixture of *cis*- and *trans*-2-aminocyclohexanol derivatives (**2a**) it is possible to separate both diastereoisomers into enantiomers simultaneously in one single analysis, the chromatogram containing two doublets owing to

[a] The acid I is known to be completely optically stable at room temperature. Its racemization, together with decomposition, is reported [29] to take place only under drastic conditions (140–160°C) in a strongly alkaline medium. No data on racemization of the acid II are available, nevertheless the fact that over 8 months the performance (retention times and separation coefficients) of the CSPs remained unchanged shows that at room temperature both the CSPs are stable in all respects.

Table 1
Chromatographic data for separation of enantiomeric benzamido alcohols on CSP 1 and CSP 2

Compound	CSP 1		CSP 2	
	k_1' (sign)	α	k_1' (sign)	α
cis-**1a**	4.12 (+)	1.09		
trans-**1a**	9.00 (−)	1.14 (B)		
trans-**1d**	8.12 (+)	1.18 (B)	4.75 (−)	1.18
trans-**1e**	5.50	1.0[a]		
cis-**2a**	4.12 (+)	1.15	2.25	1.0
cis-**2b**	6.50 (+)	1.27 (B)	4.62	1.0[a]
cis-**2e**	8.75	1.0[a]	1.87	1.0[a]
trans-**2a**	6.12 (−)[b]	1.36 (B)	2.62 (+)[c]	1.24
	5.75 (−)	1.35 (B)[d]	2.87 (+)	1.26[d]
trans-**2b**	9.37 (−)	1.50 (B)		
trans-**2e**	3.25	1.0[a]	0.87	1.0[a]
			1.75[e]	1.0[a]
trans-**2f**	2.12	1.0[a]		
cis-**3a**	4.12 (+)	1.15		
cis-**4a**	3.62 (+)	1.12		
trans-**4a**	4.25 (+)	1.35 (B)		
trans-**5a**	3.37 (+)	1.22 (B)		
cis-**6a**	3.12 (+)	1.12 (B)	1.62 (−)	1.15
trans-**6a**	3.12	1.24 (B)	1.75	1.0[a]
cis-**7a**	2.75 (+)	1.14 (B)	1.50 (−)	1.08
trans-**7a**	2.87 (+)	1.22 (B)	1.62	1.0[a]
cis-**8a**	2.50 (+)	1.20 (B)		
trans-**8a**	2.62 (+)	1.18 (B)		
trans-**9a**	2.62 (+)	1.14 (B)		
cis-**10a**	2.25 (+)	1.11 (B)		
trans-**10a**	2.37 (+)	1.21 (B)	1.12	1.0[a]
cis-**11a**	2.00 (+)	1.12		
trans-**11a**	1.87 (+)	1.20		
cis-**12a**	1.50 (+)	1.17	0.75	1.0[a]
			2.25[e]	1.0[a]
trans-**12a**	1.50 (+)	1.17	0.75	1.0[a]
			2.25[e]	1.0[a]
trans-**13a**	2.87 (−)	1.13	1.37	1.0[a]
cis-**14a**	2.50 (+)	1.10	1.25	1.0[a]
			3.62[e]	1.0[a]
trans-**14a**	2.37 (+)	1.05	1.25	1.0[a]
			3.75[e]	1.0[a]
erythro-**15b**	9.62 (+)	1.16 (B)	7.00	1.0[a]
threo-**16b**	3.12 (+)	1.16 (B)	2.12	1.0[a]
erythro-**16b**	3.12 (+)	1.24 (B)	1.87	1.0[a]
erythro-**16c**	1.25 (+)	1.20 (B)		
threo-**17a**	2.12	1.0[a]	1.12	1.0[a]
erythro-**17a**	2.00	1.0[a]		
threo-**18a**	8.12 (−)	1.17 (B)	3.50 (+)	1.07
erythro-**18a**	5.25 (−)	1.12	2.62	1.0[a]
threo-**18e**	5.00	1.0[a]		
erythro-**18e**	8.75	1.0[a]		
erythro-**18g**[f]	9.50	1.0[a]		
19a	2.50 (+)	1.10	1.50	1.0[a]
19b	3.75 (+)	1.23 (B)	2.75	1.0[a]

Table 1 *(continued)*

Compound	CSP 1		CSP 2	
	k_1' (sign)	α	k_1' (sign)	α
20b	5.25 (−)	2.05 (B)	2.62 (+)	1.24 (B)
20e	15.75	1.0[a]		
21a	4.87 (−)	1.05	1.50	1.0[a]
21b	11.87 (−)	1.07	4.62 (−)	1.05
22b	6.00 (−)	1.52 (B)	3.37 (+)	1.18
23a	2.87	1.0[a]		
24b	5.00 (+)	1.55 (B)	2.50	1.0[a]
25a	3.75	1.0[a]	1.75	1.0[a]
26a	7.62 (+)	1.15 (B)	3.12 (−)	1.08
27a	7.75	1.0[a]	2.12 (−)	1.11
cis-**28a**	3.25	1.0[a]	1.87	1.0[a]
trans-**28a**	8.37 (+)	1.10 (B)	3.25	1.0[a]
trans-**28e**	3.62	1.0[a]	1.00	1.0[a]
29a	4.25 (−)	1.26	1.75 (+)	1.21
30a	5.87	1.0[a]		
30b	14.62	1.0[a]		
31a	3.50 (−)	1.32 (B)	1.75 (+)	1.21

Mobile phase: 10% 2-propanol in *n*-heptane, flow-rate 0.5 ml/min. The capacity factor (k_1') refers to the first enantiomer eluted (its sign of rotation is given in parentheses), and the separation factor (α) is the ratio of capacity factors of the enantiomers (B denotes baseline separation). No attempts were made to optimize the analytical conditions for the individual compounds.

[a] No separation.

[b] (1*R*)-*trans*-2-Benzamidocyclohexanol.

[c] (1*S*)-*trans*-2-Benzamidocyclohexanol.

[d] Measured after 8 months of performance.

[e] Mobile phase: 5% 2-propanol in *n*-heptane.

[f] (+/−)-N-Benzoylephedrine.

separated enantiomers of the *cis*- and *trans*-isomer (Fig. 3).

The effect of conformation on the separation behaviour can be followed by comparison of compounds **19–32**, which have fixed geometry and exist in one conformation. As can be seen, on CSP 1 the diequatorial *trans*-isomers **22b**, **24b**, **29a** and **32a** are well separated, whereas the *trans*-diaxial isomers (**21a**, **21b**, **23a**, **30a**, **30b**) are separated poorly, if at all. Also, the twistane *trans*-isomer (**27a**), in which the dihedral angle between the functional groups is 150°, is not separated on CSP 1, whereas with the *trans*-90° isomer (**26a**) we observed a baseline separation. Interestingly, the generally less effective CSP 2 separates not only the twistane derivative (**26a**) but also the isomer (**27a**), being thus complementary to CSP 1. The bicyclo[2.2.2]octane derivative *trans*-**28a** (dihedral angle 120°) is baseline separated on CSP 1, whereas the epimeric *cis*-**28a** (about 0°) shows only an inflex (neither of them was separated on CSP 2). No separation was observed for the twistane *cis*-isomer (**25a**) with 30° dihedral angle. On the other hand, the *tert.*-butylcyclohexane *cis*-derivatives (**19b** and **20b**) (with approximately 60° dihedral angle) are separated completely. Summing up, CSP 1 resolved all benzamido alcohols of our set that have dihedral angles from about 60° to about 120°.

The elution order of enantiomers separated on CSP 1 is reversed (with the exception of **21b**) as compared with the order observed on CSP 2 (which is of opposite chirality). This suggests the same type of chiral recognition on both columns, which in the case of CSP 1 is apparently enhanced by polar operation of the nitro groups.

The analytes have two potential hydrogen

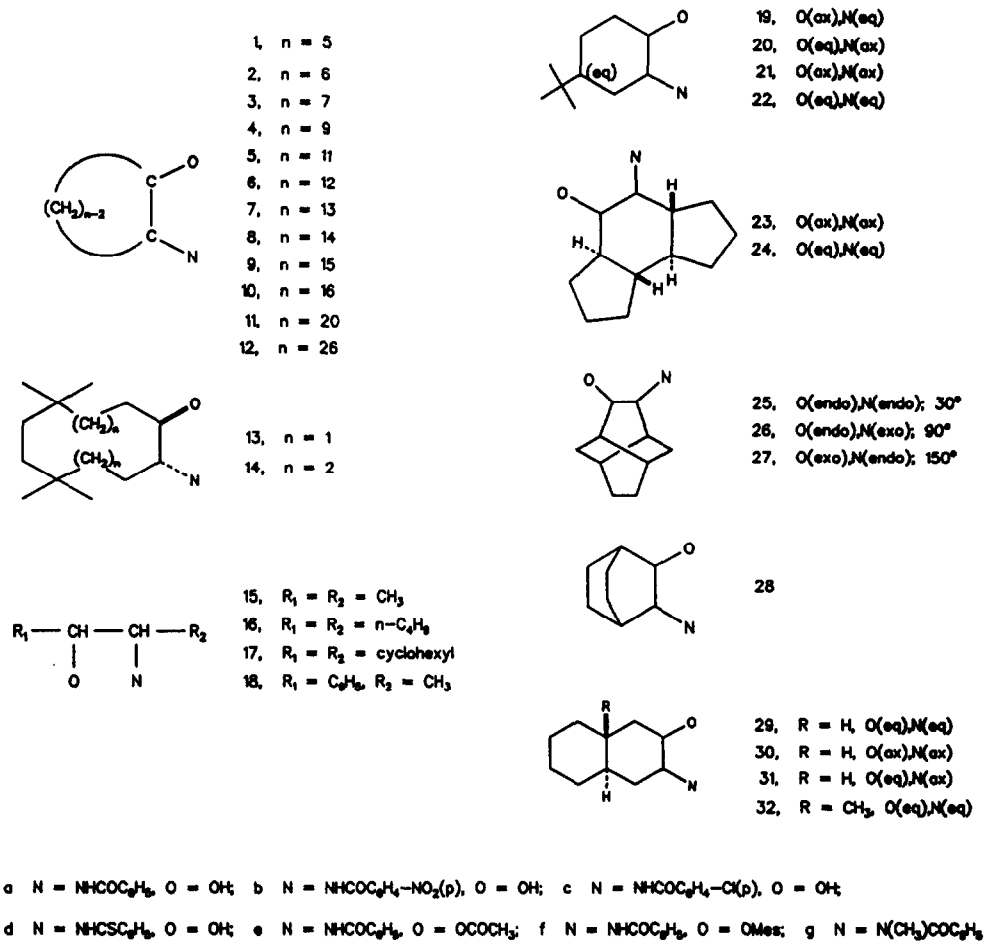

Fig. 2. Amino alcohol derivatives studied.

bonding sites —the hydroxy and the benzamido groups— which can play an important role in chiral recognition. In order to assess the importance of hydrogen bonding between the CSP and the hydroxyl group in the analyte, we converted some of the benzamides into benzamido acetates (*trans*-**1e**, *cis*- and *trans*-**2e**, *threo*- and *erythro*-**18e**, **20e**, *trans*-**28e**, **29e**). None of them was separated on CSP 1 or CSP 2. The same result was obtained with the mesylate *trans*-**2f**. Evidently, the presence of a free hydroxyl group is essential for separation. The inseparability of racemic N-benzoylephedrine (*erythro*-**18g**) indicates that the presence of a hydrogen on the amidic nitrogen atom is equally important.

Although it would be at present premature to draw any conclusions about the mechanism of chiral recognition on the CSPs described, it is evident that CSP 1 represents a wide-ranging chiral phase for separation of vicinal benzamido alcohols of various structural types. Further investigation in the direction of separator systems based on C_2 symmetry may thus be fruitful and is under way.

4. Acknowledgements

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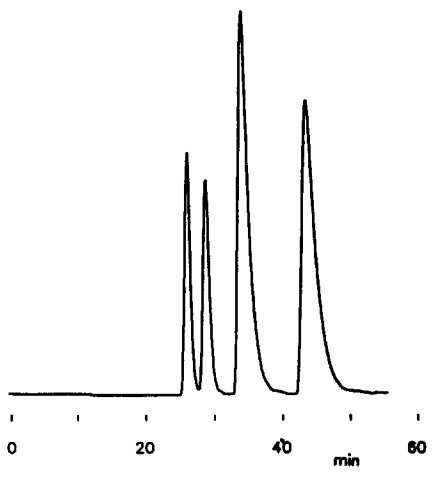

Fig. 3. Simultaneous separation of enantiomers of *cis*- and *trans*-2-benzamidocyclohexanols (*cis*- and *trans*-**2a**) on CSP 1. For chromatographic conditions see Table 1.

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